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ORIGINAL RESEARCH PAPER

Significance of stigma receptivity in intergeneric cross-pollination of *Salix* × *Populus*

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Abstract
The pollen–stigma interaction plays an important role in reproductive process and has been continuously studied in many interspecific and intergeneric crossing experiments. The aim of this study was to investigate stigma receptivity (SR) of willow in order to determine the most suitable period for its pollination with poplar pollen and improve the effectiveness of *Salix* × *Populus* crosses. Tissue samples were examined histologically using light, epifluorescent, scanning, and transmission electron microscopy. Willow SR was determined by stigma morphological traits, test of pollen germination rate, Peroxtesmo test of peroxidase and esterase activity on stigma surface as well as papilla ultrastructure at anthesis. We have ascertained that the SR duration in willow is short, lasting from 1 to 2 DA. The poplar pollen germination rate on willow stigmas on 1 DA ranged from 26.3 to 11.2%.

Keywords
Salix; *Populus*; stigma receptivity; dry papillae; pollen tube germination

Introduction

In Europe, there are 65 to 70 members of the genus *Salix* L. (Salicaceae), depending on the system of classification. The dioecious species, with separate male and female plants, flower abundantly in early spring (March, April). Their inflorescences form catkins, contain numerous reduced pistillate or staminate flowers [1–3]. Among the most frequent woody species in Poland are *Salix cinerea*, *S. fragilis*, *S. purpurea*, and *S. viminalis*, that differ in morphology and their role in ecosystems. These species often naturally hybridize interspecifically and represent a high degree of phenotypic plasticity [3]. *Salix purpurea* and *S. viminalis* in particular, are widely grown in Europe for traditional usage (stabilization of river banks, fences, furniture, basketry, and valuable medicaments as tannin and salicin [4]). Recently, the common willow (*S. viminalis*) has become an industrial crop, important for biomass production within the framework of European Willow Breeding Programs. Breeding of willows involves interspecific hybridization or selection of natural hybrids [5–8]. Some research on willows has already been carried out in Poland to increase the degree of genetic variability within the species [9–12]. However, some aspects of the flowering biology and stigma receptivity in *Salix* are still insufficiently recognized.

A comprehensive study of the stigma as an area assuring pollen adhesion [13,14], pollen identification [15–17], hydration [18], germination, and guidance of pollen tube [17–19] has been underway for about one hundred years. All stigma features, morphology, anatomy, ultrastructure, and physiological aspects of its growth and development have been described for agricultural crops as well as model species [20–23]. However, the information is scarce on the duration of stigmatic papillae

receptivity and on their effect on the length of effective pollination period [17]. Duration of stigma receptivity (SR) differs among species and can be affected by environmental conditions [20,21]; under low temperature the extension of SR and pollination period occurs, under high, acceleration of stigma disintegration takes place [24–26]. Short life-span of stigmas preserves them from microbiological infections [20,21]. Considering SR in ecological aspects, its "secondary sexual character" is important for efficient sexual selection as they play a role in the choice of mating partners [27]. SR affects male competition during pollination and female choice by suitable reception of pollen [25,28,29]. A special case of SR prolongation represents a multi-pistillate inflorescence with developmental shift of anthesis and thus with obvious stigmatic asynchrony [17].

Morphological traits of stigmas as markers of their receptivity have been assessed for some species, however, the only accurate method to prove SR is pollen germination test and seed-set registration [27–31]. Other methods of determining receptivity focus on indication of the activity of enzymes such as esterases, peroxidases, and acid phosphatases, on the stigma surface [32–35]. Stigma receptivity should be particularly analyzed in order to: (***i***) find out precisely the anthesis stage of inflorescence/flower/pistil for hand cross-pollination; (***ii***) indicate the pre- or post-receptive stage of stigmas for improved pollination efficiency; (***iii***) study the pollen/stigma incompatibility; (***iv***) propose a new/special breeding system [34,35].

In the present paper, the analysis of SR was carried out in four willow species at consecutive stages of catkin anthesis. This study was to increase the knowledge of SR in willow during experimental mating between *Salix* and *Populus* in order to plan future steps towards the successful breeding of hybrids. This was important in the light of increasing interest in the breeding system of interspecific or intergeneric hybrids that could become improved cultivars for biomass production.

Material and methods

Plant material

Female flower-bearing shoots from approximately ten-year-old willows of four species (*S. cinerea* L., *S. fragilis* L., *S. purpurea* L., *S. viminalis* L.) and shoots of male poplar (*P. tremula* L.) were harvested from the end of January to the end of March from 2010 to 2014. The experimental material was taken from the shrubs and trees growing on the natural sites in the grounds of the university campus, Poznań, Poland. To synchronize their flowering period all shoots were forced in a culture room (22–23°C, RH 50%, 16/8 h photoperiod, and 250–300 μmol m^{-2} s^{-1} using cool white fluorescent tube light).

Morphological characters of inflorescences

Samples of female catkins of willow species were randomly collected each day of the 3 days of inflorescence anthesis for morphometric measurement including: (***i***) number of pistils per catkin, (***ii***) size of the pistil, style, lobes, and papillae, (***iii***) min–max size of pollen. Three pistils per selected catkins of four species were excised to evaluate the morphological traits of the stigma from the first to the third day of anthesis.

