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Genetic variation for *in vitro* sesame pollen germination and tube growth

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Abstract *In vitro* pollen germination and tube length studies are valuable in elucidating mechanisms (germination capacity and rate, tube growth rate) possibly associated with genetic differences in male transmission. On each of two collection dates, the percentage germination and tube length of the binucleate pollen grains from five diverse sesame (*Sesamum indicum* L.) genotypes were determined at eight times (30, 60, 90, 120, 150, 180, 240, 300 min) after inoculation on a semisolid medium containing 10% (100 $g l^{-1}$) sucrose ($C_{12}H_{22}O_{11}$), 0.4% (4 $g l^{-1}$) purified agar (Fisher Lot 914409), 0.1% (1 $g l^{-1}$) calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$] and 0.01% (100 $mg l^{-1}$) boric acid (H_3BO_3). Before heating, the pH of the medium was adjusted to 7.0 with a 0.1 *N* potassium hydroxide (KOH) solution. Over the five genotypes, 5% germination was found 30 min after inoculation and a maximum of 37% germination 120 min after inoculation with no significant changes thereafter. As indicated by the highly significant genotype × time after inoculation interaction, the genotypes differed in the time at which germination was initiated and maximum germination attained. Over all five genotypes, the tube length was 91 μm 30 min after inoculation, reaching a maximum of 1000 μm 300 min after inoculation. As shown by the highly significant genotype × time after inoculation interaction, the genotypes differed in the time at which tube length was observed and the maximum tube length was attained. Little or no relationship between percent germination and tube length was observed among the genotypes. For both percent germination and tube length, the statistical significance of collection date and its interactions with genotype and time after inoculation indicated that environment in the form of collection date was also an influencing factor. These results indicated that genetic differences among genotypes were present for *in vitro* germination capacity, germination rate and tube growth rate and that these factors singly or in combination could alter male transmission of genetic elements.

Key words *In vitro* pollen germination · Sesame · *Sesamum indicum* L · Male transmission

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Introduction

In vitro pollen germination and tube-growth studies are valuable in identifying the effect of environmental factors on pollen viability, the physiological and biochemical processes involved in this critical first step in fertilization and genotypic differences within a species. Media requirements for *in vitro* pollen germination have been reported for a large number of species, with considerable variation among and within species (Stanley and Linskens 1974; Shivanna and Johri 1985). No medium for *in vitro* sesame (*Sesamum indicum* L.) pollen germination has been described.

The purpose of this study was to develop a medium which would produce satisfactory germination and tube growth of sesame pollen grains. The response of pollen grains from five diverse genotypes was examined to determine genotypic differences.

Materials and methods

Extensive preliminary studies testing pollen grains from a number of genotypes and collection dates were conducted using conditions and various combinations and concentrations of chemical compounds

which were reported to enhance pollen germination in other species (Brewbaker and Kwack 1963; Stanley and Linskens 1974; Shivanna and Johri 1985). From these studies, the most desirable and consistent germination and tube growth were obtained on a semisolid medium containing 10% (100 $g\,l^{-1}$) sucrose, 0.4% (4 $g\,l^{-1}$) purified agar, 0.1% (1 $g\,l^{-1}$) calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$] and 0.01% (100 $mg\,l^{-1}$) boric acid (H_3BO_3). Before heating, the pH of the medium was adjusted to 7.0 with a 0.1 *M* potassium hydroxide (KOH) solution. This medium was heated to 100°C and poured into 95 × 15-mm petri dishes to a depth of 4–6 mm. After cooling to room temperature, the covers were placed on the dishes to prevent drying of the medium. Pollen inoculations were made approximately 18 h after the dishes were covered.

Five diverse homozygous and homogeneous genotypes (1, 3, 6, 15, 21) described in a recent article (Pfahler et al. 1996) were field-planted in Gainesville, Florida (29°38′N lat, 82°20′W long) in 1995. Pollen grains from each genotype were inoculated on this medium at each of two collection dates (23 August, 18 September). The pollen collection procedure involved removing a large number of unopened corollas (with attached stamens) from the flowers of each genotype between 1500 and 1700 hours the day before corolla expansion and pollination would normally occur. At 0900 hours the next day (ca. 16–18 h after removal from the flower), pollen grains from the dehisced anthers were sprinkled on the surface of the medium. With this collection procedure, the anthers dehisce normally and in the same time period as would occur if the corollas remained attached to the plant. On each collection date, 16 petri dishes were inoculated with pollen grains from each genotype. At each of eight times (30, 60, 90, 120, 150, 180, 240 and 300 min) after inoculation, the activity of 2 dishes was stopped by flooding the surface of the medium with a killing and preservative solution (62 parts water, 5 parts formaldehyde, 3 parts glacial acetic acid, 30 parts glycerol). The flooded dishes were then stored at 2°C.

