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Gametophytic expression of genes controlling endosperm development in maize

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Summary. An indirect approach was adopted to select viable mutants affecting the male gametophytic generation in maize. This approach consists of a selection of endosperm defective mutants followed by a test for gametophytic gene expression, based on the distortion from mendelian segregation and on the measurement of pollen size and pollen sterility. The material used consisted of 34 endosperm defective viable mutants introgressed in *B37* genetic background. Complementation tests indicated that the mutation in the collection of mutants affected different genes controlling endosperm development. The study of the segregation in F2 revealed four classes of *de* (defective endosperm) mutants: (1) mutants in which the mutation does not affect either gametophytic development or function; (2) mutants in which the effect on the gametophyte affects pollen development processes; (3) mutants showing effects on both pollen development and function, and (4) mutants where only pollen tube growth rate is affected. Positive and negative interactions between pollen and style were detected by means of mixed pollination (pollen produced by *de/de* plants and pollen from an inbred line used as a standard and carrying genes for colored aleurone), on *de/de* and *de/+* plants. Positive interactions were interpreted as methabolic complementation between defective pollen and normal styles.

Key words: Gametophytic gene expression – Defective endosperm mutants – Pollen-style interaction

Introduction

Data accumulated during the last few years have shown that in higher plants there are a large number of genes expressed during the male gametophytic phase, and that a large portion of these genes are also expressed in the sporophytic phase (Tanksley et al. 1981; Sari-Gorla et al. 1986; Willing et al. 1984; Mascarenhas et al. 1986). Selection experiments have revealed that a large amount of genetic variability is found for most of the components of male gametophytic competitive ability, and that gametophytic selection affects both gametophytic and sporophytic generations (Ottaviano et al. 1982; Ottaviano and Sari-Gorla 1986; Ter-Avanesian 1978; Mulcahy et al. 1975; Searcy and Mulcahy 1985; McKenna et al. 1983; Zamir et al. 1981, 1982; Zamir and Vallejos 1983).

While gametophytic gene expression and gametophytic selection results reveal the importance and the role of gametophytic genetic variability due to post-meiotic gene expression, the genetic dissection of the male gametophytic phase can be efficiently approached on the basis of gametophytic mutants for pollen development in the anthers and for pollen function, i.e., pollen germination and tube growth. This type of study permits investigation of the genetic control of development in a very simple model system: pollen has a haploid genome and the structure includes only three cells.

There are few gametophytic mutants already available and suitable for this type of research. In maize all cases described of chromosomal gene male sterility (Albertsen and Phillips 1981), and cytoplasmic male sterility and restoration of cytoplasmic male sterility (Laughnan and Gabay-Laughnan 1983), are sporophytically controlled, with the exception of the restoration of *S* cytoplasm due to *Rf3* gene, which is expressed in the gametophyte (Laughnan and Gabay 1973). A gametophytic mutant – *sp: small pollen* – affecting pollen development was described by

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Singleton and Mangelsdorf (1940), while mutants for pollen tube growth rate are represented by the *gametophytic factors* (*ga*), whose expression is conditioned by the genotype of the style (Nelson 1952; Schwartz 1960; Bianchi and Lorenzoni 1975).

The screening of viable mutants affecting pollen function and/or pollen development presents difficulties of different order: (1) within species morphological variability of the pollen grains is very limited and largely controlled by the sporophyte (Heslop-Harrison et al. 1973), (2) genetical analysis of viable mutants is difficult and time-consuming. For these reasons, our selection of gametophytic mutants has been based on an indirect approach. The idea is mainly based on the observation that components of pollen deevelopment and function show positive correlations with endosperm development (Mulcahy 1971; Ottaviano et al. 1980), and that some alleles determining defective endosperm in maize (Jones 1928) and lethal embryo in *Arabidopsis* (Meinke 1982, 1985; Meinke and Baus 1986) are expressed in the male gametophyte. The detection of defective endosperm mutant genes affecting the male gametophytic generation (*de-ga* mutants) is mainly based on distortion from the expected mendelian segregation. Quantitative analysis of pollen size and pollen sterility is used for gametophytic characterization of the mutants, while the analysis of the progeny produced by means of mixed pollinations is used for the detection of pollen-style interaction.