Viability of poplar pollen

Poplar shoots with male inflorescences were temporarily kept in vials filled with tap water. After 3 weeks of forcing, freshly released mature pollen grains were collected at 9:00 p.m. to evaluate pollen shape, ornamentation, aperture, and viability by staining with DAPI or 2% solution of tetrazolium chloride TTC [30]. At the same time, viability of poplar pollen was tested for germination efficiency in vitro and incubated under dark condition at 25°C on a modified BK liquid medium [31] (saccharose 5%, H_3BO_3

100 mg L^{-1}, $Ca(NO_3)_2$ 300 mg L^{-1}, pH 6.5). Pollen germination was analyzed quantitatively under light microscope for at least 100 grains taken from each sample.

Peroxtesmo KO test of stigma receptivity

For a swift determination of peroxidase activity, a rapid qualitative Peroxtesmo KO test (Machery-Nagel, Dren, Germany) was used according to recommended procedure [32]. A droplet of the test solution was applied to stigmas on both the preanthesis day and the next 2 days of anthesis. After 3–5 min at 22°C the papillae of the collected pistils stained dark blue and were regarded as receptive when most of stigma surface was dyed. Each treatment was carried out with ten pistils in three replicates. Stigma surface was photographed using an Olympus stereomicroscope. The catkins were considered as receptive when more than half of all pistils was stained.

A second, simpler and less expensive procedure, showing stigma receptivity through the presence of hydrogen peroxide (H_2O_2), was the Perex Test (Merck Chemical 16206), in which the appearance of air bubbles and the pale-yellow color of the solution droplet indicated a presence of peroxidases [33] and esterases [34,35].

Pollination test

To monitor the duration of SR, we used delayed pollination (1 DA – 3 DA) for checking the ability of stigma to induce pollen germination. Hand cross-pollination was performed by depositing fresh pollen of *Populus* over the stigma of willow species including four sections within the genus *Salix*: *S. cinerea* sect. *Cinerella*, *S. fragilis* sect. *Salix*, *S. purpurea* sect. *Helix*, and *S. viminalis* sect *Viminella*. Using a two-factor randomized design (four willow species × one poplar donor of pollen), pollen germination after four combinations of experimental design was evaluated. Following the Randall procedure of one-stop pollination [36], the mature pistils were hand-pollinated, when half of every willow catkin came into anthesis, i.e., at the "*Salix* growth stage 6.65" according to Saska and Kuzovkina [37]. As a control, the stigmas of *Populus tremula* treated by *P. tremula* pollen was evaluated.

Poplar pollen (adapted to wind transportation) was smooth, had a loose consistency and was easy to use in hand pollination. After 24 h – 1 DA, 36 h – 2 DA, and 48 h – 3 DA stigmas were removed from pistils, squashed in 0.05% aniline blue or DAPI, and examined using epifluorescence microscopy (Zeiss Axioscope, Jena, Germany). Pollen was considered as germinated when the pollen tube had a length that was greater than the diameter of the poplar pollen grain [30,31]. The percentage of pollen grains germinating was calculated on the stigmas of willow and poplar.

Histochemical analysis of papillae

Isolated stigmas (1 DA) were fixed in FAA (formalin / glacial acetic acid / 50% ethanol, 5:5:90 v/v/v) for 24 h, dehydrated in a graded ethanol series (70–100%) followed by butanol treatment, next infiltrated and embedded in Paraplast Plus (Sigma, St Louis, MO, USA). Samples (12 μm thick) were sectioned with a rotary microtome (Leica, Nussloch, Germany) and double-stained with safranine O (1% v/v) and fast green FCF (0.5% v/v; Merck, Darmstadt, Germany), then mounted into entelan. Examination of the slides was made under light microscope (Zeiss Axioscope, Jena, Germany). Histochemical characteristics of samples had been investigated after staining with periodic acid–Schiff's (PAS) reagent (insoluble polysaccharides), Sudan B (lipids), or toluidine blue (proteins) according to Souza et al. [24]. The morphology of stigmas was observed in scanning electron microscope (SEM) and papillae ultrastructure was examined by using transmission electron microscopy (TEM). Samples were fixed with 2% (v/v) glutaraldehyde and 2% (v/v) formaldehyde (pH 6.8; Polysciences, Warrington, USA) for 2 h at room temperature and rinsed three times with cacodylate buffer (0.05 M). Material was then post-fixed in 1% osmium tetroxide and dehydrated in a graded acetone series. For TEM observations, samples were embedded in Spurr's resin and

ultra-thin sections (0.1 µm) were obtained with an Ultracut S Ultramicrotome (Leica-Reichert, Bensheim, Germany). Sections were mounted on formvar-coated copper grids and analyzed with a JEM 1200 EX II transmission electron microscope (Jeol, Tokyo, Japan). For SEM analysis, fixed and dehydrated specimens were critical-point dried (CPD drier, Balzers, Lichtenstein), mounted on metal stubs and sputter-coated with gold particles using a SPD-050 sputter coater. Samples were examined with an Evo 40 Scanning Electron Microscope (Carl Zeiss, Jena, Germany).

Statistical analysis

The mean values of such parameters as pollen viability, stigma receptivity, pollen germination, and range of variation as a standard deviation were calculated.

