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Pollen-pistil interaction in maize: effects on genetic variation of pollen traits

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Abstract Various factors (pollen diameter, in vitro germination and tube length, in vivo growth rate in selfed and nonselfed styles) which could possibly contribute to the competitive ability of pollen were investigated on 30 *Zea mays* L. inbred lines. The only factor with which pollen diameter was positively correlated was in vitro pollen-tube growth. Traits related to the early stages of growth (in vitro germination, in vitro tube length, early in vivo pollen growth rate) were all positively correlated with each other, and these early characteristics were negatively correlated with late in vivo tube growth rate, which is largely influenced by the stylar genotype.

Key words Maize · *Zea mays* L. · Pollen competitive ability · Pollen germinability Pollen-tube growth · Pollen-pistil interaction

Introduction

Pollen fitness is a trait of great biological significance since, as a component of plant fitness, it can affect the genetic structure of plant populations and has important agronomic implications in that it conditions the reproductive biology of crops. The reproductive success of a pollen grain is the result of diverse pre- and post-shedding components, among which germination time and rate, tube growth rate, pollen-pistil interaction are the most relevant in determining the variability in pollen fitness since, after shedding, pollen grains are directly exposed to the effects of the external and stylar environment.

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Although primary metabolic systems should be common to most developmental stages, there should also be different sets of genes expressed during the diverse stages of pollen development and function. Recently, many pollen-specific genes have been detected and isolated, and different classes of stage-specific genes, expressed during early or late phases of microspore development, have also been identified (Mascarenhas 1992). Very little is known about the function of these genes or about their possible involvement in the control of pollen traits. On the other hand, the influence of genotype on pollen development and function has been demonstrated in a number of plant species (Ottaviano et al. 1991), but little information is available about the specific genes responsible for this influence.

The findings of Kindiger et al. (1991) indicate the existence of genes specifically controlling the different stages of microsporogenesis. Using B-A translocations of maize, these investigators demonstrated that the developmental modifications of the microspore induced by the presence of different chromosomal deficiencies vary according to the particular chromosome fragment lost. Information on the genetic systems involved in the control of pollen function has been produced by linkage analysis with restriction fragment length polymorphism (RFLP) markers of post-shedding pollen competitive ability (PCA) components: pollen grain germinability in vitro and in vivo, pollen-tube elongation in vitro, and tube growth rate in vivo (Sari-Gorla et al. 1992, 1994). These experiments identified quantitative trait loci (QTLs) controlling each component of trait variability and indicated that different sets of genes control the early stages of pollen growth and the late growth stage, when pollen tube is well-established into the style, up to fertilization.

The pollen-style interaction is an important component of pollen fertilization ability: in many plant species, such as maize (Pfahler 1967; Sari-Gorla et al. 1976; Ottaviano et al. 1980), pearl millet (Sarr et al. 1988; Robert et al. 1991) and barley (Pedersen 1988), it has been shown that pollen performance varies according to

the pollen/style genetic combination. An attempt to detect loci specifically related to the pollen-pistil interaction was made in maize by means of molecular markers; pollen of a recombinant inbred (RI) line population was used to pollinate female plants of two different genotypes: the F_1 from which the population was derived ("self-female") and a genetically unrelated hybrid genotype ("cross-female"). Linkage analysis allowed the identification and chromosome localization of genetic factors involved in the pollen-style interaction (Sari-Gorla et al. 1995). The data revealed, on average, a greater growth of pollen on the self-female in the early stages and a greater tube growth on the cross-female during the later stage. Moreover, a significant inverse correlation was observed within females: RI lines with high germinability revealed a low growth rate, and those with low germinability revealed a high growth rate. These were quite unexpected observations. However, it should be taken into account that, in this experiment, the segregating genes from only two genotypes were examined – those of the two parental lines from which the RI population was produced.

The purpose of the investigation described here was to determine if the pollen-pistil interaction effects indicated from these results may have a general biological significance.

Materials and methods

Plant material and experimental design

Thirty inbred lines (A632, A660, A69Y, B14, B37, B73, C103, CG15, F7, H95, H99, H117, H3025, Hp302, K41, K55, Ky226, L289, Lo876, Mo17, NI72, OH43, Pa33, Tx323, Va85, W22, WF9, W64A, 33.16) of different genetic origin and representative of maize germ plasm were used as pollen parents.

Female parents were of two types: "self-female", that is, the same as the pollen source, and an hybrid genotype (A62 × Mo17). Pollen diameter, germination percentage and tube elongation in vitro was determined on pollen from each inbred. On each of the two females, self and cross, pollen germinability and early tube growth (PGG) as well as pollen-tube growth rate (PTGR), explained below, were also measured.

The experimental design was a complete randomized block with two replications (days).