Materials and methods

The material used in this study consisted of 34 single-gene endosperm viable mutants. Twenty of these were kindly furnished by Prof. F. Salamini (Istituto di Cerealicoltura di Bergamo); hereafter they will be referred to by their original laboratory numbers: *de*-B1, de*-B3–de*-B246.* Fourteen mutants of spontaneous origin obtained from various material will be referred to by identification numbers: *de*-M1, de*-M2 –de*-M15.* As a check for genetical analysis a *bt2* mutant line was also included in the experimental group. All the mutants used were introgressed in the same homogeneous inbred (*B37*) background: seven backcross generations were carried out for the material referred to as *de*-B*, and five generations for all the other mutants. Hereafter, when not referring to a specific mutant line, the symbol *de* and + will be used to indicate, respectively, mutant and normal alleles or phenotypes.

A complementation test between 13 *de*-B* mutant lines (*1, 3, 15, 18, 22, 34, 37, 50, 69, 116, 122, 126* and *127*) was carried out on the basis of a partial diallel design including 58 *F1* combinations.

Heterozygous plants (*de*/+) were selfed and the resulting ears (*F2* kernels) were scored for *de* and normal phenotypes. Before scoring, kernels at the extreme top and base parts of the ears were removed in order to avoid mis-classifications. Then the ears were divided into three sectors of equal length (number of kernels per row divided by three), to obtain the proportion of *de* phenotypes in the top, middle, and base sectors of the ear. For the majority of the mutant lines studied, five well-developed and grained ears were used. Gametophytic expression of *de* allele was tested as deviation from the expected mendelian segregation and as heterogeneity between sectors (see next section).

The mutants showing significant distortions from mendelian expectation were analyzed for pollen size and pollen sterility. For this analysis, the main branch of the tassel of *de/de, de/+* and *+/+* plant genotypes, obtained by selfing the most advanced backcross generation, was removed and fixed in 3:1 ethanol/acetic acid (Farmer's fluid) solution one day before pollen shedding. The genotype of each plant was established on the basis of the segregation in the selfed progeny. In a few cases, some genotypes were not recovered. Samples of pollen from anthers in the same position on the tassel branch were collected and stained in a solution of blue aniline dye. Pollen size evaluated as grain area was measured by means of a graphic digitalizer (Videoplan Kontron) and pollen sterility as a percentage of empty unstained grains. Sample size was 100 and 400 per each parameter, respectively, and three replications were considered for each genotype of each mutant. Differences between plant genotypes of the same mutant were evaluated by means of analysis of variance. For this statistical analysis the proportion (p) of pollen sterility was transformed into *arc sen p.*

Pollen-style interaction was studied by means of mixed pollination technique in seven *de*-B* mutants (*1, 3, 18, 22, 116, 126* and *127*). Pollen collected from *de/de* plants was mixed with an equal amount of pollen of an unrelated inbred line (*W22*) with colored aleurone, and used to pollinate *de/de* and *de/+* plants of the same *de* mutant. To avoid differences due to sampling variation, the two plant genotypes of each mutant were pollinated by pollen of the same mixture simultaneously. Ears produced were divided in three sectors as reported above and scored for colored and uncolored kernels.

Results

The progeny produced by crossing the 13 different *de* mutants showed normal endosperm phenotypes in all 60 combinations, indicating that the mutation is based on 13 different genes involved in endosperm development. Considering the fact that all of the *de*-M* mutants were of independent origin and the results of the complementation test carried out by Manzocchi et al. (1980a) on a set of *de*-B* mutants, *mn* (*miniature*) and *cp* (*collapsed*), these results indicate that 33 mutants included in this study in most cases carry a mutation for a different gene affecting endosperm development.

Table 1a summarizes the results of the *F2* segregation analysis. For most of the mutants 4–6 ears were considered; only for a few of them was the analysis based on the progeny of 2–3 ears. Statistical analysis tests: the deviation from the expected ratio 3:1 of the overall *F2* progeny of each mutant ($\chi^2_{(1)}$) and the heterogeneity between the ear sectors ($\chi^2_{(2)}$). The latter value is obtained as $\sum_1^3 \chi^2_{(1)i} - \chi^2_{(1)} = \chi^2_{(2)}$, where $\chi^2_{(1)i}$ is the value referred to each ear sector: apex, centrum and