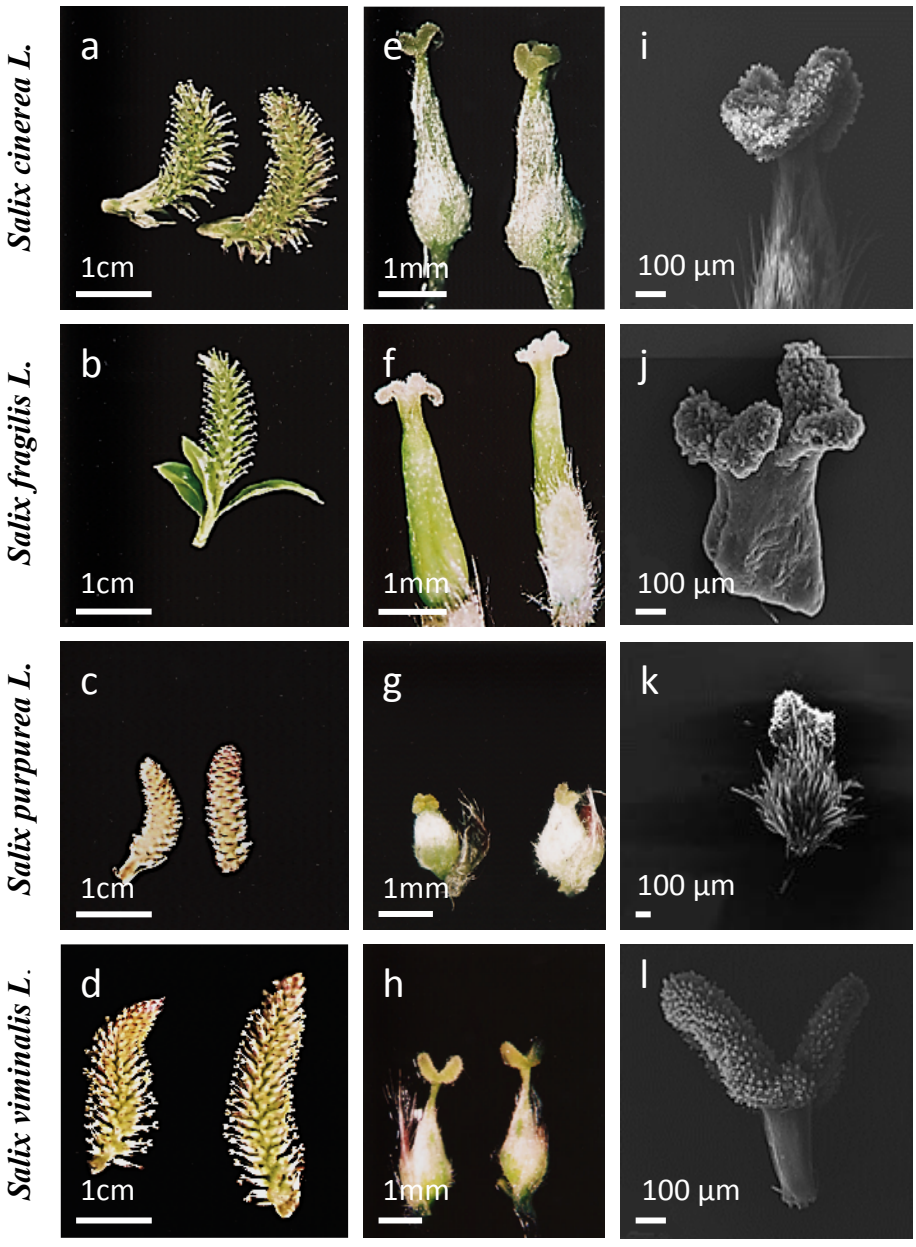

Fig. 1 *Salix* species morphology of inflorescences (**a–d**), pistils (**e–h**), stigmas (**i–l**) as seen in stereomicroscope (**a–h**) and SEM (**i–l**).

Results

Willow flower characteristics

Female inflorescences of willows are unisexual catkins with spiral arrangement of numerous pistillate flowers attached to the central axis (Fig. 1a–d, Tab. 1). Individual flower is built of a single pistil with a short hollow style, dry stigmas and a transparent appendage with nectariferous tissue located at their base (entomophilous type of pollination [2]). The pistil of the genus *Salix* sp. represents a syncarpous, bicarpellate gynoecium, which contains a unilocular ovary with a parietal placentation, on which 6–8–12 anatropous ovules develop, with reduced or absent endosperm. Stigmatic papillae form a continuum with the transmitting tissue covering the inside of the hollow style. The papillae cells occasionally divide and become bi- or three-celled. Structural morphology of stigmas was observed in SEM (Fig. 1i–l).

Morphometric analysis of inflorescences revealed that the female catkins of selected willow species differed reciprocally in their size (Fig. 1a–d) and the size and number of pistils on a single axis (Fig. 1e–h, Tab. 1). The largest catkins with numerous pistils were present in

Tab. 1 Characteristics of selected features of components of cross-pollination of *Salix* × *Populus*.

