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Effect of pollen-style interaction on the pollen tube growth of *Gossypium hirsutum* *

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Summary. All possible crosses among 5 strains of *Gossypium hirsutum* were made, and the pollen tubes were grown in vivo for 4 h before being fixed, stained and measured. Temperatures ranging from 18.5 to 40.0 °C were tested for pollen germination and pollen tube growth. The optimal temperature for pollen tube growth was 30.0 °C. Relative humidity levels of 0 to 100% were used as a pre-pollination treatment of the pollen. Significant differences among the mean pollen tube length of the strains occurred due to pollen × style interactions. The strains also differed in the number of styles which did not support pollen tube growth. These differences were also due to pollen × style interactions. Pollen and style strains could be ranked according to their relative contribution to pollen tube length.

Key words: Gamete competition – Pollen tube growth – Pollen germination – Pollen-style interaction

Introduction

Pollen, the male gametophyte, has been the subject of relatively little genetic research compared to the sporophyte. However, studies have been done which demonstrate that pollen can be considered not only as a part of the sexual reproduction process in plants, but as a separate, metabolically active organism with half the genetic complement of the parent plant. Pollen from genetically diverse plants have genetically different vegetative nuclei, and hence discernable differences in pollen tube growth (Barnes and Cleveland 1963; Sari Gorla et al. 1975). The expression of genes at this gametophytic level could provide an additional stage (in addition to the sporophytic level) at which genotypes could be selected for research or breeding programs.

Genes which are expressed in the sporophytic stage of a plant have been shown to be expressed in the gametophytic stage as well. Ter-Avanesian (1978) suggested a link between pollen tube competition and variability of resulting progeny in *Gossypium hirsutum*. In *Zea mays* (Mulcahy 1974) and *Dianthus chinensis* (Mulcahy and Mulcahy 1975), seedlings produced from faster growing pollen tubes were more vigorous than those from slower growing tubes. Zamir et al. (1981) demonstrated that pollen produced by cold tolerant *Lycopersicon hirsutum* plants was better adapted to functioning at cold temperatures than was pollen from *L. esculentum*, a non-cold tolerant species. In other studies with *L. esculentum*, Tanksley et al. (1981) found that 60% of the structural genes coding for 9 sporophytic enzyme systems were expressed in the gametophyte.

To develop methods of screening genotypes for desirable traits at the cellular level, it is important to understand the effects of pollen and style genotype and various environmental factors on pollen tube growth rate. In the present study, the objective was to determine the contribution of both pollen and stylar genotype on pollen tube growth rate. The effects of different levels of temperature and relative humidity were also examined.

Materials and methods

Five *Gossypium hirsutum* strains were chosen on the basis of their relatively different sporophytic characteristics. They were: T25 (non-wilting), T169 (readily-wilting), T141 (slow-growing), T147 (fast-growing), and Texas-Marker-1 (TM1), a long-term inbred strain of 'Deltapine 14'.

All possible self and cross pollinations were performed in vivo. To determine the optimum temperature for pollen tube growth, the pollinated styles were incubated in petri

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dishes for 4 h at temperatures ranging from 18.0 to 40.0 °C. The styles were then placed in a fixative of 7 parts 95% ethanol: 3 parts glacial acetic acid. The stylar tissues were softened in 1 N NaOH for 12 to 16 h and stained with an aniline blue stain (100 mg aniline blue, 7 g K_3PO_4, 1 l H_2O, Martin 1958) for 12 to 16 h. Using fluorescence microscopy, measurements were taken from the tip of the style to the point where the majority of the pollen tubes had stopped growing. In a normal style there were hundreds of pollen tubes. However, approximately 40% of the styles had no pollen germination or very few (1–5) pollen tubes present. These styles were scored as having no germination.

To determine the effect of relative humidity (RH) on pollen tube growth, pollen was incubated prior to pollination at 30.0 °C in a closed container with one of the following humidity-controlling substances: P_2O_5 powder (RH = 0%), or saturated solutions of $CaCl_2$ (RH = 35%), $CaNO_3$ (RH = 55%), NH_4SO_4 (RH = 80%), or tap water (RH = 100%).

The relative contributions of the pollen type and style type on pollen tube length were determined through a series of Duncan's Multiple Range (DMR) tests (Steel and Torrie 1980). To determine differences in pollen tube length attributable to the type of pollen used, DMR tests were done within each style type to compare differences in the pollen's contribution to pollen tube length. Similarly, to discern differences in pollen tube length attributable to the style type, DMR tests were performed within each pollen type to compare differences in the style's contribution to pollen tube length. These analyses used means of pollen tube lengths obtained by averaging across humidity levels. Regression analysis was used to determine if the humidity pre-treatment affected pollen tube growth. In the temperature experiments, 889 styles were examined for pollen tubes, while in the relative humidity experiments, 4,078 styles were examined.