Table 1a. F2 segregation of 34 defective endosperm (*de**) mutants

Mutants	No. of kernels	% of *de* phenot.	$\chi^2_{(1)}$	$\chi^2_{(2)}$	Mutants	No. of kernels	% of *de* phenot.	$\chi^2_{(1)}$	$\chi^2_{(2)}$
de-B1*	2,039	20.70	**	**	*de*-B127*	1,691	16.38	**	**
de-B3*	1,447	19.07	**	**	*de*-B163*	791	15.30	**	ns
de-B15*	2,269	26.75	ns	ns	*de*-B246*	1,827	17.52	**	ns
de-B18*	1,990	23.47	ns	**	*de*-M1*	393	9.16	**	ns
de-B22*	1,825	27.23	*	ns	*de*-M2*	1,477	25.66	ns	ns
de-B34*	1,029	17.59	**	ns	*de*-M3*	893	21.87	**	ns
de-B37*	1,779	22.60	**	**	*de*-M4*	281	24.20	ns	ns
de-B39*	1,737	27.00	ns	ns	*de*-M5*	223	76.68	**	ns
de-B40*	2,327	23.25	ns	ns	*de*-M6*	373	12.33	**	ns
de-B50*	705	19.57	**	ns	*de*-M7*	829	24.60	ns	ns
de-B69*	1,687	23.37	ns	ns	*de*-M8*	392	26.79	ns	ns
de-B70*	1,744	27.52	**	ns	*de*-M11*	893	35.83	**	**
de-B76*	662	34.14	**	ns	*de*-M12*	1,945	22.26	**	ns
de-B116*	959	13.97	**	ns	*de*-M13*	251	25.90	ns	ns
de-B122*	1,835	19.07	**	ns	*de*-M14*	817	25.70	ns	ns
de-B123*	2,065	22.37	**	ns	*de*-M15*	408	46.68	**	ns
de-B126*	1,404	9.47	**	ns	*bt 2*	2,731	26.00	ns	ns

ns, * and ** stand for not significant ($P > 0.05$), significant ($P < 0.05$) and highly significant ($P < 0.01$) differences, respectively

Table 1b. Classes of *de* mutants according to distortions from 3 : 1 ratio (classes are indicated according to the proportion of *de* kernel in F2)

	$P \simeq 0.25$	$P < 0.25$	$P > 0.25$	$P_1 > P_2 > P_3$
No. of mutants	11	12	5	6

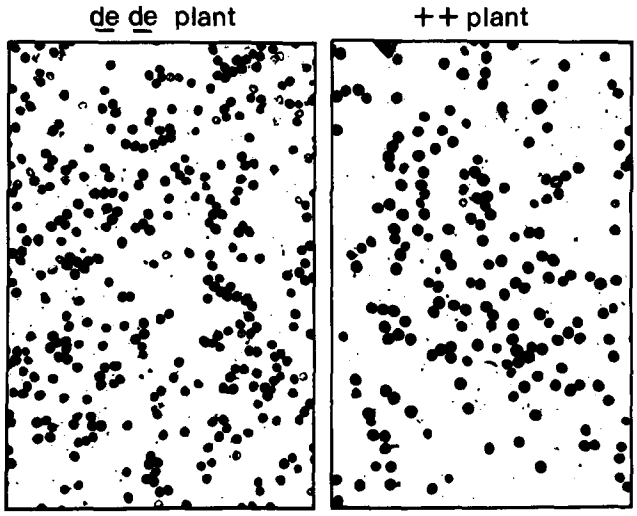

Fig. 1. Pollen size and sterility in *de**-B246

base. This value ($\chi^2_{(2)}$) tests for differences in competitive ability between *de* and + pollen due to tube growth rate: the length of the ear at pollination time increases from the apex to the base of the ear, therefore if the allele is expressed during the gametophytic phase and affects the character, the competition of several pollen tubes in the same silk leads to differential fertilization. When *de* pollen is less competitive than that carrying the normal allele, the proportion of *de* kernels decreases from the apex to the base of the ear.

On the basis of this analysis the mutants can be classified into four different types: (1) mutants non-significant in both tests, showing that the mutation does not affect gametophytic development and function; (2) mutants for which only the first test ($\chi^2_{(1)}$) showed a significant reduction of the kernel frequency: because differences between sectors are not detected, the deviation from 3:1 is attributable to less competitive ability of *de* pollen during pollen development in the anther, pollen sterility or pollen germination time; (3) mutants showing an overall increase of *de* kernels, indicating that *de* pollen has higher competitive ability than the normal counterpart, although in at least one of them (*de*M-25*), for which a very large deviation was observed, a more complex genetical basis cannot be excluded; and (4) mutants showing a significant reduction of *de* kernels from the apex to the base of the ear, a pattern which can be a result of a reduction of pollen competitive ability due to a reduction of pollen tube growth. The conclusions (Table 1 b) are in accordance with the preliminary results already published (Ottaviano et al. 1986): in a large number of observations, only three mutants attributed to class (1) in the previous report revealed a significant gametophytic effect on this basis.