Feature examined	Female species				Male species
	Salix cinerea	*Salix fragilis*	*Salix purpurea*	*Salix viminalis*	*Populus tremula*
Number of pistils per catkin	164.0 ±21.12	140.7 ±10.15	112.5 ±20.62	179.6 ±20.88	-
Pistil length (mm)	3.84 ±1.27	2.68 ±0.65	2.54 ±0.68	4.22 ±1.92	-
Ovary shape	Pyriform	Obclavate	Obturbinate	Pyriform	-
Style size (mm)	0.88 ±0.114	0.59 ±0.09	0.33 ±0.164	1.78 ±0.29	-
Lobes size (mm)	0.53 ±0.12	0.32 ±0.04	0.13 ±0.02	1.21 ±034	-
Stigma shape	Broad-cylindrical	Flat with rounded tips	Flat with rounded tips	Slender-cylindrical	-
Average papillae size (µm)	42.3 × 14.2	33.7 × 12.2	23.3 × 0.81	38.7 × 14.1	-
Ovules per ovary	12.4 ±1.57	8.6 ±1.89 – 10 ±1.88	6.2 ±2.20	11.4 ±1.35	-
Pollen shape	Subprolate	Prolate-sphaeroidal	Subprolate	Prolate-sphaeroidal	Sphaeroid
Average, min–max size of pollen	28.4, 22.3–29.2	21.1, 16.1–23.2	18.4, 15.8–21.5	23.5, 20.1–24.2	38.4, 33.1–42.6
Pollen ornamentation	Thickly reticulate with tiny tubercles	Deeply reticulate with spiny tubercles	Deeply reticulate with tiny tubercles	Weakly reticulate with tiny tubercles	Smooth, tiny echinulate
Pollen aperture	Tricolporate	Tricolporate	Tricolporate	Tricolporate	Lack

S. viminalis and *S. caprea*, and the smallest, with the lowest number of pistils, in *S. fragilis* and *S. purpurea* (Tab. 1). Therefore, the reproductive potential of willows depends on the catkin size, the number of pistils, and the number of ovules in the ovary (Tab. 1). The morphometric analysis revealed differences in shape, size, and ornamentation between *P. tremula* pollen and the pollen of studied *Salix* species. The pollen of *S. cinerea* was the largest and that of *S. purpurea* the smallest among the willow species.

Viability of poplar pollen

Fresh, smooth, non-aperturate pollen grains of *P. tremula* were larger than these of the four species of willows (Tab. 1). Staining pollen with DAPI revealed 84.61% ±17.1 viable grains and with TTC 68.07% ±14.6 viable grains. Some differences in pollen size were observed. Whilst the normal-sized pollen grains stained, the small ones did not. After sowing on BK 1963 medium [32], poplar pollen germination in vitro started with high percentage (incubation of 8 h – 59.25 ±9.8 and 12 h – 86.6 ±16.4). The pollen tubes were long, straight, and their tips did not burst. The germination rates were high among the normal-size pollen grains; small ones did not germinate. Pollen stored at 4°C maintained viability and high germination percentage for 2 weeks. At room temperature, their viability decreased gradually across the time.

Signaling of stigma receptivity by morphpological traits

Willow stigmas were more or less deeply bilobed at the distal ends. The structure displayed a diversity of forms, ranging from folded to flat, and represented two types, "convolute" – rolled/folded along the lobes (Fig. 1i,l) and "coralliforme" – resembling polyps in colonial corals (Fig. 1j,k, Tab. 1). At the pre-anthesis stage (0 DA), stigma lobes remained twisted and their papillae were pale, short, and adhered to "bent-over" lobes. When pollinated at that time, papillae retained pollen grains but by means of their adhesion only. On the first day of anthesis (1 DA), straightened lobes separated into four parts [morphological marker of willow stigma maturation; (Fig. 2a–h)]. The histochemical analysis performed at that time showed that papillae were covered with a thin cuticle, underneath which the presence of lipids, proteins, and carbohydrates could be detected with different staining methods (Fig. 3a–d). Mature papillae became elongate, turgid, light green, cylindrical, and thin-walled. At this stage they densely covered the stigmas