Results and discussion

Pollen tubes grew longest at 30.0 °C. The lowest temperature at which pollen would consistently germinate was 22.0 °C. Below 22.0 °C pollen germination was erratic, and when germination did occur it was only at the extreme apical area of the stigma. The highest temperature with consistent pollen germination was 35.0 °C. Pollen grains which germinated at this higher temperature did not do so in any one specific area of the style.

The regression analysis showed that the level of relative humidity applied in the pre-treatment significantly affected pollen tube length ($P < 0.05$). Pollen tube length increased with increasing levels of relative humidity for all strains except T169. The T169 pollen had no significant changes in pollen tube length due to relative humidity level.

An analysis of variance of the relative humidity pre-treatment experiments revealed the significant ($P < 0.05$) effects of pollen, style, and humidity as well as all 2-way interactions on pollen tube length. Since pollen × style interactions were significant, all comparisons among pollen tube lengths of different pollen strains were made within the same strain of style and vice-versa. Duncan's Multiple Range tests were performed for each type of style to compare pollen tube lengths (Table 1). Pollen tubes of T147 grew as fast, or faster than any other pollen tubes in every type of style. The pollen strains can be ranked from fastest to slowest as follows: T147, T169, TM1, T141, and T25.

The type of style had a marked effect on the length of the pollen tubes growing in it. Duncan's Multiple Range tests were performed within pollen strains to detect these differences (Table 2). Styles from T25 and T147 plants consistently supported faster pollen tube growth than did T141 and TM1, regardless of the strain of the pollen used. Pollen tubes grown in T169 styles were always slower than those grown in other styles.

Comparison of Tables 1 and 2 reveal some interesting contrasts among the behavior of the strains. For example, T169 pollen tubes grew relatively fast, but the ability of a T169 style to support pollen tube growth was by far the poorest of any strain. The T25 pollen and style behaved the opposite of T169. The pollen tubes from T25 pollen were the slowest of any in this study, yet the T25 style was among the best in supporting pollen tube growth. This contrast is interesting because T25 and T169 were chosen for their different

Table 1. Comparison of pollen strains using DMR tests within style strains (vertical comparisons). Numbers presented are mean pollen tube lengths in mm

Pollen strain	Style strain					
	TM1	T25	T141	T147	T169	Mean
TM1	6.39 b	7.34 bc	6.59 a	7.54 ab	5.56 a	6.89
T25	6.25 b	7.00 c	6.00 b	6.84 c	4.84 b	6.78
T141	6.32 b	7.46 bc	6.34 a	7.33 b	4.70 b	6.64
T147	7.45 a	8.29 a	6.63 a	7.75 a	5.79 a	7.22
T169	6.87 ab	7.84 ab	6.53 a	7.75 a	5.61 a	7.00

Means followed by different letters are different at 0.05 probability level

Table 2. Comparison of style strains using DMR tests within pollen strains (horizontal comparisons). Numbers presented are mean pollen tube lengths in mm

Pollen strain	Style strain				
	TM1	T25	T141	T147	T169
TM1	6.39 b	7.34 a	6.59 b	7.54 a	5.56 c
T25	6.25 b	7.00a	6.00 b	6.84 a	4.84 c
T141	6.32 b	7.46 a	6.34 b	7.33 a	4.70 c
T147	7.45 b	8.29 a	6.63 c	7.75 b	5.79 d
T169	6.87 b	7.84 a	6.53 b	7.75 a	5.61 c
Mean	6.72	7.63	6.47	7.47	5.38

Means followed by different letters are different at 0.05 probability level

sporophytic characteristics: T169 wilts readily under stress and T25 does not.

Not all strains with fast growing pollen tubes have styles which are poor supporters of pollen tube growth and vice-versa. The T147 style provides a good environment for pollen tube growth, nearly equal to that of the T25 style, and the pollen tubes from T147 were the fastest. The T141 style provided a relatively poor environment for pollen tube growth, and the T141 pollen tubes were equally poor performers. At the sporophytic level, T147 is a fast-growing plant and T141 is a slow-growing plant. These contrasts illustrate how these strains differ at both the gametophytic and sporophytic level.

