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Stylar water potential and unilateral interspecific incompatibility in *Solanaceae*

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Abstract

Water and osmotic potentials were measured in young and mature styles of selfcompatible (SC) and selfincompatible (SI) species of *Solanaceae*. For mature flowers, the stylar water and osmotic potentials were considerably lower in SI species *Nicotiana alata* and *Petunia hybrida* than in SC species *N. sylvestris* and *N. tabacum*. Stylar water content (in % fresh mass) was also significantly lower in SI species (*N. alata, P. hybrida,* diploid clones of potato) than in SC species (*N. acuminata, N. glauca, N. paniculata, N. sylvestris, N. tabacum*). For flower buds younger than 2 d before anthesis, no differences in stylar water potential between *N. alata* (SI) and *N. tabacum* (SC) were observed. During further flower bud development the stylar water potential in *N. alata* decreased more expressively than in *N. tabacum*. Pollen culture in media with various concentrations of sucrose or polyethylene glycol revealed that optimal water potential for pollen tube growth was lower in *N. alata* than in *N. tabacum*. The differences were similar as for differences in stylar water potential. The results are considered in relation to possible involvement of stylar water potential in unilateral interspecific incompatibility in *Solanaceae*.

Introduction

Sexual incompatibility between self-compatible (SC) and self-incompatible (SI) species of the same genus usually occurs unilaterally. In the unilateral interspecific incompatibility pollen of SI species is mostly accepted by pistils of SC species, while in reciprocal crosses pollen germination or tube growth are inhibited. It is often postulated that the S-locus controlling self-incompatibility is in some way participating in the genetic control of interspecific incompatibility. Possibilities of an involvement of some additional factors in rejection of SC pollen by SI pistils is, however, also considered (for review see De Nettancourt 1977).

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In *Solanaceae*, self-incompatibility is governed gametophytically and the reaction of both self- and interspecific incompatibility is expressed in the style. Studies on the reaction of self-incompatibility in several members of the *Solanaceae* indicated that incompatibility inhibition of pollen tube growth may result from their interaction with S-specific glycoproteins exhibiting ribonuclease activity and secreted by the cells of the style-transmitting tract (for review see Franklin-Tong and Franklin 1993). Some difficulties, however, emerged in demonstrating S-specific activity of the glycoproteins against germinating pollen *in vitro* (Harris *et al.* 1989, Jahnen *et al.* 1989, Gray *et al.* 1991). In *Nicotiana alata* S-glycoproteins isolated from the style could only partly inhibit pollen tube growth *in vitro* which may indicate that some additional factors are needed for total inhibition (Harris *et al.* 1989). This is also suggested by the observation of normal compatible growth of both self-pollen of *N. tabacum* and S_2-pollen of *N. alata* in styles of transgenic *N. tabacum* expressing S_2-glycoprotein of *N. alata* (Murfett *et al.* 1992). These results together with the unilaterality of interspecific incompatibility suggest that some unspecific physiological background in the style could be needed for pollen tube arrest through specific reactions of incompatibility.

Germination of pollen grains and elongation growth of pollen tubes requires a fairly high water uptake and is therefore dependent on water potential gradient between the developing pollen tube and the transmitting tissue of the style. Interaction of growing pollen tube with different molecules or factors present in transmitting tissue should be greatly influenced by water relations. Involvement of stigma osmotic potential in self-incompatibility was first proposed by Lewis (1943) in *Linum grandiflorum* with heteromorphic type of incompatibility. He suggested that a ratio of 4:1 in the osmotic potential of pollen and stigma cells is required for compatible pollination. However, detailed analysis of osmotic potentials of pollen and stigmatic cells in different *Linum* species (Murray 1986) did not confirm this hypothesis. Recently crucial importance of pollen coat for pollen-stigma interaction enabling water uptake required for pollen germination was showed in pop1 mutant of *Arabidopsis thaliana* (Preuss *et al.* 1993).

In the gametophytic type of incompatibility with wet stigmas and stylar arrest of pollen tubes the aspect of water relations was not yet considered. Therefore with the aim to contribute to the elucidation of putative unspecific factors controlling unilateral interspecific incompatibility we have studied: (*1*) stylar water content, (*2*) stylar water and osmotic potentials and (*3*) optimum medium water potential for pollen tube growth *in vitro* in some SI and SC species of *Solanaceae*. We believe that insight in the mechanism of interspecific unilateral incompatibility could also help to better understand intraspecific self-incompatible interaction.

Materials and methods

Self incompatible plants of *Nicotiana alata* Link et Otto, *Petunia hybrida* A.L. Juss and of diploid clones of *Solanum tuberosum* L. as well as self-compatible species *Nicotiana sylvestris* Spegazzini et Comes, *Nicotiana tabacum* L., *Nicotiana* *acuminata* (Graham) Hooker, *Nicotiana glauca* Graham and *Nicotiana paniculata* L. were grown in the greenhouse under sufficient water supply. The unilaterality of incompatibility was verified by fluorescent microscopy of aniline blue-stained styles 2 d after pollination in combinations of all SC and SI species except for combinations of *Solanum tuberosum* with *N. acuminata, N. glauca* and *N. paniculata*. Flowers or flower buds in different ontogenic stages were collected during morning hours to prevent eventual temporary daily wilting of plants which can affect floral water relations (Schussler and Westgate 1991). SC and SI species were always measured simultaneously and great attention was paid to the collection of flowers and further handling as temperature and air humidity can affect stylar water relations as well as viability and germinability of pollen *in vivo* and *in vitro* as showed for *Nicotiana tabacum* pollen Ciampolini *et al.* (1991) and Shivanna *et al.* (1991).