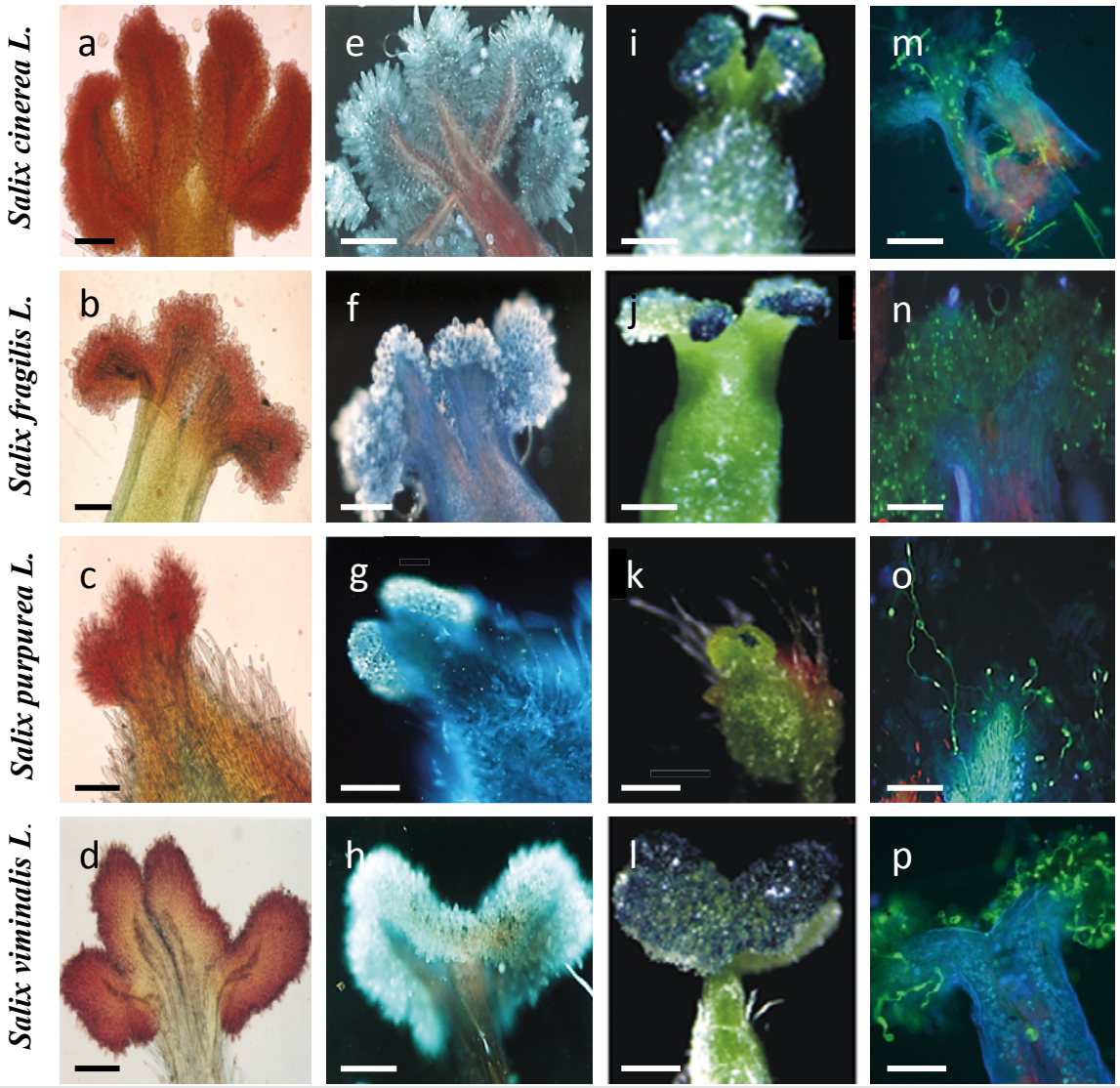

Fig. 2 Stigma structure of four species of *Salix* at the anthesis. **a–h** Straightening and separation of stigma into four lobes at anthesis – in the light and epifluorescence microscopy, stained subsequently with acetocarmine and aniline blue. **i–l** Localization of peroxidase activity in receptive stigmas; binocular microscopy. **m–p** Germination of *Populus tremula* pollen grains on the stigmas of *Salix* at 24 h after hand-pollination. Scale bars: 100 μm.

in all the studied species (Fig. 3e–h). On the second day of anthesis (2 DA), papillae were maximally elongated, vacuolized, and their polyphenolics content increased (Fig. 3i–l). On the third day, papillae lost their turgor and shortly disintegrated. Pollination at this stage resulted in low and impaired germination of pollen grains. To sum up, the morphological traits of willow stigma such as properties of papillae (enlarged, straight, light green, irregular in size, and turgid) positively correlated with receptivity and sufficiently indicated an appropriate time for pollination.

Confirmation of stigma receptivity

The unspecific peroxidase test used to display the maturity stage of stigmas of the four willow species showed that SR lasted there for 2 days. The Peroxtesmo KO test showed a positive and immediate reaction and revealed only slight differences in the intensity of stigma staining among the species. The whole stigmatic surface was stained blue (Fig. 2i–l) only on the first day of anthesis (1 DA 98.2% ±6.2). After this time, the staining intensity became weak (2 DA 43.7% ±4.4). On the 3 DA the lack of stain indicated a significant decrease of receptivity. The Perex Test confirmed that SR in willows lasted 1–2 DA.

