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EFFECT OF SEASON, PLANT AGE AND TEMPERATURE DURING PLANT GROWTH ON COMPATIBLE AND INCOMPATIBLE POLLEN TUBE GROWTH IN PETUNIA HYBRIDA

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SUMMARY

In *Petunia hybrida* the length of pollen tubes was measured after growth in the style for 24 h. The length attained depends upon the temperature regime under which the pollen as well as the styles developed prior to pollination. The temperature regimes used were 25.5/18°C and 19.5/18°c respectively, both in a 16/8 h day/night regime.

A high temperature regime during the progamic phase causes a greater length of the tubes than the low temperature regime; this difference is larger after cross- than after self-pollination.

The length after 24 hours' growth of incompatible pollen tubes is larger when the pollen developed, prior to pollination, under the high temperature regime than under the low one, but their length is not affected by the temperature at which the styles developed. With compatible pollen tubes, on the other hand, that length is larger when the styles developed under the high temperature regime than under the low one and it is also enhanced by a pretreatment of styles, developed under a low temperatue regime, with the higher temperature for 24 h. The enhancement is largest when the treatment precedes pollination at the low temperature regime immediately and is absent when the time between treatment and pollination exceeds four weeks.

The length of incompatible pollen tubes after 24 h is in general lower in plants cultivated from summer- than from winter-cuttings; the length of compatible pollen tubes in plants cultivated from winter-cuttings decreases with the age of the plant, if style development and tube growth take place under the low temperature regime.

Compatible pollen germination is affected by a low temperature regime during style development and progamic phase.

The incompatible pollen germination rate is lower in pollen from summer- than from winter-cuttings, when pollen development and pollination occur at the low temperature regime.

1. INTRODUCTION

In several plant species the growth of the pollen tubes in the style is much slower after an incompatible pollination than after a compatible one. This growth inhibition weakens towards the end of the flowering period of the plant producing the styles (LINSKENS 1973). In young flower buds the growth inhibition capacity of the style is weak, but during maturation it increases (LINSKENS 1964, HERRERO & DICKINSON 1980). In old and wilting flowers this growth inhibition reaction decreases again (ASCHER & PELOQUIN 1966a). Age is an important physiological factor not only in relation to the incompatible pollen tube – style interaction, but also in relation to the compatible one. ASCHER & PELOQUIN (1966a) found in *Lilium* that compatible pollen tube growth velocity varies with the physiological age of the styles. LINSKENS (1977) investigated compatible

pollen tube lengths during the flowering period of *Petunia* plants, but it is not clear whether the differences observed were caused by the advancing age of the plants or by the advancing growth season.

The temperature is important during the progamic phase (LEWIS 1942, ASCHER & PELOQUIN 1966b, TOWNSEND 1968, STRAUB 1958, LINSKENS 1973), but also before: in *Oenothera* a partial decrease of the incompatibility reaction was obtained by either a low temperature during, or a high temperature prior to the progamic phase (KWACK 1965). Low temperatures before pollination had no effect on the incompatible pollen tube growth in *Oenothera* (LEWIS 1942), but changed the compatibility reaction in *Trifolium* to self-incompatibility (TOWNSEND 1968). However, in *Medicago sativa* pollen germination was hardly affected by incubation temperatures, but more by the temperature at which the plants were grown (DANE & MELTON 1973).

Still less is known about the impact of temperature pretreatments on the pollen tube – style interaction. We decided therefore to investigate the effect of temperature treatment during the pre-progamic phase and of aging and season in relation to the pollen tube – style interaction after compatible or incompatible pollinations.

2. MATERIALS AND METHODS

The heterozygous clone W166K (S_1S_2) of *Petunia hybrida* was used only as pollen provider and the homozygous clone T_2U (S_3S_3) for the pollen and styles.

Unless otherwise stated both self-incompatible clones were grown, from the moment of cutting, in two plant growth chambers, one at 19.5 ± 0.2°C, the other at 25.5 ± 0.2°C during the light period, and both at 18.0 ± 0.1°C in the dark. The light regime was 16 h light (starting at 7.00 a.m.), lighting intensity 25 klx, and 8 h dark.

The plants were grown from cuttings made in August (summer cuttings) and January (winter cuttings) from mother-plants grown in the greenhouse under day-light conditions, in the winter months supplemented with light from a Philips mercury lamp HLRG 400 W, lighting intensity 25 ± 5 klx.