To investigate whether the gametophytic gene expression of *de* mutation is detectable by means of direct observations on pollen grains, each mutant was studied for effect on pollen size and pollen sterility. To avoid residual background effects, pollen from three plant genotypes was studied in sib plants obtained by selfing heterozygous plants of the last backcross generation

(*BC7*). For the same reason, comparisons were made only between plants of the same family, i.e. not between mutants. Although both pollen traits showed a large sampling error, for some mutants very clear differences were detected (Fig. 1).

Table 2 gives details of the segregation analysis already reported in Table 1 together with the mean values for pollen size and pollen sterility for most of the mutants affecting gametophytic development (class 2). The mutation in *de*-B34* and *de*-B246* increases pollen sterility; in the latter the effect on pollen size is also very clear. Considering that for these two mutants the proportion of *de* kernels, although not significant, is reduced from the base to the apex of the ear, it is possible to conclude that grains carrying the recessive allele have higher sterility and, when viable, are less competitive than those with the normal allele. In *de*-B126* and *de*-B163* the differences are clearly associated with a reduction of pollen size, and the reduction of competitive ability is shown only in the early stages (germination and initial tube growth) of pollen function. More difficult to understand is the larger size of pollen grains from *de/+* than from *+/+* plants found in *de*-B123*.

Table 3 gives the data of mutants affecting pollen competitive ability due to tube growth rate. In all of these cases the early stages are not affected: competitive ability of *de* pollen measured at the apex of the ear is equal to, or even higher than, that of the pollen carrying the normal allele. The reduction of pollen competitive ability due to pollen tube growth in the second phase of pollen function is positively associated with pollen size in *de*-B3*, while the opposite situation is found in *de*-37*, where the frequency of fertilization of *de* pollen at the apex of the ear is also higher than normal. No differences in pollen size were detected for the mutants *de*-B1*, *de*-B18*, and *de*-B127*. Apart from *de*-B3* mutants, pollen sterility does not show a clear trend. In some cases (*de*-B1*, *de*-B127*), the higher sterility in *de/+* plants may be due to pollen competition during development in the anthers, although an opposite situation is also found in *de*-B18*.

Table 4 reports the data obtained from the analysis of pollen-style interaction. Proportion of uncolored kernels produced by mixed pollination technique measures the competitive ability of *de* pollen compared with that of *the W22* inbred line used as standard. The difference between the proportion of uncolored kernels on *de/de* plants and that on *de/+* plants estimated the amount of pollen style interaction. This data cannot be used for comparisons between mutants, however, because the pollination of each group was not carried out on the same day: it has been shown that this factor can affect differentially the fertilization ability of pollen of different genetic backgrounds (Pfahler 1965). In three cases (*de*-B3*, *de*-B18* and *de*-116*), a positive pollen style interaction was detected: competitive ability of *de* pollen is enhanced when the stylar tissue carries a normal allele. In two cases (*de*B1* and *de*-B127*), the opposite situation occurred, while in the other two mutants, silk genotype did not affect pollen fertilization ability at all.

Table 2. Pollen dimension and sterility of *de* (defective endosperm) mutants showing reduced gametophytic competitive ability

Mutants	% of *de* kernels in F2 ears				Plant genotype	Pollen	
	Apex	Centrum	Base	Overall		Dimension* μm^2	Sterility* %
de-B34*	21.05	15.13	16.67	17.59	*de/de*	2,590.3 a	27.03 a
					de/+	2,603.5 a	20.51 a
					+/+	2,906.6 a	15.68 b
de-B116*	14.93	14.37	12.83	13.97	*de/de*	3,212.2 a	15.06 a
					de/+	2,935.3 a	20.49 b
de-B123*	20.77	20.87	25.87	22.37	*de/+*	3,619.5 a	16.99 a
					+/+	3,218.7 b	17.99 a
de-B126*	10.24	9.11	9.11	9.47	*de/de*	3,154.6 a	17.95 a
					de/+	3,554.2 b	9.27 b
					+/+	3,634.9 b	18.25 a
de-B163*	18.29	14.47	13.28	15.30	*de/de*	3,277.4 a	28.32 a
					de/+	4,115.1 b	13.56 b
					+/+	4,162.6 b	23.57 a
de-B246*	19.16	16.90	16.64	17.52	*de/de*	2,846.4 a	36.68 a
					de/+	3,066.3 a	20.27 b
					+/+	3,787.7 b	14.50 b

