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IN VITRO GERMINATION AND POLLEN TUBE GROWTH OF MAIZE (ZEA MAYS L.) POLLEN. X. POLLEN SOURCE GENOTYPE AND GIBBERELLIN A_3 INTERACTIONS*

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SUMMARY

Pollen grains from four pollen source genotypes (H55, Oh43, K64 × K55, Ky49 × Ky27) were cultured on an artificial medium (15% sucrose, 0.6% bacto-agar, 0.03% calcium nitrate, 0.01% boric acid) supplemented with four concentrations (0, 5, 25, 100 ppm) of gibberellin A_3 (GA_3). At 1, 2, and 3 h after inoculation, germination percent and pollen tube length were measured. A highly significant concentration × pollen source genotype interaction for germination percent was obtained. Depending on the pollen source genotype, increasing GA_3 concentrations either substantially increased or did not alter germination percent. For pollen tube length, a highly significant concentration × pollen source genotype interaction was also obtained. However, depending on the pollen source genotype, increasing GA_3 concentrations either increased, decreased, or had no effect on pollen tube length. Any stimulatory effect of GA_3 on pollen tube length was expressed early in the germination process or between inoculation and one h after inoculation. The results suggested that heterozygosity level could be a contributing factor in the GA_3 response and within each pollen source genotype, the effects of GA_3 on germination percent and pollen tube length were independent.

1. INTRODUCTION

Gibberellins are known to play a decisive role in all phases of plant growth and development. Their specific role in the male reproductive process is not completely understood but it has been established that considerable variation in endogenous gibberellin levels are present at different stages of this complex process (BARENDSE et al. 1970, FUKUI et al. 1958, KAMIENSKA & PHARIS 1971, STANLEY & LINSKENS 1974). Substantial variation among species in endogenous gibberellin levels during *in vitro* germination was reported (KAMIENSKA & PHARIS 1971). Also, the effect of exogenous gibberellin on *in vitro* germination and tube growth was found to be greatly influenced by species (CHANDLER 1957) and gibberellin concentration (BOSE 1959, CARMICHAEL 1970). At the present time, little is known about genetic factors within a species which may alter the effect of exogenous gibberellins on *in vitro* pollen germination and tube growth. This study reports the combined effects of pollen source genotype and exogenous gibberellin A_3 on the *in vitro* germination characteristics of maize pollen. Four pollen source genotypes representing two extreme heterozygosity levels were included.

*Dedicated to Professor Dr. H. F. Linskens on the occation of his 60th birthday in 1981.

2. MATERIAL AND METHODS

Pollen grains from two inbreds (H55, Oh43) and two single cross hybrids (K64 × K55, Ky49 × Ky27) were cultured on an artificial medium (15% sucrose, 0.6% bacto-agar, 0.03% calcium nitrate, 0.01% boric acid) supplemented with four concentrations (0, 5, 25, 100 ppm) of 94% gibberellin A_3 (GA_3). At 1, 2 and 3 h after inoculation, germination percent and pollen tube length measurements were taken. The complete experiment was repeated on each of two dates.

General methods of pollen collection, medium preparation, inoculation procedures, killing and preservation, data collection, statistical procedures, and statistical techniques have been presented in previous papers (PFAHLER 1965, 1967). A slight variation in the preparation of the medium was used to meet the requirements of this particular experiment. To reduce the loss of GA_3 activity resulting from the necessary heating in preparing the medium, the appropriate amounts of freshly-prepared aqueous GA_3 solutions were thoroughly mixed into the basal medium during cooling but before the solution attained a semi-solid condition. Then, pollen inoculations were made within 2 h after the medium had become semi-solid and had reached ambient temperature.

For both the percent and length data, an analysis of variance in the form of a complete factorial was performed with pollen source genotype, concentration, and hour after inoculation as the main effects. For the percent data, the arcsin transformation was applied before analysis.

Minimum differences for significance in *tables 2 and 3* were obtained by means of the revised Duncan's ranges using for p only the maximum number of means to be compared (HARTER 1960).