Water content in styles was determined gravimetrically as percent decrease of fresh mass of 20 styles after drying for 1 h at 130 °C. Stylar water and osmotic potentials were measured in laboratory under constant temperature 25 ± 0.002 °C by thermocouple psychrometer jointed with a *Keathley Microvolt Ammeter 150 B*. Water potential was determined on living styles and osmotic potential was determined after the same styles had been frozen (-18 °C, 16 h) and thawed. As one sample for this measurement needed more than 10 styles from mature flowers and about 30 styles from flower buds, *Wescor Dew Point Hygrometer HR-33T* with a miniature chamber was used for measurement of water potential when material was not abundant.

Pollen of SC and SI species was cultivated *in vitro* on media with different water potentials prepared from 0.01 % boric acid by addition of different amounts of sucrose or polyethylene glycol (PEG) 400 or 4000. Values of osmotic potentials of media used were also measured by thermocouple psychrometer. Pollen suspensions (1.5 mg per 1 cm^3 of medium) were shaken at 25 °C for 3 h or 5 h and the cultures were evaluated by measurement of 100 or 200 pollen tubes.

Results and discussion

There are differences in overall style morphology and anatomy between SI species *Nicotiana alata* and *Petunia hybrida* and SC species *N. sylvestris* and *N. tabacum*. In SI species the styles are more narrow and have distorted longitudinal axis (Fig. 1). Cross sections of transmitting tissue show larger intercellular spaces in SC species than in SI species and the cells of this tissue are more narrow in *N. alata* (6 ± 1 µm) than in *N. sylvestris* (9 ± 2 µm).

Water content (in percentage of fresh mass) was 5 - 6 % lower in SI species (*N. alata, P. hybrida*, potato dihaploids) than in SC species (*N. acuminata, N. glauca, N. paniculata, N. sylvestris, N. tabacum*, Table 1). This lower water content corresponds with the above mentioned morphological and anatomical characteristics. The SC species *N. acuminata, N. glauca* and *N. paniculata* have similar style morphology as *N. tabacum*. Stylar water potential in mature flowers (after anthesis) of SI species *N. alata* and *Petunia hybrida* was considerably lower (in average 0.35 MPa) in comparison with water potential of SC species *N. sylvestris* and *N. tabacum* (Fig. 2). Similar difference in osmotic potential was found (data not given).

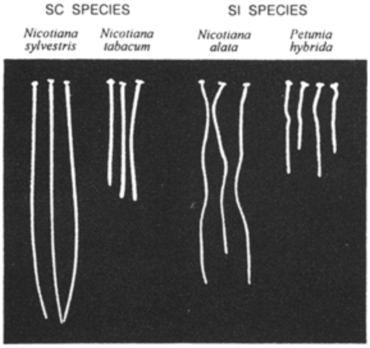

Fig. 1. Stylar morphology of SC species (*Nicotiana sylvestris* and *N. tabacum*) and SI species (*N. alata* and *Petunia hybrida*).

Table 1. Water content in styles of SC and SI species. Means ± S.E. of four (*Solanum tuberosum* dihaploids) or five (other species) sets of 20 styles.

SC species	Water content [%]	SI species	Water content [%]
Nicotiana acuminata	86.3 ± 1.3	*N. alata*	79.1 ± 0.6
N. glauca	83.6 ± 0.8	*Petunia hybrida*	79.9 ± 0.7
N. paniculata	83.0 ± 0.1	*Solanum tuberosum*	77.3 ± 0.3
N. sylvestris	85.7 ± 0.8		
N. tabacum	84.7 ± 0.4		

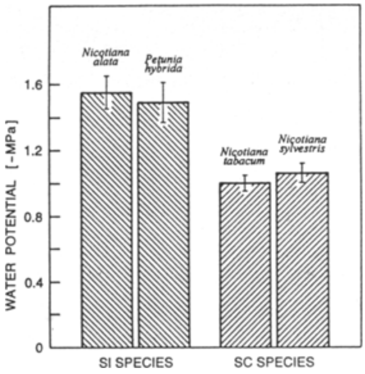

Fig. 2. Comparison of stylar water potential of mature flowers of representatives of SI species (*Nicotiana alata* and *Petunia hybrida*) and SC species (*N. tabacum* and *N. sylvestris*). The results presented here were measured in June. Means of 10 measurements ± S.E.