The anthers were collected from flower buds just before anthesis, and dried at 23°C for 24 h in the dark. The pollen was separated from the anther tissue by sieving; it was used immediately for pollination by applying an excess on the stigma. Styles of emasculated flowers were pollinated in both growth chambers at 9.00 a.m. and after 24 h (progamic phase), both for cross- and self-pollinations the length of the pollen tubes was measured with an U.V. fluorescence microscope according to the method of LINSKENS & ESSER (1957). Eight styles were selected at random and the average length of 90% of the pollen tubes was determined.

Experiments could be carried out from the 10th till the 25th and the 15th till the 25th week for summer and winter cuttings respectively.

3. RESULTS

The vegetative vigour of the plants in the growth chambers was better than that of plants in the greenhouse of the same age. The plants grown under the low temperature regime were more vigorous than those grown under the high temperature regime.

3.1. Self-pollination

After self-pollination ($S_3S_3 \times S_3$) we got the following results depending on the applied pretreatment.

Temperature during style development: The length of incompatible pollen tubes is not correlated with the temperature at which the style developed (*fig. 1*), not even when the temperature during pollination is high (*fig. 2*).

Temperature during pollen development: The tube is longer when the pollen developed at the high temperature regime (*fig. 1, 2*).

Temperature during the progamic phase: A comparison of *figs. 1* and *2* shows that the high temperature has an equal impact on the two curves in figure 1 except that there is no decrease in length after the 21st week.

Aging of the plant: The pollen tube length and germination percentage appear independent of the age of the plant except for the 19.5/18 °C temperature regime during pollen development and progamic phase (*fig. 1*) at which pollen tube length is smaller and the germination percentage lower after the 21st week.

Growth-season: In summer cuttings the pollen tube length is in general lower than in winter cuttings. The percentage of germination is lower in the former than in the latter, but only after the low temperature regime during pollen development and progamic phase.

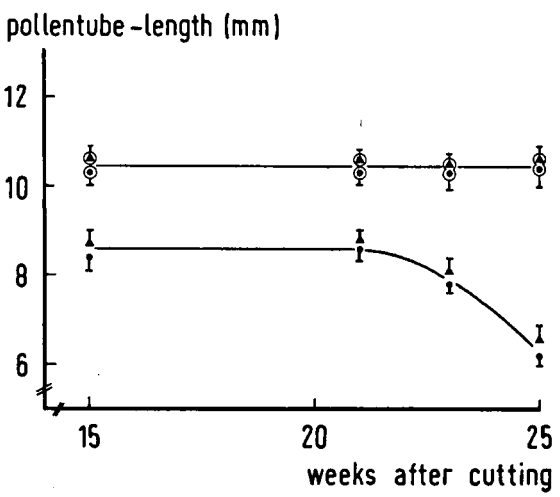

Fig. 1. Length of incompatible pollen tubes, from pollen developed at 25.5/18°C (▲ ⊙) and 19.5/18°C (▲, ⊙), after 24 hours growth at 19.5/18°C in styles developed at 19.5/18°C (▲, ▲) and 25.5/18°C (⊙, ●). Pollen and styles are from plants developed from winter cuttings.

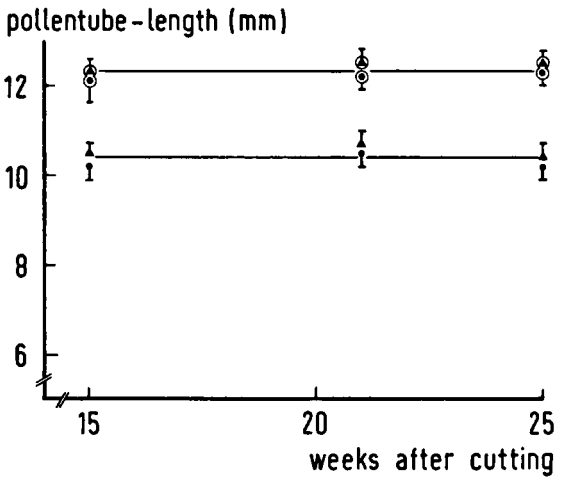

Fig. 2. Length of incompatible pollen tubes, from pollen developed at 25.5/18°C (◬, ⊙) and 19.5/18°C (▲, ●), after 24 hours growth at 25.5/18°C in styles developed at 19.5/18°C (◬, ▲) and 25.5/18°C (⊙, ●). Pollen and styles are from plants developed from winter cuttings.

Figs. 1 and *2* are similar for the summer cuttings except that experiments could be started five weeks earlier when summer cuttings were used. Tubes, from pollen developed at the high temperature regime, attain a greater length than tubes from pollen developed at the low temperature regime no matter if we used summer- or winter-cuttings.