3. RESULTS

Germination percent: Highly significant mean squares for the main effects, pollen source genotype and concentration, and the concentration × pollen source genotype interaction were obtained (*table 1*).

Major differences among pollen source genotype means were found ranging from 93% for H55 to 34% for Oh43 (*table 2*).

Over all pollen source genotypes, an increase in concentration from 0 to 5 ppm resulted in a highly significant increase from 62 to 65%, respectively (*table 2*). No difference was obtained between 5 and 25 ppm but at 100 ppm, the percent increased sharply to 71%.

The response of each pollen source genotype to increasing concentrations differed considerably (*table 2* and *fig. 1*). For either of the two inbreds (H55, Oh43), no significant differences among concentrations were found. In contrast, the two single cross hybrids (K64 × K55, Ky49 × Ky27) increased with increasing concentrations. In general, the pattern of the increase by the hybrids followed the same pattern shown by the concentration means, ie., a highly significant increase from 0 to 5 ppm, no change from 5 to 25 ppm, and a highly significant increase from 25 to 100 ppm.

Table 1. Mean squares from the variance analysis of each character

Character	Source of variation	Degrees of freedom	Mean square
Germination percent	Pollen source genotype (PSG)	3	27468.13**
	Concentration (C)	3	620.32**
	C × PSG	9	147.58**
	Hour after inoculation (HI)	2	540.06**
	HI × PSG	6	17.80
	HI × C	6	10.13
	HI × C × PSG	18	15.85
	Error	336	20.35
Pollen tube length	PSG	3	2071487**
	C	3	21371
	C × PSG	9	73940**
	HI	2	26257398**
	Hi × PSG	6	636088**
	HI × C	6	65246**
	HI × C × PSG	18	24771
	Error	3792	16273

** F value significant at the 1% level.

A highly significant mean square for the main effect, hour after inoculation, was also present (*table 1*).

Over all pollen source genotypes and concentrations, the percent was 62, 67 and 68 at 1, 2, and 3 h after inoculation, respectively. A highly significant increase was obtained only between 1 and 2 h after inoculation.

Pollen tube length: Highly significant mean squares for the main effect, pollen source genotype, and the concentration × pollen source genotype interaction were found (*table 1*).

Major differences among pollen source genotype means were found ranging from 596 μm for Oh43 to 501 μm for K64 × K55 (*table 2*).

Considerable variation among the pollen source genotypes to increasing concentrations was observed with no distinct pattern emerging (*table 2* and *fig. 1*). Increasing concentrations either had no effect (Ky49 × Ky27), increased the length only at the highest concentration (K64 × K55), or altered the length in an irregular pattern (H55, Oh43). In general, no consistent relationship between GA_3-induced changes in the germination percent and pollen tube length within each pollen source genotype was apparent suggesting that the effect of GA_3 on germination percent and pollen tube length was independent.

A highly signficant mean square for the hour after inoculation × concentration interaction was present (*table 1*).

At 1 h after inoculation, the length at 5, 25, and 100 ppm were approximately equal but considerably longer than 0 ppm (*table 3*). At 3 h after inoculation, the lengths at all concentrations were essentially equal. Apparently, over all pollen source genotypes, the effect of GA_3 was not associated with concentration levels above 0 ppm and was expressed primarily during the early germination phase.