* Differences between values indicated by the same letter are not significant ($P > 0.05$); comparisons are made only between plant genotypes of the same mutant

Table 3. Pollen dimension and sterility of *de** (defective endosperm) mutants showing reduced gametophytic competitive ability proportional to the length of the silks

Mutants	% of *de* kernels in F2 ears				Plant genotype	Pollen	
	Apex	Centrum	Base	Overall		Dimension* μm²	Sterility* %
de-B1*	25.98	19.03	17.26	20.70	*de/de*	3,163.2 ab	12.35 a
					de/+	3,262.4 a	28.08 b
					+/+	2,893.4 b	11.78 a
de-B3*	24.77	16.41	16.77	19.07	*de/de*	2,470.2 a	15.95 a
					+/+	3,518.8 b	9.41 b
de-B18*	29.70	22.09	18.97	23.47	*de/+*	3,155.7 a	14.01 a
					+/+	3,003.5 a	20.60 b
de-B37*	31.85	19.97	16.52	22.60	*de/+*	3,971.0 a	13.23 a
					+/+	3,073.3 b	12.09 a
de-B127*	26.80	14.50	9.69	16.38	*de/+*	3,605.5 a	15.98 a
					+/+	3,345.7 a	8.11 b

* Differences between values indicated by the same letter are not significant ($P > 0.05$); comparisons are made only between plant genotypes of the same mutant

Table 4. Proportion of uncolored kernels produced by *de/de* and *de/+* ears fertilized by pollen mixtures (Δ)

Ear genotype		No. of kernels	% Uncol. kern.	$\chi^2_{(1)}$
de-B1,*	*de/de*	1,606	57.91	**
	de/+	1,213	35.70	
de-B3,*	*de/de*	812	71.80	**
	de/+	1,452	82.71	
de-B18,*	*de/de*	1,031	68.19	**
	de/+	1,092	77.75	
de-B22,*	*de/de*	1,377	29.56	ns
	de/+	1,997	29.54	
de-B116,*	*de/de*	1,302	82.95	**
	de/+	1,465	87.85	
de-B126,*	*de/de*	1,354	51.85	ns
	de/+	1,826	52.64	
*de*B127,*	*de/de*	1,935	63.10	*
	de/+	2,447	58.19	

Δ: 50% (in volume) from *de/de* plants and 50% from the inbred W22 with colored aleurone
ns, * and ** stand for not significant ($P > 0.05$), significant ($P < 0.05$) and highly significant differences ($P < 0.01$), respectively

Discussion

The main questions in this research concerned the efficiency of the procedure of selection of mutants for male gametophyte development and the function and evaluation of the extent of endosperm-gametophyte gene expression. A positive answer to the first question was obtained: the indirect approach – selection of defective viable endosperm mutants followed by a test for gametophytic gene expression – permits detection of gametophytic mutants, and important data for their characterization are obtained by means of a very simple and inexpensive procedure. The efficiency of the method, which resulted in the detection of a large set of *de-ga* mutants, enabled also the second question to be answered. Apart from *bt2*, included in the experiment as a control and already described by Nelson (1978) for its non-detectable effect on the gametophyte, of 33 mutants studied a large proportion also revealed effects on gametophyte fitness. Taking into account that the mutation of each mutant essentially regarded different genes and that the number of genes controlling endosperm development is expected to be very large (Neuffer and Sheridan 1980), a large set of viable mutants can easily be obtained on the basis of this procedure. However, this approach does not allow for identification of mutants specifically expressed in the gametophytic phase. The existence of these genes, although their number is not very large, is indicated by isozyme analysis (Tanksley et al. 1981; Sari-Gorla et al. 1986), heat shock protein analysis (Frova et al. 1986), and mRNA analysis (Willing et al. 1984; Willing and Mascarenhas 1984). The quantitative evaluation of endosperm-gametophyte gene expression (endosperm-gametophyte genetic overlap) is given by the ratio $21/32 = 0.656$ (in this computation *de*-B25*, the characterization and genetical basis of which needs further investigation, and *bt2*, used as a check, have not been considered). This ratio, although affected by a large sampling error because the number of mutants used was not very large, proves that there is a large number of genes expressed both in endosperm and in the gametophytic phase. This interpretation is not in contrast with the results obtained by Nelson (1978),

who showed that of five endosperm starch mutant genes, well-characterized at enzymatic level, two revealed gametophytic expression. With regard to this it should be noted that only the analysis of mature pollen (and not of developing microspores and pollen tubes) can underestimate the amount of gene expression (Frova et al. 1986).