Each day freshly shed pollen from the 30 inbreds was collected. Samples were dusted onto artificial medium (100 mg H_3BO_3, 300 mg $Ca(NO_3)_2$, 170 g sucrose, 7 g agar/l), solidified in a glass cylinder, cut in slices of 1–2 mm and put in petri dishes. After 3 h of incubation at 27 °C, activity was stopped by additing a few drops of fixative solution (Farmer liquid: ethanol, acetic acid 3:1) to the dishes, and the dishes were stained with aniline blue. A sample of the same pollen was mixed with an equal quantity of pollen of a standard line (W22), genetically marked for coloured aleurone and used to pollinate the self-female and the cross-female (5 plants for each). Thus, a total of ten ears per 60 male-female combinations was planned. However, the Hp302 line was discarded since it was revealed to carry a *Ga* (*Gametophytic factor*) allele; moreover, because of flowering time synchronization, it was only possible to make 25 pollinations per female.

Pollen grown under artificial conditions was used to measure grain diameter, percentage of germination and tube length. On the ears resulting from the mixed pollinations on the two females, pollen characters expressed in vivo, PGG and PTGR, were evaluated.

Character evaluation

Pollen diameter, germination percentage and tube elongation in vitro were evaluated with the aid of a graphic digitizer (Videoplan). Grain diameter was measured on 40 grains per dish, per 2 days, for a total of 80 grains. Percentage of germination was assayed on 500 grains per dish, and 40 pollen tubes was measured per dish.

Pollen germinability and tube growth rate in vivo were evaluated on the ears resulting from the mixed pollinations of the two females. Each ear, according to its length, was divided into four or five segments each eight kernels long, and the proportion of uncoloured kernels was determined for each segment. Since the style length in maize varies according to the position of the flowers on the ear, increasing from the apex to the base, relative pollen-tube growth rate (PTGR) can be expressed as the increase in the proportion of uncoloured kernels from the apex to the base, i.e. as a regression coefficient of the uncoloured kernels on the ear segments. Furthermore, the proportion of uncoloured kernels in the apical segment gives a measure of pollen germinability and early tube growth (PGG); these two components cannot be evaluated separately since the proportion of kernels produced by the two pollen types at the apex of the ear represents either higher in vivo germinability and/or faster tube growth of those germinating.

Statistical analysis

The association between traits were assessed by the Pearson correlation coefficient (SAS 1985).

With respect to the analysis of the in vivo components of PCA, in the case of PTGR the vector of observed proportions was linearly transformed by means of an operator matrix in which rows correspond to the orthogonal polynomial coefficients that define the linear component (Ottaviano et al. 1988). Both PTGR, after transformation, and PGG were subjected by the SAS GLM procedure (SAS 1985) to a weighted least square analysis, the weights being the reciprocals of the proper variances. Two models were adopted: (1) Model A – analysis of variance (ANOVA); (2) Model B—analysis of covariance (ANCOVA), including regression on in vitro germinability for PGG, and regression on PGG for PTGR. Thus, the following models were used:

Model A: $E(y_{ijk}) = \mu + \beta_i + \phi_j + \gamma_k + (\phi\gamma)_{jk}$

Model B: $E(y_{ijk}) = \mu + \rho x_{ijk} + \beta_i + \phi_j + \gamma_k + (\phi\gamma)_{jk}$

where μ = general mean, β_i = effect of i-th block (day), $i = 1,2$, ϕ_j = effect of j-th female, $j = 1,2$, γ_k = effect of the k-th male, $k = 1, \ldots, 25$, $(\phi\gamma)_{jk}$ = interaction effect of j-th female and k-th male, x_{ijk} = observation of in vitro germinability (or PGG), ρ = coefficient of regression of PGG on in vitro germinability, or of PTGR on PGG.

Results

The analysis of the traits was carried out in a sequential manner according to the structure of the experiment. First, the pollen traits (grain diameter, in vitro germination and growth) were measured as they can be considered to be indicators of pollen quality, independent of the effect of the female environment. Then pollen was used for pollinating the two females. PGG was measured on the first segment of the ears resulting from the mixed pollination, and PTGR was computed as the regression of frequencies of uncoloured kernels of the inbred lines in all segments, including the first. A positive regression coefficient would indicate that the frequency of marker kernels decreased from the tip to the base of the ear. The

higher this coefficient was, the greater the presumed PTGR of the inbred pollen being tested.

First of all, in order to obtain a general picture of the possible relationships between all of the pollen characters, a correlation analysis was performed. The correlation coefficients between the PCA components, expressed both in vitro and in vivo, computed on the entire set of data, are reported in Table 1.

Pollen diameter was significantly associated only with tube length in vitro, a finding already reported by Kumar and Sarkar (1980). This suggests that larger pollen grains have more reserve material to be used in the autotrophic early stages of growth in vitro, whereas this advantage is lost when the heterotrophic growth continues into the stylar tissues.