A microprojector was used to determine germination percentage and tube length at ×67.5 and ×400 magnifications, respectively. For germination percentage, fields of 30–35 well-separated grains were classified. Three counts of 100 grains each were taken on each dish so that six percentage values were obtained for each genotype per time after inoculation per collection date combination. For tube length, 20 randomly selected tubes from each petri dish were measured, giving a total of 40 measurements for each genotype per time after inoculation per collection date combination.

An analysis of variance in the form of a complete factorial with genotype, time after inoculation and collection date as the main effects was performed for each variable. To reduce variance heterogeneity, we transformed the germination percentage and tube-length data to the arcsin × square root of the percent and square root, respectively (Snedecor 1956). The minimum differences for significance presented in the tables were obtained using the Duncan's range values for the maximum number of means to be compared (Harter 1960).

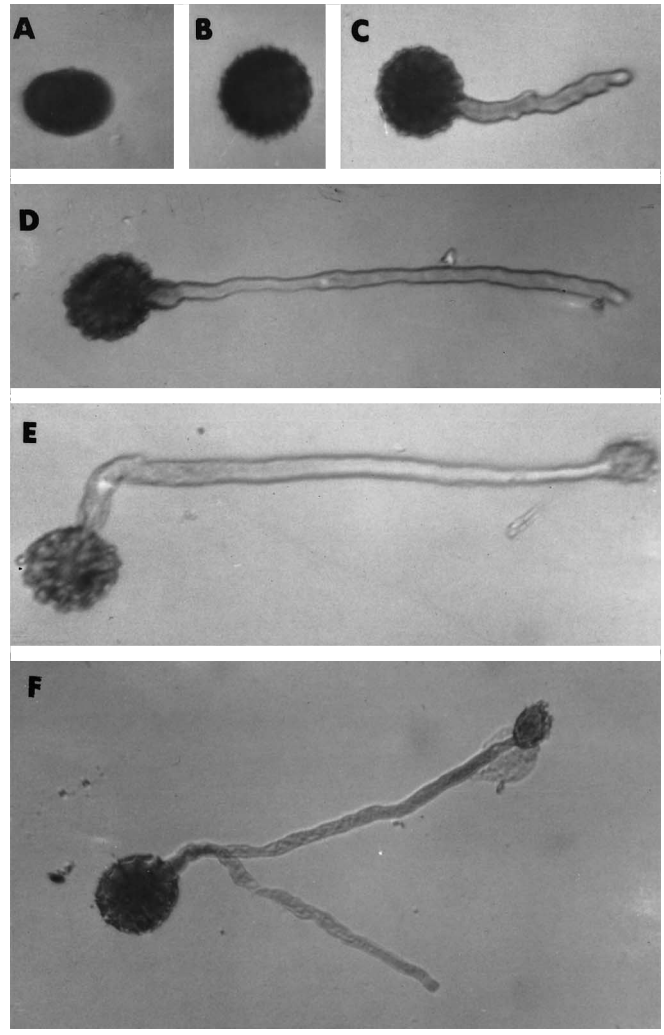

Fig. 1A–F Photomicrographs of ungerminated and germinated grains on the surface of the *in vitro* medium. **A** Polar view of an ungerminated grain, **B** equatorial view of an ungerminated grain, **C, D** tube emergence and development during germination, **E** germinated grain with tube end burst, **F** germinated grain with branched tube. Magnification: ×170

Results

Photomicrographs of ungerminated and germinated grains on the surface of the *in vitro* medium are shown in Fig. 1. Figure 1A, B shows the general shape of the ungerminated grain which has been reported to be a flattened (oblate) sphere with a mean polar diameter of approximately 44 μm and a mean equatorial diameter of approximately 64 μm (Pfahler and Pfahler 1991). The exine wall pattern consists of a series of furrows passing through the poles and intersecting the equatorial plane at right (90°) angles (Pfahler and Pfahler 1991). The pollen tube emerges at the equatorial plane from a pore or opening between the furrows (Fig. 1C, D). After considerable tube growth (2000 + μm), the ends of the tubes in a relatively small percentage (20–30%) of germinated grains burst, expelling their contents on the surface of the medium (Fig. 1E). A relatively small percentage (less than 1%) of the germinated grains have branched tubes (Fig. 1F).