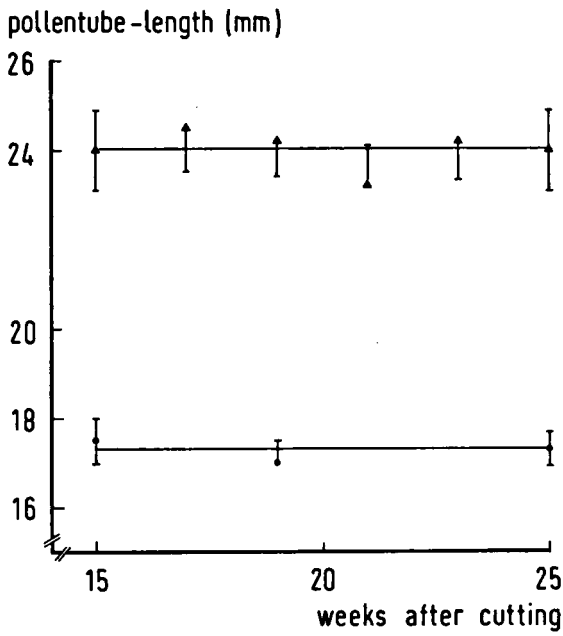

Fig. 3. Length of compatible pollen tubes, from pollen developed at 25.5/18°C, after 24 hours growth at 25.5/18–C (▲) and 19.5/18°C (●), in styles developed at 25.5/18°C. Pollen and styles are from plants developed from winter- or summer-cuttings.

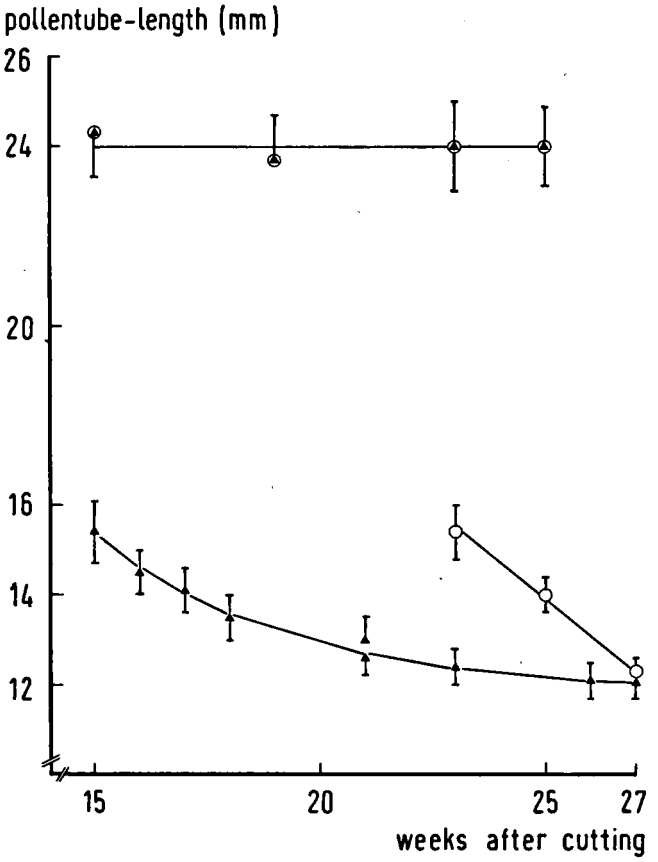

Fig. 4. Length of compatible pollen tubes, from pollen developed at 25.5/18 °C, after 24 hours growth at 25.5/18 °C (▲) and 19.5/18 °C (▲, ○) in styles developed at 19.5/18 °C (▲, ▲) and 19.5/18 °C + in the 23the week 24 hours 25,5/18 °C just before the start of the progamic phase (○). Pollen and styles are from plants developed from winter cuttings.

3.2. Cross-pollination

After cross-pollination (S_3S_3 × S1,2) the following observations for the different pretreatments are made.