Approximately 40% of the styles examined had no pollen germination, even though abundant pollen was present on the stigmatic surface. As indicated in Table 3, a lower percentage of styles with no pollen germination generally occurred when T147 and T169 pollen were used than when TM1, T141, and T25 pollen were used. The T25 pollen was the poorest, failing to germinate on 45% of the styles upon which it was applied. Styles of T169 supported the least germination, with 28 to 59% of the styles examined having no pollen tubes (Table 4). Among the best supporters of growth were TM1 and T147; only 16 to 38% of these styles were without pollen tubes.

Comparison of Tables 3 and 4 reveals contrasts among the behavior of the strains which show a marked resemblance to the contrasts observed for pollen tube length. Pollen of T169 usually germinates on any style, but the T169 styles often fail to support growth. On the other hand, pollen of T25 fails to germinate on many styles, while T25 styles show moderate support. Both styles and pollen of T141 account for a high proportion of the pollinations with no germination, while pollinations involving T147 styles or pollen usually had germination.

A quantification of the effects of both pollen and style type on pollen tube growth can be obtained by dividing the mean pollen tube length by the fraction of styles with no germination for that cross (Table 5). The higher the compatibility coefficient, the more compatible the cross. Crosses using T147 and T169 pollen always had higher compatibility coefficients than crosses using TM1, T25, or T141 pollen. This suggests that T147 and T169 pollen is adapted to a wider range of styles. Crosses that used T147 styles had the highest coefficients, and T169 styles always had the lowest.

The ability to rank pollen strains by pollen tube growth rate, and to rank stylar strains by ability to support pollen tube growth has also been observed in *Nemesia strumosa,* a species with a weakened self-incompatibility system (Robacker and Ascher 1982). Breakdown of self-incompatibility is often gradual, suggesting that many genes govern the inhibition or promotion of pollen tube growth. Perhaps many self-compatible species, such as *Gossypium hirsutum,* were once self-incompatible, and therefore retain genes which govern interactions between pollen and style.

Some of the genes responsible for sporophytic characteristics may also be those responsible for the expression of pollen tube growth characteristics, and further studies may establish correlations between sporophytic characteristics and pollen tube growth. With this link between pollen behavior and whole plant

Table 3. Comparison of pollen strains using DMR tests within style strains (vertical comparisons). Numbers presented are fractions of styles with no pollen germination

Pollen strain	Style strain					
	TM1	T25	T141	T147	T169	Mean
TM1	0.327 a	0.393 ab	0.360 a	0.268 a	0.507 ab	0.375
T25	0.336 a	0.456 a	0.497 a	0.381 a	0.599 a	0.454
T141	0.303 a	0.338 ab	0.443 a	0.314 a	0.540 a	0.388
T147	0.163 a	0.240 b	0.138 b	0.238 a	0.278 c	0.211
T169	0.226 a	0.252 b	0.316 ab	0.259 a	0.326 bc	0.284

Means followed by different letters are different at 0.05 probability level

Table 4. Comparison of style strains using DMR tests within pollen strains (horizontal comparisons). Numbers presented are fractions of styles with no pollen gemination

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T141	0.303 b	0.338 b	0.443 ab	0.314 b	0.540 a
T147	0.163 a	0.240 a	0.138 a	0.238 a	0.278 a
T169	0.266 a	0.252 a	0.316 a	0.259 a	0.326 a
Mean	0.279	0.336	0.351	0.296	0.450

Means followed by different letters are different at 0.05 probability level

Table 5. Compatibility coefficients (mean pollen tube length (mm)/fraction of styles with no pollen germination)

Pollen strain	Style strain					
	TM1	T25	T141	T147	T169	Mean
TM1	19.52	18.68	18.32	26.37	10.96	18.77
T25	18.62	15.35	12.07	17.95	8.08	14.42
T141	20.88	22.07	14.31	23.36	8.70	15.86
T147	45.74	35.54	48.07	32.58	21.48	36.68
T169	25.84	31.11	20.67	29.94	17.22	24.96
Mean	26.12	24.55	22.69	26.04	13.28	

characteristics, the possibility to use pollen tube growth as a screening tool in research and breeding programs exists.

Conclusions

Considerable variability in the behavior of *Gossypium hirsutum* pollen tubes was demonstrated. Differences were detected in pollen tube length due to pollen strain, stylar strain, temperature, humidity, and number of styles with no pollen germination due to pollen strain and style strain. Just as each strain has its own set of sporophytic characteristics, each also has a unique set of gametophytic characteristics.

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