Germination percentage

The analysis of variance is shown in Table 1. All sources of variation were significant at the 1% level except the main effect, collection date, which was significant at the 5% level.

Over the five genotypes, 5% germination was found 30 min after inoculation, and a maximum of 37% germination was reached 120 min after inoculation (Table 2). No significant changes above 120 min after inoculation were present.

As indicated by the highly significant genotype × time after inoculation interaction (Table 1), the pollen grains from the five genotypes differed in the time after inoculation at which germination was observed and maximum germination attained (Table 2). For example, genotype 15 had 0% germination 30 min after inoculation and attained a maximum of 43% germination 150 min after inoculation. No significant changes were found above 150 min after inoculation. Genotype 6 had 9% germination 30 min after inoculation but reached a maximum of 36% germination 120 min after inoculation. No significant changes were found above 120 min after inoculation. A somewhat different pattern was observed in genotypes 1, 3 and 21. These genotypes had almost identical germination 30 min after inoculation, with each reaching a maximum germination 120 min after inoculation. However, the maximum germination attained by these genotypes differed considerably (range = 42% for genotype 1 to 30% for genotype 3).

The statistical significance (Table 1) of the main effect, collection date and its interactions with genotype and time after inoculation indicated that germination can be influenced by collection date within the same year.

Table 1 Mean squares and significance levels from the variance analysis of the transformed (arcsin × square root of the percent) germination percentage results and the transformed (square root) pollen tube length data

Source of variation	*df*	Germination percentage	Pollen tube length
Genotype (G)	4	677.0**	1731.8**
Time after inoculation (TI)	7	6321.5**	27784.3**
G×TI	28	132.3**	347.0**
Collection date (CD)	1	94.3*	336.2**
G×CD	4	299.9**	114.2**
TI×CD	7	46.3**	313.8**
G×TI×CD	28	39.7**	97.7**
Error	[a]	15.1	22.9

*,** *F* values are significant at the 5% and 1% levels, respectively
[a] Error *df* were 400 for germination percentage and 3120 for pollen-tube length

Pollen tube length

The analysis of variance is shown in Table 1. All sources of variation were significant at the 1% level.

Over the five genotypes, the tube length was 91 μm 30 min after inoculation, reaching a maximum of 1100 μm 300 min after inoculation (Table 3).

The highly significant genotype × time after inoculation interaction (Table 1) indicated that tube growth rates among the germinated grains of the five genotypes differed. Among those four genotypes (1, 3, 6, 21) which had low levels of germination 30 min after inoculation (Table 2), no significant differences in tube length was found 30 min after inoculation (Table 3). However, substantial differences in their growth rate over time were present. For example, the mean length of genotype 1, which was 124 μm 30 min after inoculation, reached a maximum of 967 μm 240 min after inoculation, with no significant change 300 min after inoculation (Table 3). At the other extreme, genotype 3, which had a mean length of 97 μm 30 min after inoculation, attained a maximum length of 1261 μm 300 min after inoculation. The increase in length of genotype 15, which had 0% germination 30 min after inoculation (Table 2), was delayed about 30 min compared to the other genotypes but reached a maximum length of 1129 μm 300 min after inoculation.

Table 2 Germination percentage means. The transformed mean is included in parenthesis immediately below each mean for statistical comparisons[a]

Genotype	Time (min) after inoculation							
	30	60	90	120	150	180	240	300
1	6	13	28	42	39	42	37	41
	(13.6)	(20.3)	(31.7)	(40.5)	(38.3)	(40.0)	(37.0)	(39.5)
3	5	11	16	30	26	32	28	34
	(12.1)	(18.1)	(23.1)	(32.8)	(30.5)	(34.5)	(31.7)	(35.4)
6	9	10	17	36	30	30	32	32
	(17.1)	(17.9)	(24.1)	(36.7)	(32.6)	(33.0)	(34.3)	(34.2)
15	0	7	14	35	43	40	36	38
	(0.0)	(14.4)	(21.8)	(35.7)	(40.6)	(39.3)	(36.4)	(37.7)
21	5	14	31	40	41	47	37	39
	(12.1)	(21.4)	(33.0)	(39.2)	(39.6)	(43.4)	(37.2)	(38.4)
Mean	5	11	21	37	36	38	34	37
	(11.0)	(18.4)	(26.7)	(37.0)	(36.3)	(38.0)	(35.3)	(37.0)