Traits related to the early stages of growth (in vitro germination percentage, in vitro tube length, PGG) were all positively correlated with each other, negatively correlated with late tube growth (PTGR). Lines that performed well during the first stages of pollen function exhibited a lower tube growth rate in the later stages, whereas genotypes that grew slowly in the early phase had a higher performance later on. When considering pollen characteristics within females, the picture is about the same, but greater effects were observed for pollen growth features on the cross-female. In particular, the correlation coefficient between PGG and PTGR in the cross combination was -0.70, but only -0.19 between PGG and PTGR on the self-female.

Correlation analysis revealed that of the three pollen traits (pollen diameter, in vitro germination and in vitro tube length) in vitro germination had the strongest association with PGG. Therefore, two models were adopted for PGG analysis: an ANOVA model (Model A) and a model (Model B) including regression on in vitro germinability. The results (type III mean squares) are summarized in Table 2. The model which should be preferred, since it better fit the data, was model A; it indicates, as expected, a wide variability among the pollen genotypes, significant differences between females and a slightly significant interaction.

On the basis of this model, predictions of PGG (predicted values of uncoloured kernels in the first segment of the ear) were 35% for the self-female and 31% for the cross. Thus, it can be concluded that, as previously stated, in vitro germinability has no effect on in vivo performance and, in general, the self environment is more adaptive for early growth than is the cross environment.

Similarly, for PTGR only the association with PGG was found to be of potential meaning. Thus, for the analysis of this trait two models were considered: standard ANOVA (Model A) and Model B, which included PGG as the concurrent variable. The results (see Table 3) indicated that Model B should be preferred, since it decreased the error MS and the regression on PGG was significant. The analysis indicated high variability between pollen sources, whereas differences between females were not significant, even though the mean value of PTGR on the cross-female was greater than that on the self, -1.5 and -2.3, respectively, clearly indicating a more negative trend with respect to the standard pollen for pollen growing into the self stylar environment. The interaction effects were highly significant, thus indicating that the performance of the pollen tube during the late stages of growth, though depending on the pollen genotype, was largely affected by the female stylar genotype. The relative influence of the pollen-

Table 2 Mean square values from the ANOVA of PGG of 25 inbred lines on the self- and the cross-females

Source	Model A	Model B
In vitro germinability	–	0.03
Female	52.93*	59.96*
Male	311.29*	212.13**
Female*male	19.61*	19.61
Error	10.98	11.22

*, ** $P < 0.05$ and 0.01, respectively

Table 3 Mean square values from the ANOVA of PTGR of 25 inbred lines on the self- and the cross-females

Source	Model A	Model B
PGG	–	40.01*
Female	1.47	0.12
Male	31.85**	15.60**
Female*male	13.35*	13.58**
Error	6.52	5.80

*, ** $P < 0.05$ and 0.01, respectively

Table 1 Coefficients of correlation between pollen traits

	In vitro germination percentage	In vitro tube length	PGG on self-female	PGG on cross-female	PTGR on self-female	PTGR on cross-female
Pollen diameter	−0.05	0.38*	0.22	0.05	−0.13	−0.08
In vitro germination %		0.57**	0.49**	0.57**	0.05	−0.53**
In vitro tube length			0.38*	0.39*	0.03	−0.27
PGG on self-female				0.80**	−0.19	−0.72**
PGG on cross-female					0.20	−0.70**
PTGR on self-female						0.01

*, ** $P < 0.05$ and 0.01, respectively

pistil interaction in modulating early (PGG) and late (PTGR) tube growth is graphically illustrated in Figs. 1 and 2, where the performances of the inbred lines with respect to PGG and PTGR on self- and cross-females are reported.

Discussion

PCA expressed when there is pollen-tube growth into a self stylar environment and in an unrelated female geno-

Fig. 1 Single inbred line performance for PGG (proportion of uncoloured kernels in the apical segment of the ear) on self- and cross-females