Endosperm-gametophytic mutants can be used for a genetic dissection of male gametophytic development and function. A first step in this direction is the characterization of the mutants according to the main stages of the male gametophytic phase, e.g., pollen grain development within the anther, germination on the stigma, tube growth in the stylar tissues, and fertilization. For this purpose, the data on pollen size and pollen sterility can be used for a better understanding of the effect on fertilization ability. In fact, differences in pollen size, pollen sterility, and pollen tube growth in the very early stages (in this experiment revealed by fertilization ability at the apex of the ear), are to be referred to the effect of the mutation during pollen development, whereas differences in tube growth during later stages are attributable to the effects of genes specifically connected with tube growth.

This model is justified by the fact that storage material contained in pollen grains lasts only for the first stage of pollen tube growth. Thereafter, male gametophyte development is strictly dependent on stylar tissue i.e., it changes from autotrophic to auxotrophic nutrition. Normal pollen tube growth is the result of normal complementation between male gametophyte and stylar tissues, in which enzyme activation and nutritional components are involved (Heslop-Harrison 1975; Ottaviano et al. 1980). Evidence of this biphasic pattern has been obtained by Bergamini-Mulcahy and Mulcahy (1986) in binucleated pollen species and in our laboratory (unpublished data) as regards maize, a three-nucleated species.

According to this reasoning, three classes of *de-ga* mutants can be distinguished: (1) mutants for genes affecting pollen developmental processes which can affect pollen size, pollen sterility and early stages of pollen tube growth (*de*-B126* and *de*-B163*); (2) mutants affecting both pollen development and tube growth in early and in late stages (*de*-B3, de*-B37* and probably *de*-B34* and *de*-B246*); and (3) mutants affecting pollen tube growth rate (*de*-B1, de*-B18* and *de*-B127*). Mutations affecting starch synthesis and accumulation could modify pollen size and competitive ability during pollen development in the anthers, and at the early stage of the pollen autotrophic phase, suggesting a hypothesis concerning the nature of the mutants here described as class (1).

As far as the mutants belonging to class (2) are concerned, methabolic lesions involving basic processes (such as energy production, protein synthesis and so forth), fundamental for both grain development and pollen tube growth, could explain our results. This observed unspecific effect is also suggested by the results obtained by Manzocchi et al. (1980a), which show for these mutants effects also on plant development. Finally, the physiological effects in the mutants of the third group could imply the synthesis of the tube growth components (callose, cellulose, etc.). It is important to note that a constant reduction of dry matter accumulation for these mutants has also been found in the endosperm (Manzocchi et al. 1980b) and for two of them (*de*-B18* and *de*-B127*), a very high reduction of indol-acetic-acid (IAA) in the endosperm has been detected; indeed, the same authors (Torti et al. 1986) found that *de*-B18,* where IAA is almost absent, contains a normal number of cells, while cell size is reduced.

Experiments based on pollen mixtures reveal pollen style interaction due to single gene differences: positive, negative, or absence of interaction reflect the type of biochemical effect of each mutation. Positive interactions, i.e., the faster growth rate of *de* pollen in *de/+* than in *de/de* styles, involves mutants with a reduced pollen tube growth. Pollen tube growth in the auxotrophic phase is dependent on the style for its nourishment: several methabolites are transferred from stylar tissues to pollen tubes. Accordingly, if a methabolic function is active in both style and pollen tube, a methabolic deficiency in the gametophyte could be partially repaired in a normal stylar tissue (Ottaviano et al. 1980). Data supporting this hypothesis has been obtained by Linskens and Pfahler (1977), who found evidence for physiological complementation between pollen tubes and style. For *de*-B18,* Torti et al. (1986) have shown that the mutant is defective in IAA, and that endosperm development can be normalized by naphtalenic-acetic acid when applied to developing kernels. In the gametophyte of this mutant, the effect of the mutation is not expressed during pollen development in *de/+* plants and in *de/+* styles: normalization could be produced by normal tapetal cells and normal stylar tissues. It is difficult to propose a model for negative interaction. Although this type of effect due to single genes or to more complex situations has been found in different plant species and has been interpreted as a mechanism favoring self impollination (for a review, see Ottaviano and Mulcahy 1986), it is difficult to propose a model which also includes the effect on endosperm development.

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