[a] Minimum differences among the transformed means for significance at the 5% and 1% level, respectively: time after inoculation = 1.6 and 2.1, and any combination of genotype-time after inoculation = 4.0 and 5.2

Table 3 Pollen tube length means (μm). The transformed mean is included in parenthesis immediately below each mean for statistical comparisons[a]

Genotype	Time (min) after inoculation							
	30	60	90	120	150	180	240	300
1	124	439	470	626	743	785	967	914
	(10.7)	(20.1)	(21.0)	(24.6)	(26.8)	(27.8)	(30.8)	(29.9)
3	97	414	571	637	872	1125	1221	1261
	(9.6)	(19.7)	(22.7)	(24.7)	(29.0)	(33.3)	(34.5)	(35.0)
6	124	326	606	798	778	918	1002	1009
	(10.9)	(17.5)	(23.8)	(27.7)	(27.5)	(29.7)	(31.3)	(31.5)
15	0	218	298	534	680	988	955	1129
	(0.0)	(14.0)	(16.4)	(22.3)	(25.6)	(31.2)	(30.6)	(33.1)
21	108	333	431	654	712	923	1072	1189
	(10.1)	(17.6)	(20.1)	(25.0)	(26.3)	(30.0)	(32.5)	(34.2)
Mean	91	346	475	650	757	948	1043	1100
	(8.3)	(17.8)	(20.8)	(24.9)	(27.0)	(30.4)	(31.9)	(32.7)

[a] Minimum differences among the transformed means for significance at the 5% and 1% level, respectively: time after inoculation = 0.7 and 1.0, and any combination of genotype-time after inoculation = 1.9 and 2.5

The statistical significance (Table 1) of the main effect, collection date, and its interactions with genotype and time after inoculation indicated that growth rate and final length can be influenced by collection date within the same year.

Discussion

The results of this study indicated that the pollen grains from the genotypes or pollen sources differed greatly in germination and tube length. The differences were expressed not only in the maximum germination and tube length attained but also in the rate of germination and tube growth. The specific physiological and biochemical processes associated with germination and tube growth are not fully understood. Studies have indicated that alleles at various endosperm mutant loci in maize can alter not only *in vitro* germination characteristics (Pfahler and Linskens 1972) but also the biochemical composition (Pfahler and Linskens 1970, 1971; Linskens and Pfahler 1977) of the mature grains. Other studies have indicated that certain isozymes and nucleic acids of the pollen grains are altered by their genotype (Hormaza and Herrero 1992). Apparently, the genotype of the pollen grain influences many physiological and biochemical processes that could influence *in vitro* germination.

The relationship between *in vitro* germination and *in vivo* fertilization capacity has not been studied extensively. In a limited number of studies, pollen grains which had no *in vitro* germination were found to be capable of *in vivo* fertilization (Stanley and Linskens 1974; Shivanna and Johri 1985). Occasionally, the reverse was the case, with grains germinating *in vitro* but not being capable of fertilization. Most pollen tubes cultured *in vitro* stop growing before they reach the length normally required to fertilize *in vivo*, and the rate of pollen-tube growth is seldom as rapid as *in vivo* (Stanley and Linskens 1974). In sesame, *in vivo* fertilization requires the tubes to grow approximately 11 000 μm (Pfahler et al. 1996), and the time between pollination and fertilization was estimated to be 4–6 h (Yermanos 1980). In the study reported here, the mean length of the tubes was approximately 1100 μm 300 min after inoculation, which is about 10% of the length required for fertilization. Obviously, the pistil contributes nutrients and conditions not supplied in the *in vitro* medium.

Interest in the potential advantages of pollen genotype selection was generated by the pioneering studies of Mulcahy (1971, 1974). From studies with maize pollen transmission, he found that the ability to fertilize was related to pollen genotype, with the most competitive grains producing progeny which were superior in various important traits. Before pollen genotype selection can be used extensively to supplement sporophytic selection in plant improvement programs, additional studies on both the *in vitro* and *in vivo* levels are necessary to develop appropriate breeding strategies.

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