Temperature during style development: Compatible pollen tubes grown at 19.5/18 °C are longer in styles developed at 25.5/18 °C than in styles developed at 19.5/18 °C (*fig. 3, 4* and *5*). In styles developed at 25.5/18 °C and those developed at 19.5/18 °C the behaviour of the tubes raized after pollination at 25.5/18 °C, are with regard to the length of the compatible tubes identical (*fig. 3, 4*). When an exposure of only 24 hours of the high temperature regime was given to the styles before the progamic phase, the length of the compatible tubes is larger in those styles compared with non-exposed styles (*fig. 4, 5*) provided that the temperature during style development and pollination is low. The time lag between exposure

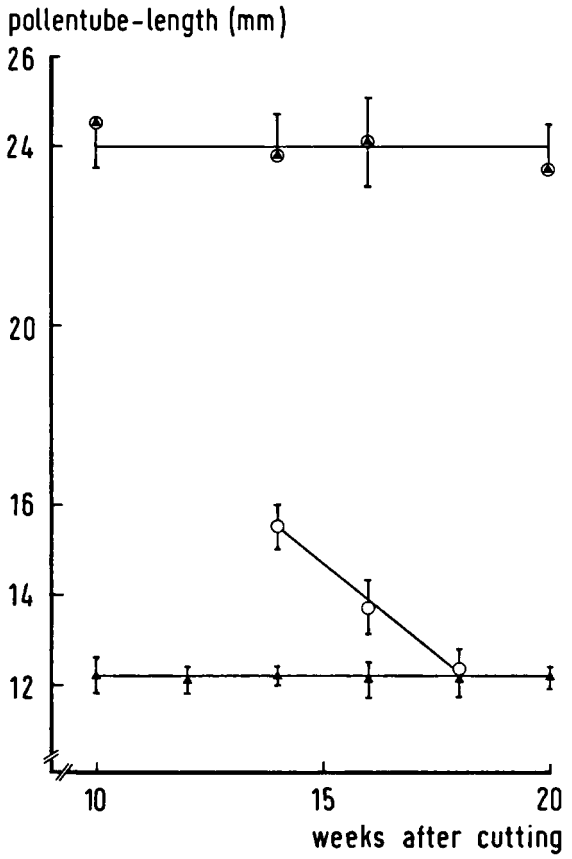

Fig. 5. Length of compatible pollen tubes, from pollen developed at 25.5/18 °C, after 24 hours growth at 25.5/18 °C (◮) and 19.5/18 °C (▲, ○), in styles developed at 19.5/18 °C (▲, ◮) and 19.5/18 °C + in the 13th week 24 hours 25.5/18 °C just before the start of the progamic phase (○). Pollen and styles are from plants developed from summer cuttings.

and the moment of pollination is important: the length of the tubes becomes shorter when time between pretreatment and start of the progamic phase increases (*fig. 4, 5*).

Temperature during pollen development: The pollen tube length after cross-pollination is not significantly different from pollen developed at different temperature regimes.

Temperature during the progamic phase: The compatible tube length is larger and the germination percentage higher in styles developed and pollinated at 25.5/18 °C or developed at 25.5/18 °C and pollinated at 19.5/18 °C than in styles developed and pollinated at 19.5/18 °C (*fig. 3, 4, 5*).

Aging of plants: The effect of aging is only visible in winter cuttings when both temperatures during style development and progamic phase are 19.5/18 °C (*fig. 4*).

Season effects: In winter cuttings the pollen tubes grow a shorter distance with increasing age of the plant (*fig. 4*) when both temperatures during pollination and style development are 19.5/18 °C. The length after 25 weeks is equal to the length in summer cuttings after 10 weeks (*fig. 4, 5*).

4. DISCUSSION

Pollen tube growth depends not only on the temperature regime during the progamic phase but also on the temperature regime during the preceding flower and plant development. The effect of temperature during pollen or style development on the lengths of incompatible or compatible pollen tubes respectively can be discussed in relation to the most fitting gene action models of self-incompatibility put forward by ASCHER (1966) and VAN DER DONK (1975). The process of recognition after the landing of pollen on the stigma leads to a response in the pistil (LINSKENS 1975) and the time lag between the start of the progamic phase and rejection or acceptance of the pollen in the pistil can be controlled by the temperature during the progamic phase (LEWIS 1952). We think that the low temperature regime during style development and progamic phase does not only prolong the recognition process in *Petunia*, but also alters it in such a way that acceptance of the compatible pollen does not occur, and that this probably explains why the compatible pollen tubes have approximately the same length as the incompatible ones (see also ASCHER & PELOQUIN 1966b for *Lilium*). According to the model of VAN DER DONK (1975), the style specific polypeptides are probably not synthesized by the stylar S-gene, because either there is no activation of the style or the style does not have preformed mRNA, and thus, as in the case of an incompatible pollination, the pollen genome is not activated (since there are no style specific polypeptides), and subsequently the pollen tube growth stops when its resources, depending on the kind of pollen (T_2U or W166K) and the temperature during pollen development, are consumed. The observation that incompatible pollen, developed at a higher temperature, grows farther due to its resources seems to be confirmed by the fact that its protein content is higher than in pollen developed at a lower temperature regime (VAN HERPEN in prep.). According to the model of ASCHER (1966), the high velocity operon is not repressed, but the high velocity growth system cannot move into action because the necessary stylar metabolites are not available because of the low temperature regime during style development and progamic phase, thus growth of the pollen tube occurs at the slow rate until compatible pollen reserves have been depleted.