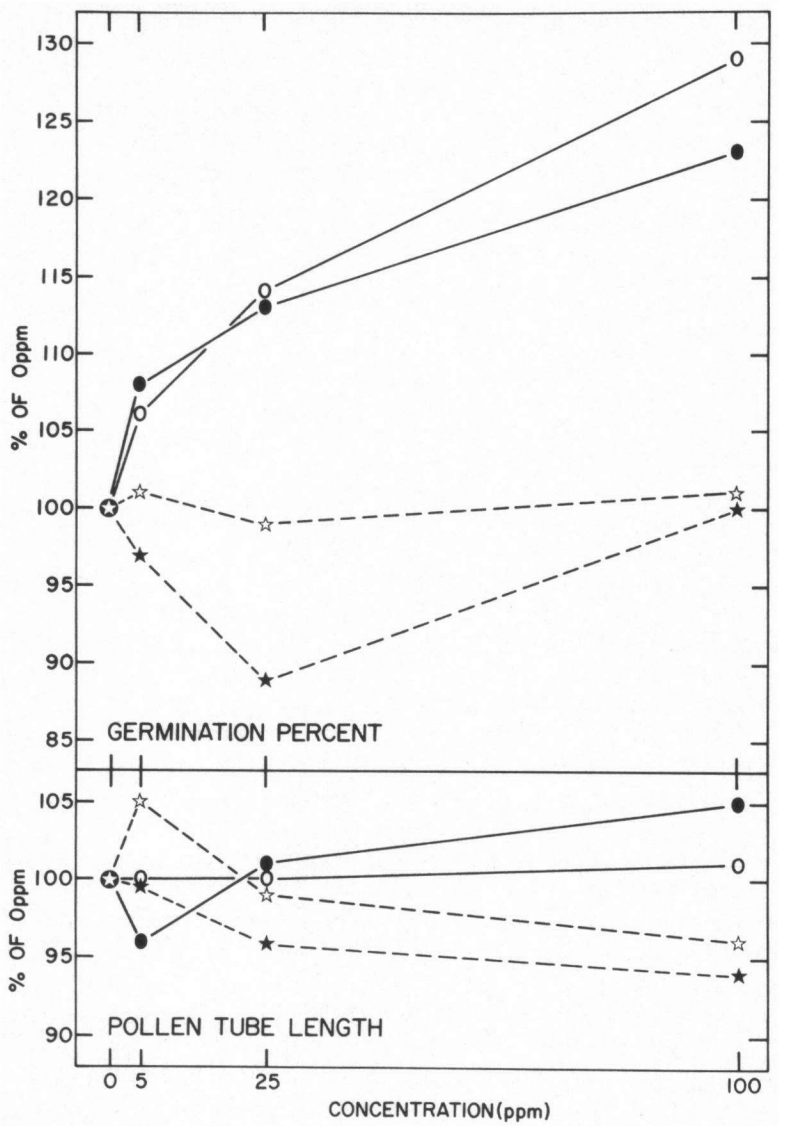

Figure 1. The effect (in % of 0 ppm) of increasing concentrations of GA_3 on the germination percent and pollen tube length of four pollen source genotypes. Open stars = H55; closed stars = Oh43; closed circles = K64 × K55; and open circles = Ky49 × Ky27.

Table 2. The effect of various concentrations of GA_3 on the germination percent and pollen tube length of four pollen source genotypes

Character	Pollen source genotype	Concentration (ppm)				Pollen source genotype mean
		0	5	25	100	
Germination percent[a]	H55	92(73.3)	93(75.6)	91(72.5)	94(75.9)	93(74.3)
	Oh43	35(35.7)	34(35.4)	31(34.0)	35(36.1)	34(35.3)
	K64 × K55	71(57.4)	77(61.2)	80(62.8)	87(69.5)	79(62.7)
	Ky49 × Ky27	51(45.3)	54(47.0)	58(49.3)	66(54.2)	57(49.0)
	Concentration mean	62(52.9)	65(54.8)	65(54.7)	71(58.9)	
Pollen tube length (μm)[b]	H55	558	584	552	538	558
	Oh43	612	612	585	576	596
	K64 × K55	498	480	504	521	501
	Ky49 × Ky27	499	501	501	505	502

[a] Each value associated with each combination of pollen source genotype and concentration represents the mean of 24 measurements. Minimum differences for significance among means in parenthesis (arcsin transformation) at the 5 and 1% level, respectively are as follows: (1) Pollen source genotype or concentration = 1.4 and 1.8; and (2) Any combination of pollen source genotype and concentration = 3.2 and 4.1.