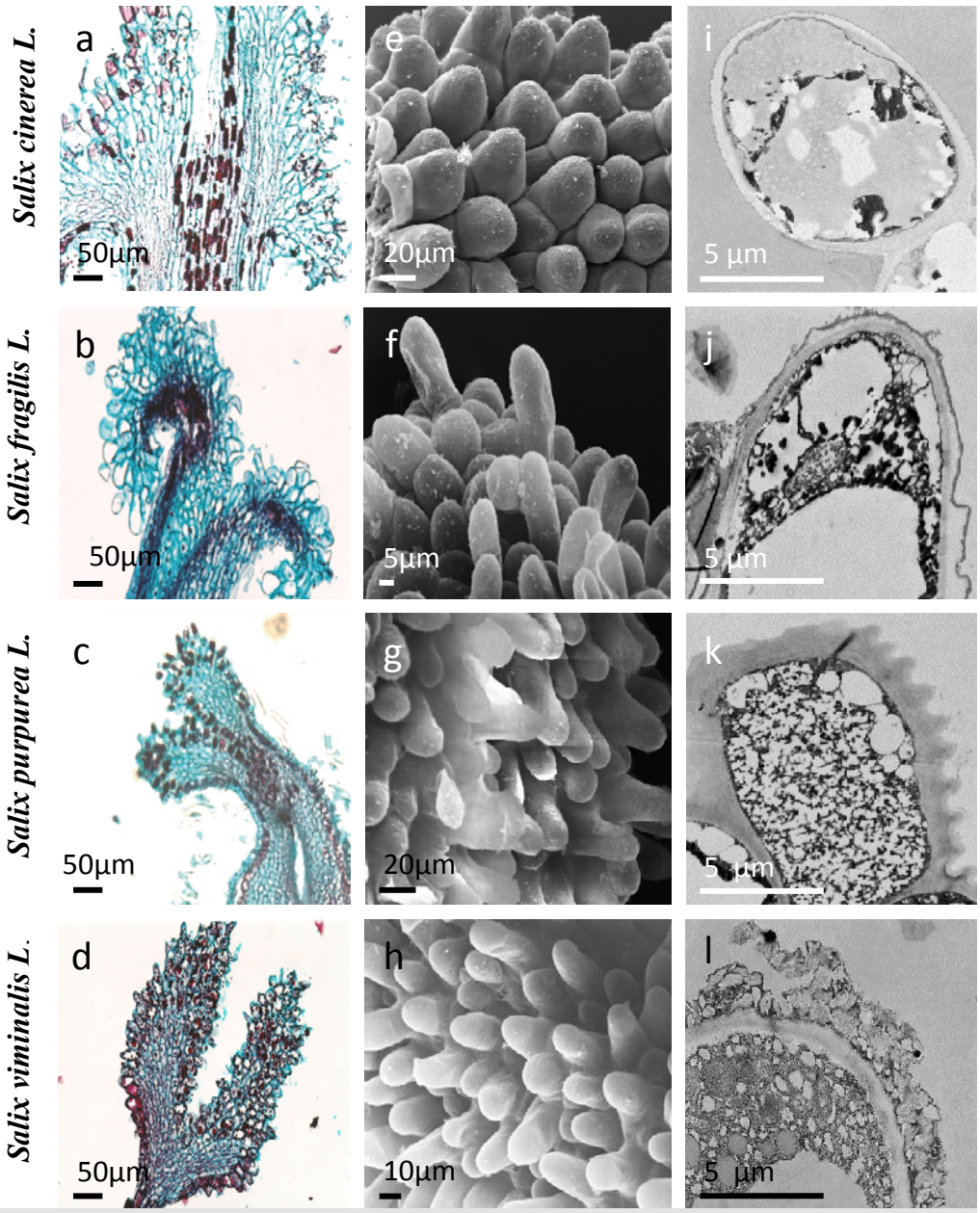

Fig. 3 The *Salix* stigmas at anthesis. **a–d** Longitudinal section of stigmas after histochemical staining. **e–h** SEM view of the receptive surface of mature, unpollinated stigmas, showing differences in elongation of papillae at the receptivity stage. **i–l** Ultrastructure of the upper tip of four willow species in different stages of development (from immature to mature stages) sectioned longitudinally, seen in TEM.

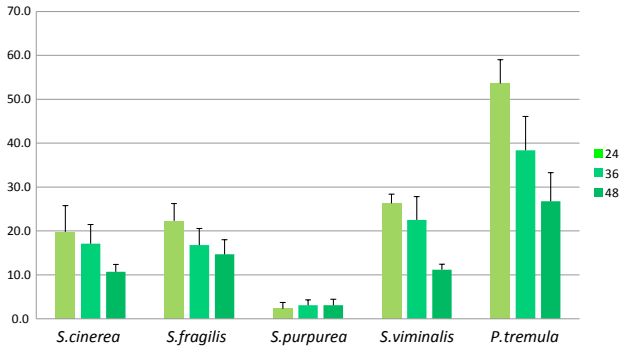

Fig. 4 The poplar pollen germination rate on the stigmas of four willow species during 3 days of anthesis. As a control, female *P. tremula* was pollinated with *P. tremula* pollen.

Using delayed pollination (1–3 DA) we demonstrated the decreasing ability of stigma to support pollen germination (Fig. 2m–p). Moreover, the frequency of poplar pollen germination, expressed as a percentage on the first, second, and third day of willow anthesis, was considerably different among willow species. The highest rate of germination was on the stigmas of *S. viminalis* (1 DA – 26.3%; 2 DA – 22.5%; 3 DA – 11.2%), the medium on the stigmas of *S. fragilis* (1 DA – 23.3%; 2 DA – 16.8%; 3 DA – 14.7%) and *S. cinerea* (1 DA – 19.8%; 2 DA – 17.1%; 3 DA – 10.7%), and the lowest on the stigmas of *S. purpurea* (1 DA – 2.3%; 2 DA – 3.1%; 3 DA – 3.1%). The data recorded at 1–3 DA intervals could prove gradual decrease of germination rate (Fig. 4). However, through comparison of the values of poplar pollen germination

rate on willow stigmas (closely related, but representing different species), with a very high rate of its germination on poplar stigmas (1 DA – 53.6%; 2 DA – 38.4%; 3 DA – 26.8%), the importance of compatibility of the both partners of pollination became apparent. Willow genotype and time of anthesis had significant influence on poplar pollen germination rate during hand cross-pollination of *Salix* × *Populus*.