The difference in compatible tube lengths in plants cultivated at the low temperature regime either from cuttings made in summer or in winter cannot be explained by a difference in pollen germination percentage (see TER-AVANESIAN 1978), but perhaps by a different stylar gene activity (VAN DER DONK 1975, LINSKENS 1975) towards fast repair of the damage caused by the growing pollen tube.

All styles mentioned above are developed and pollinated at the low tempera-

ture regime, higher temperatures during the progamic phase induce longer incompatible pollen tubes in *Petunia* (LINSKENS 1973), *Trifolium* (CHEN & GIBSON 1973, TOWNSEND 1968), *Lilium* (ASCHER & PELOQUIN 1966b) and *Brassica* (GONAI & HINATA 1971). Our experiments in the growth chambers give similar results for compatible pollen tubes as found by ASCHER & PELOQUIN (1966b), except that compatible tubes have approximately the same length as incompatible tubes at the low temperature regime and twice as long at the high temperature regime during pollination. According to the model of VAN DER DONK (1975), the style specific polypeptides are probably synthesized very quickly by the high pollination temperature and either do not or do react whether it is a compatible or incompatible pollination, with the pollen specific polypeptides. A differential reaction takes place (VAN DER DONK 1974) leading to a large difference in the respective pollen tube lengths. In the model of ASCHER 1966) the high velocity operon is not repressed, as in the case where low temperatures during style development and progamic phase are used, but the high-velocity system can move into action now because the stylar metabolites are synthesized very quickly by the high temperature regime during the progamic phase.

When styles are developed under the low or high temperature regime and pollinated at the low temperature regime, the tube lengths are only different for compatible and not for incompatible pollinations. This difference in compatible pollen tube lengths occurs also when styles developed at 19.5/18°C are treated with 24 hours of the high temperature regime before the start of the progamic phase as compared with styles which are not, and this difference lasts for about four weeks when pollination is still performed at the low temperature regime. VAN DER DONK (1975) claims the synthesis of style specific polypeptides during the progamic phase. Our results suggest either temperature dependent S-gene activity and subsequent polypeptide synthesis before the progamic phase or S-gene activity during the progamic phase but affected by temperatures before the progamic phase, the mechanism of this last possibility is, however, not yet clear. In the model of ASCHER (1966), enough stylar metabolites are synthesized during the high temperature pretreatment to enable the action of the high-velocity system even when the pollination temperature is low. The length of incompatible pollen tubes is independent of the temperature during style development, and dependent on the temperature during pollination but not to such an extent as the compatible tubes are. In the model of ASCHER (1966) the latter observation can be explained by the fact that there are different operons at work for the different pollinations, and in the model of VAN DER DONK (1975) by a gene, responsible for the repair of the damage caused by the growing pollen tube, which works only during incompatible pollinations and better at a high temperature.

Germination of alfalfa (*Medicago sativa*) pollen is dependent upon the clone from which the pollen is collected and influenced by the temperature at which the plants are developed (DANE & MELTON 1973). HAYASE (1955) found maximum germination of pollen from male flowers of cucumber at 20–25°C, lower and higher temperature treatments given from 2 days before anthesis decreased the pollen viability. The decrease in the percentage of pollen germination and pollen

tube length in *Petunia* especially at the low temperature regime is perhaps caused by a change in the calcium concentration (BREWBAKER & KWACK 1963) in the style. The pollen tube length is also determined by the number of germinated pollen grains (TER-AVANESIAN 1978).

After self- or cross-pollination a change in the saturation degree of the fatty acids is found, and the distribution of the fatty acids among the various glycosphingolipids in self- and cross-pollinated styles is different (DELBART et al. 1980). Bacteria, plants and animals can all adapt to changes in environmental temperature by altering the degree of saturation in their fatty acid side chains (FURTH 1980). It is possible that environmental conditions before and during the progamic phase have their influence on the pollen tube – style interaction via a change in structure and/or distribution of lipids in the membranes.

Environmental factors are not only decisive *during* the progamic phase but also *before*. Thus the use of controlled conditions for the cultivation of plant material is absolutely necessary (LINSKENS 1975).

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