[b] Each value associated with each combination of pollen source genotype and concentration represents the mean of 240 measurements. Minimum differences for significance among means at the 5 and 1% level, respectively are as follows: (1) Pollen source genotype = 13 and 16; and (2) Any combination of pollen source genotype and concentration = 28 and 36.

4. DISCUSSION

The results of this study indicated that the effect of exogenous GA_3 on the germination percent and pollen tube length was influenced by pollen source genotype. Although limited information is available concerning endogenous levels of gibberellins and/or growth substances in maize pollen (FUKUI et al. 1958, JONES 1964, STANLEY & LINSKENS 1974), no studies reporting differences resulting from pollen source genotype were found. Alleles at a number of loci associated with dwarfing in maize are known to interfere with different reactions in the biochemical pathway of gibberellin production and as a result, influence the endogenous gibberellin level in the sporophyte (PALEG & WEST 1972, PHINNEY 1961). This control of a number of steps in the biochemical pathway of gibberellin production in the sporophyte by simple qualitative genetic factors suggests that differences in endogenous gibberellin levels resulting either from the genotype of the haploid pollen grain or the diploid pollen source are a distinct possibility. However, all pollen source genotypes tested in this study were normal at the known dwarfing loci and as a result, these qualitative genetic factors were not involved.

The pollen source genotypes tested in this study did differ in another important aspect, heterozygosity level. Since the general vigor, productivity and environmental stability of hybrids greatly surpass the inbreds because of complex quantitative factors, differences in heterozygosity levels might influence the

Table 3. The effect of various concentrations of GA_3 on the pollen tube length (μm)[a] at each hour after inoculation

Hour after inoculation	Concentration (ppm)			
	0	5	25	100
1	369	389	385	396
2	587	569	552	553
3	670	676	669	655

[a] Each value represents the mean of 320 measurements. Minimum differences for significance among the means were 24 and 31 at the 5 and 1% level, respectively.

pollen response to GA_3. The results found in this study indicated that both hybrids responded to increasing GA_3 levels to a much greater degree than both inbreds especially for germination percent. The specific effects of heterozygosity level on this consistent response pattern cannot be determined at this time since the response to exogenous GA_3 application is associated not only with endogenous GA_3 levels *per se* but also a large number of factors which influence GA_3 effectiveness (BARENDSE 1975, MANN 1975, PALEG & WEST 1972). However, the consistency of these results suggests that heterozygosity levels should be examined further or at least considered as a potential contributing factor.

The specific mechanism by which gibberellins influence *in vitro* germination and pollen tube growth is unknown at the present time. Depending on the species, gibberellins have been reported to affect either germination percent, pollen tube length or both (CHANDLER 1957). Once again depending on the species, the gibberellin concentration was found to be an important influencing factor with any enhancing effect present at intermediate levels and an inhibitory effect observed at higher concentrations (BOSE 1959, CARMICHAEL 1970, CHANDLER 1957). Gibberellins are known to have many metabolic effects including α-amylase formation, nucleic acid synthesis, invertase production and membrane systems (MANN 1975). Maize pollen is classified as starchy (BAKER & BAKER 1979). Therefore, the effect of GA_3 on *in vitro* maize pollen germination may be related to the breakdown and utilization of starch as an energy source during the germination process. The results reported here indicate that the most pronounced effect of GA_3 on pollen tube length was observed between inoculation and 1 h after inoculation. However, when a large number of species is surveyed (BAKER & BAKER 1979) and their *in vitro* germination response to gibberellins compared (CHANDLER 1957), no consistent relationship between starch level in pollen and gibberellin effects is apparent.

Since gibberellins are closely associated with many aspects of reproduction, additional research in this area would be desirable. Studies indicating that the application of exogenous gibberellins induced male sterility in maize (NELSON & ROSSMAN 1958) and promoted pollen tube growth and ovary development in otherwise incompatible barley-rye crosses (LARTER & CHAUBEY 1965) sug-

gest the extent of gibberellin effects in the reproductive area and the potential value and benefits on a practical level.

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