Ultrastructure of receptive papillae

At the 1 DA stage, single-celled, highly vacuolized papillae (Fig. 5a,b) reached receptivity. Thin cell wall was covered by cuticle and already visible pellicle (membrane-like layer of molecular contact between stigma and pollen grains [29]; asterisk in the Fig. 5a). The cytoplasm of individual cells contained large vacuoles with electron-opaque tannin material (Fig. 5a). TEM images revealed a common glandular character of stigmatic papillae in all investigated willows. The papillae contained numerous mitochondria, plastids with starch grains, abundant ribosomes and a long strand of endoplasmic reticulum. The lipid bodies and many small vacuoles with electron-dense osmiophilic material were also present (Fig. 5c–f). In the TEM images, membrane-attached ribosomes could be seen in rough ER as well as Golgi apparatus which showed a stacking of four to five cisternae (Fig. 5f). In the mature papillae, an electron dense substance appeared in the granular zone of cytoplasm next to cell walls. Fusion of the electron-transparent globules occurred. Sections revealed occasional connections between ER and at least one cisternae of the Golgi apparatus. The ultrastructure of papillae cells appeared similar in all analyzed species.

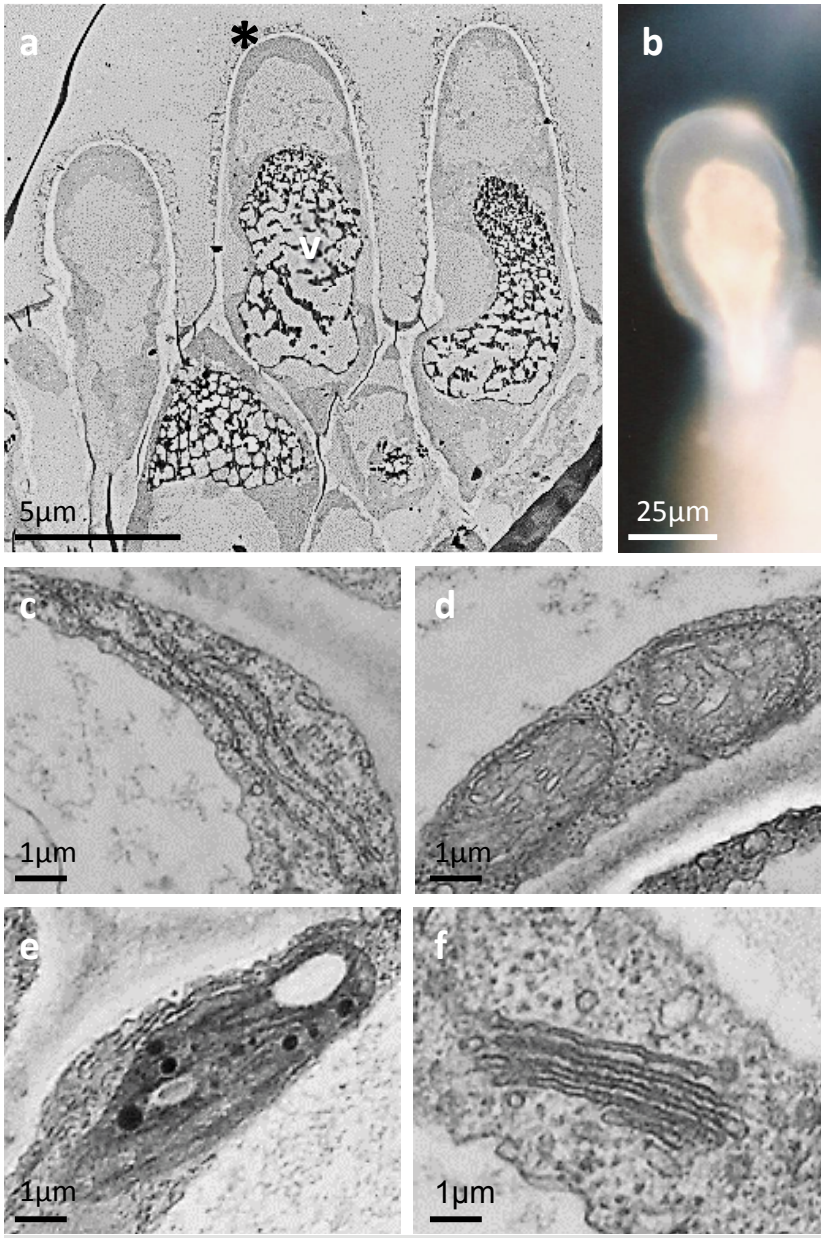

Fig. 5 The papillae of *S. viminalis* during anthesis. **a** General aspect of papillae in longitudinal section as seen in TEM; v – vacuole and pellicle (asterix). **b** Isolated very young papilla, binocular view. **c** Rough endoplasmic reticulum. **d** Mitochondria. **e** Plastid with thylakoid systems and starch granules. **f** Golgi apparatus. **c–f** TEM images.

Discussion

On the basis of three methods of stigma examination: by morphological traits, peroxidase activity stain, and poplar pollen germination test we proved that willow stigma receptivity lasts for a short time, from 1 to 2 DA. Similar methods of SR evaluation have been reported in other plant species [35,36]. Short SR time appears to be characteristic of *Salix* species, together with the pattern of stigma structure, both expressing a high degree of similarity in four willow genotypes. Among the studied species only stigmas of *S. viminalis* warranted relatively high rate of poplar pollen grain germination (Fig. 2m–p).