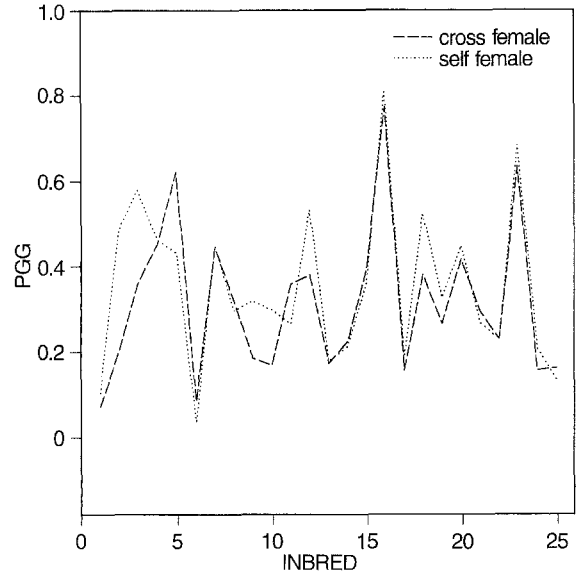
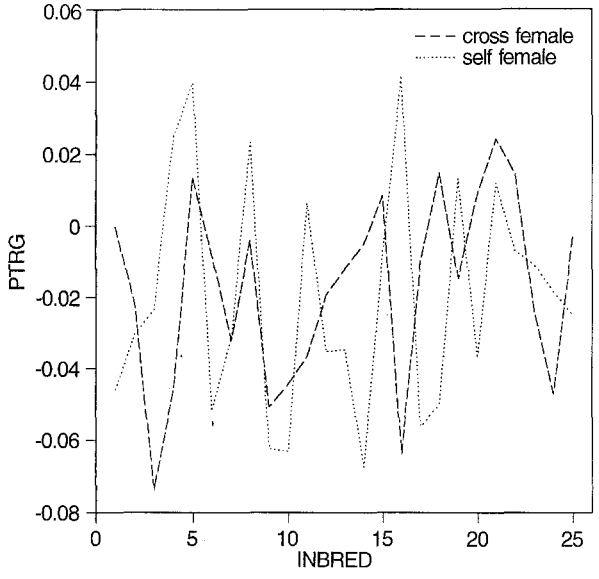


Fig. 2 Single inbred line performance for PTGR (coefficient of regression of the proportion of uncoloured kernels on the ear segments) on self- and cross-females



type revealed two aspects. (1) The characters describing early growth were all positively correlated, whereas a negative association was revealed with the late stage of growth. This negative correlation was particularly high on the cross-female. In general, considering all the data, it emerges that genotypes which performed well during the early stages of growth had a lower growth rate in the later phases, and those with greater efficiency with regard to germinability and early growth had a lower tube growth rate. (2) On average, pollen grew better on female tissues of the same genotype in the early stage, while tube growth rate during the later stages was largely dependent on the genotype of the stylar environment. Since the data reported here involved a sample of genotypes representative of maize germ plasm, they suggest that these phenomena are widespread in this species.

A possible physiological mechanism explaining this different performance in the early and late stages of pollen growth could be connected with differences in pollen size in that varying amounts of reserve material could allow a more efficient metabolism during the phase of autotrophic growth (Kumar and Sarkar 1980). However, the results reported here revealed that pollen diameter was significantly associated only with pollen elongation in vitro, whereas it appeared not to have any influence on the heterotrophic growth in vivo.

Generally speaking, it should be expected that genes for accelerated pollen-tube growth should spread throughout a population and, conversely, that mutations causing slower growth should be eliminated, thereby leading to the fixation of alleles giving a maximal pollen-tube growth rate (Walsh and Charlesworth 1992). However, variation in pollen-tube growth rates are frequently observed in natural populations of plants (Richardson and Stephenson 1992). Phenomena which maintain genetic variability in pollen competitive ability must thus exist.

It is possible that genetic factors for significant PCA would reduce fitness in other stages of the plant life cycle, resulting in a negative genetic correlation between pollen and progeny quality. However, many experimental results obtained in different plant species have demonstrated that pollen genotypes with a faster pollen-tube growth tend to produce progeny with a high fitness (see Ottaviano and Mulcahy 1989; Sari-Gorla and Frova, 1995 for reviews). Thus, most of the variation would be expected to be due to detrimental mutations, so that pollen selection acts as a process reducing the level of the genetic load (Walsh and Charlesworth 1992).

In the present study, we observed a highly significant male × female interaction in PTGR. This is possible even though the mean values for PTGR do not differ between cross- and self-pollinations. In some combinations, the self PTGR was considerably different from the cross PTGR and in others the difference was small, thus producing a significant interaction. This observation is highly relevant to the Walsh and Charlesworth question of how can genetic variation in PTGR persist despite

intense selection. Given the strong interaction between female and male in determining PTGR, the fixation of genetic factors which determine PTGR could not be easily accomplished. This suggested that the continued maintenance of genetic variation in PTGR could be a consequence of what Van Valen (1973) has described as the Red Queen hypothesis. That is, the high significant female × male interaction produces an ever-shifting optimum that prevents the fixation of specific genotypes.

The maintenance of genetic variability for PCA in natural populations could also be facilitated by the fact that different components of post-shedding PCA are controlled by different sets of genes (Sari-Gorla et al. 1992, 1994) and that frequently their allelic distribution confers an opposite advantage during the diverse phases of pollen function. Further studies, especially those including characteristics of the female components of the interaction, are needed before any final conclusions can be reached.

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