In *N. alata* the pistils become self-incompatible one or two days before flower opening and self-pollination of younger buds is used to produce self-seeds (for review see Franklin-Tong and Franklin 1993). From this aspect it is of interest that in flower buds younger than 2 d before anthesis the stylar water potential of *N. alata* was the same as in *N. tabacum* (Fig. 3). However, during the later ontogeny of flower buds the stylar water potential in *N. alata* decreased more expressively than in *N. tabacum* (Fig. 3). The measurements were repeated during two vegetative seasons and gave corresponding results with only small differences in absolute values of water potential. Even if the material was collected in morning hours the values of water potential were dependent on weather during previous days and this was the cause of differences in values of stylar water potential of mature flowers measured in June and September or August (*cf.* Fig. 2 and 3).

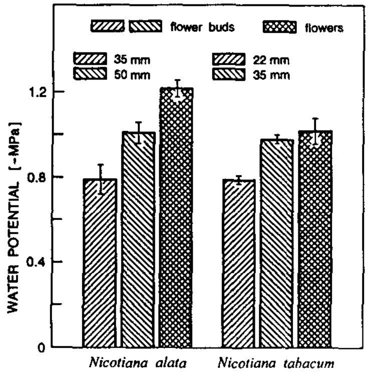

Fig. 3. Changes in stylar water potential during flower ontogeny in SI species *Nicotiana alata* and SC species *N. tabacum*. The buds were approximately 4 and 3 d before anthesis and the flowers first day after anthesis. The measurements were done during August and September. Means of 10 measurements ± S.E.

The pollen tube growth *in vitro* was dependent on the concentration of osmoticum. The highest pollen tube growth in *N. tabacum* was in medium with 6 % of sucrose, while the optimum for pollen tube growth in *N. alata* was at 13 % of sucrose (Fig. 4). The corresponding values of water potential are -0.48 MPa and -1.06 MPa, respectively. Because water potential of sucrose media may change during pollen tube growth due to extracellular activity of tube invertases (Tupý 1960) further experiments were carried out using metabolically inert PEG 400 and 4000 as osmotica. With PEG 400 (M_r near to sucrose) the optima of concentration for *N. tabacum* and *N. alata* pollen tubes were almost identical with the optima in sucrose media (Fig. 4). In medium with PEG 4000 optimum concentration for growth of *N. tabacum* pollen tubes was between 16 and 23 % and for *N. alata* 23 to 26 % (Fig. 4). Pollen tube growth was also affected by the type of osmoticum and under the same medium water potential pollen of both species produced longer tubes on medium with PEG. Similarly Zhang and Croes (1982) found that PEG 400 was more suitable osmoticum for germination of *Petunia hybrida* pollen than sucrose. They also observed that in sucrose medium a water potential of -1 MPa was optimal for tube growth of *Petunia* pollen which is similar to the optimum for *N. alata* found in our experiments. We also evaluated pollen tube growth in *N. sylvestris* at different sucrose, PEG 400 or PEG 4000 concentrations and obtained similar data as for *N. tabacum* (results not shown). In summary, it appears that optimal water potential for pollen tube growth is about 0.5 MPa lower in SI species *N. alata* and *Petunia hybrida* that in SC species *N. tabacum* and *N. sylvestris*.

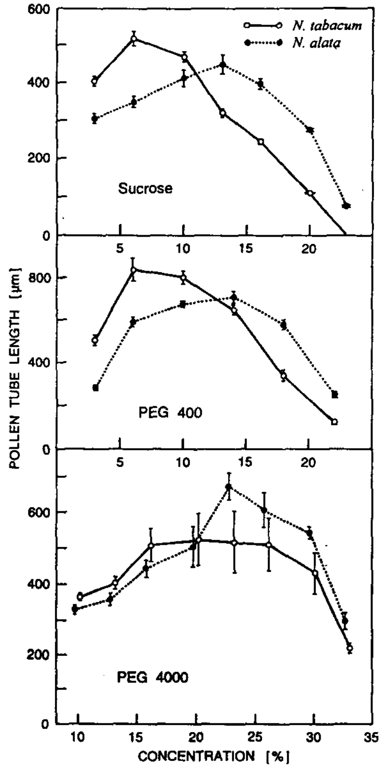

Fig. 4. Growth of pollen tubes of SI species *Nicotiana alata* and SC species *N. tabacum in vitro* under different concentrations of sucrose, PEG 400 and PEG 4000. Means ± S.E. from 100 pollen tubes after 5 h (media with sucrose and PEG 400) or from 200 tubes after 3 h (PEG 4000) of culture.

These differences between SI and SC species in optimum water potential of medium for pollen tube growth *in vitro* are in accord with the differences observed in stylar water potential. These could mean that the physiology of pollen tubes is adapted to species specific water relations in styles.

It may be that the relatively dry and probably also more compact environment in styles of SI species as compared with SC species can play a part not only in interspecific but also in intraspecific incompatibility inhibition of pollen tube growth. Apart from possible direct inhibitory effect of low water potential, this condition in SI styles could be necessary for expression and/or function of some S-gene products. This possibility would be compatible with the model of self-incompatibility and unilateral interspecific incompatibility put forward recently by Trognitz and Schmiediche (1993). This model postulates specific as well as unspecific products with or without linkage to S-locus responsible for pollen tube inhibition on style site and for ability to overcome this inhibition on pollen site.

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