Negative effect of short SR might be compensated by increasing the amount of pollen applied to the stigmas during hand cross-pollination. This factor could significantly influence the percentage of pollen germination. A strong effect of their density on pollen germination has been found for stigmas of several plant species [29,30]. These observations reflect density dependence on the pollen germination rates which have been observed to increase simultaneously with increasing pollen density on the stigma. The "pollen population effect" is also associated with secretion of small peptides which can induce pollen germination and enhance possibility of fertilization [30,36].

It is likely that the artificial type of pollination is significant. Some pollen grains may accidentally be deposited on immature stigmas, which are considered to be able to "catch" pollen by adhesion [22,23]. Some data suggest that bud pollination (or pollination of immature pistils) allows incompatibility barriers to be overcome (i.e., in intergeneric crosses [20,21,27]). Therefore, it appears reasonable to hand-pollinate catkins when the basal part of about half of flowers in an inflorescence starts to bloom.

The poplar pollen viability tested on the BK medium showed very high viability (68–84%) which lasted for about two weeks. After being stored at 4°C, poplar pollen viability was prolonged to 1 month. On the stigmas of four willow species, the test of poplar pollen germination revealed moderate viability (much lower than on the BK medium), probably due to the species incompatibility. Our results show then the poplar pollen viability depends on stigma–pollen interaction, particularly in relation to willow genotype (Fig. 4). In this case, stigmatic papillae acted as a barrier which could inhibit pollen tube germination. Similar observations have been reported for other plants [16,20,26].

Detailed aspects of pollen–stigma interactions following incompatible pollination were considered in crosses of willows and poplars. The observations revealed that dry papillate stigma of three of the four species of willows provide conditions sufficiently supporting the germination of poplar pollen grains. In general, the stigmatic area of *Salix* does not appear to be very hospitable for pollen grains of *Populus*. The size and shape of *Populus* and *Salix* pollen grains are different (Tab. 1). The larger pollen grains of *Populus* landed on top of the papillae while the smaller pollen of *Salix* fitted much better between the papillae of unequal lengths (structural coadaptation of papillae with pollen [20,21,26]). Experiments with compatible mating in *Senecio squalidus* revealed that the pollen tube penetrates between the papillae through their basal region where the cuticle is absent and does not involve enzymatic degradation [16]. Thus, stigma morphology can be used, as well as pollen morphology, for evaluation of both taxons crossing potential.

There is no doubt that germination occurred despite the limited contact of *Populus* pollen with *Salix* papillae, although the frequency of fertilization was rather low [9,10]. It is apparent that the consequence of the micro-incompatibility of both structures was a slow germination and a greater pollen tube attrition exhibited by poplar pollen.

At the receptive stage of papillae, the points of contact between pollen and stigma stain positively for esterase activity [16]. Cytochemical studies have shown that peroxidases, esterases, and glycoproteins are the major components of the pellicle of receptive stigmas in *Brassica*, *Malva*, *Hibiscus*, *Silene*, *Gladiolus*, and *Secale* [16,20]. Our analysis has shown that mature stigmas of willows also contain a high level of peroxidases which stained blue using the technique recommended by Dafni and Maues [32]. Not only peroxidase activity but overall appearance of willow stigmas and particular characters of papillae do indicate their readiness to pollination.

The phenology of flowering within large inflorescences [37,38] reveals a sequence of developmental processes leading to blossom. In willow, when the basal part of female catkins reaches maturity, the pistils on the top are still immature [11]. This is why the anthesis in willows is prolonged. In nature, flowering of the whole willow inflorescence is very short (a few hours of a sunny day) because in such conditions all pistils within one catkin reach receptivity virtually simultaneously (personal observation). On the other hand, in experimental conditions (culture room, forcing shoots, low level of light intensity) flowering longevity increases from 2 to 3 days and pistils develop along the rachis sequentially and slowly. Spirally arranged pistils differ in

their developmental stage because initiation of pistils proceeds acropetally and their maturation basipetally. As a consequence of heterochrony on one catkin, all developmental stages of pistils are present simultaneously. Stigma receptivity also "moves" from the basis towards the top of the catkins. Simultaneously, one catkin contains various stigmas from obsolete, mature, immature, to very young ones. For this reason after hand- or open-pollination all stigmas receive pollen grains at the same time despite the level of their receptivity.

Conclusions

From the present study the conclusion can be drawn that relatively low germination rate of poplar pollen in crosses of *Salix* × *Populus* is most probably due to a short time of SR in willow. Thus, for the future experiments with intergeneric crosses between willows and poplars we recommend the following good markers of SR in a willow: (***i***) peroxidase and esterase activity on the surface of willow stigma highest on the first day of willow anthesis; (***ii***) frequency of poplar pollen germination highest on the first day of anthesis; (***iii***) secretory activity of papillae at the anthesis resulting from the presence of numerous Golgi and ER structures within a cell. Useful but not unequivocally determining SR were morphological traits of stigma – erect form of bifurcate lobes and solid turgor of the light green, elongate cells of papillae.

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