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Embryo-lethal Mutants of *Arabidopsis thaliana*: Evidence for Gametophytic Expression of the Mutant Genes

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Summary. Normal and aborted seeds from two recessive embryo-lethal mutants (79A and 124D) of *Arabidopsis thaliana* were shown to be distributed non-randomly along the length of heterozygous siliques. Significantly more than half of the aborted seeds in these two mutants were located in the top half of the silique, in the region closest to the stigma surface. Segregation ratios (percent aborted seeds) were unusually low at the base of the silique, and slightly higher than expected at the tip. In contrast, aborted seeds from four other embryo-lethal mutants (87A, 123B, 50B, and 71E) were distributed randomly along the length of the silique. These results suggest that the mutant genes in 79A and 124D are expressed during both the gametophytic (n) and sporophytic (2n) phases of development. These two mutants provide further evidence for the hypothesis that many genes expressed prior to fertilization also perform a critical function during growth and development of the sporophyte. Embryo-lethal mutants of *Arabidopsis* may therefore be useful in future studies of gametophytic gene expression and the regulation of pollen-tube growth in higher plants.

Key words: *Arabidopsis* – Embryo-lethal mutants – Gametophytic gene expression – Pollen-tube growth

Introduction

Arabidopsis thaliana (Cruciferae) has been described previously as a model plant system for the isolation and characterization of embryo-lethal mutants (Meinke and Sussex 1979a). Six non-allelic recessive lethals isolated following EMS seed mutagenesis have been studied in detail (Meinke and Sussex 1979b). Developmental arrest and subsequent lethality of mutant embryos in each of the six mutants occurs at a characteristic stage of early embryogenesis (Fig. 1). Mutant embryos may enlarge slightly after the initial point of developmental arrest, but they never proceed to a later stage of embryogenesis (Meinke 1979). The altered genes in these six mutants must therefore play a critical role in the completion of specific stages of early embryo development.

Siliques on plants heterozygous for an embryo-lethal mutation contain approximately 25% aborted seeds following self-pollination (Fig. 2). The position of aborted seeds along the length of heterozygous siliques is determined by three factors: (a) the position of mutant ovules prior to fertilization; (b) the growth of mutant pollen tubes from the stigma surface to the mutant ovule; and (c) successful fusion between male and female gametes carrying the mutant allele. In this report I show that aborted seeds in mutants 79A and 124D are not distributed randomly along the length of heterozygous siliques. Approximately 60% of the aborted seeds in these two mutants are located in the top half of heterozygous siliques, and this deviation from the expected random distribution of aborted seeds becomes highly significant when data from 20 or more siliques are combined. These results suggest that in mutants 79A and 124D, the mutant gene is expressed not only during embryogenesis but also at some point prior to fertilization, most probably during pollen development or pollen-tube growth. The analysis of embryo-lethal mutants of *Arabidopsis* may therefore provide new clues to the nature of gametophytic gene expression in higher plants.

Materials and Methods

Wild-type and mutant plants of *Arabidopsis thaliana* (L.) Heynh strain "Columbia" were grown in a constant-temperature growth chamber at 25 °C in 16 h/8 h light/dark cycles (Meinke and Sussex 1979a). Six recessive embryo-lethal mutants (87A, 123B, 79A, 50B, 71E, and 124D) isolated following EMS seed mutagenesis (Meinke and Sussex 1979b) were used in the present study. All six mutants were main-

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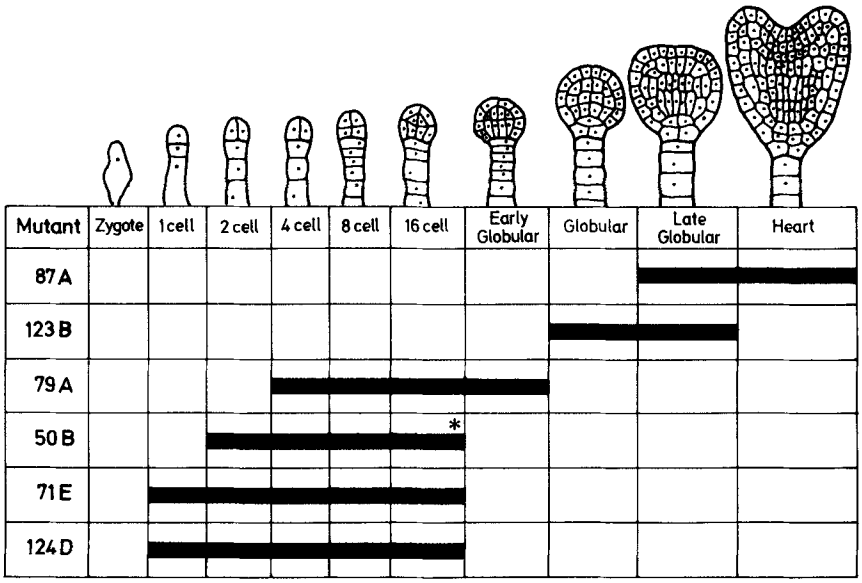

Fig. 1. Developmental arrest of mutant embryos from six embryo-lethal mutants of *Arabidopsis*. The lethal phase for each mutant is represented by a solid line beneath the corresponding stages of normal development. In mutant 50B (*), developmental arrest of the embryo proper is followed by abnormal growth of the suspensor

tained as heterozygotes, which were phenotypically normal except for the presence of siliques containing 25% aborted seeds following self-pollination. Mutant lines were maintained by planting phenotypically normal seeds (⅔ heterozygotes and ⅓ wild type) and identifying heterozygous plants in the next generation by screening siliques for the presence of aborted seeds. Once identified, each heterozygous plant produced hundreds of additional siliques for subsequent studies.

Immature siliques at a cotyledon stage of seed development (Meinke and Sussex 1979a) were removed from heterozygous plants, placed under a dissecting microscope, and split longitudinally with a razor blade to reveal two rows of seeds separated by a central septum (Fig. 2). Most of the siliques used in this study contained 40–65 seeds, all the product of natural self-pollination. Aborted seeds were identified by their characteristic size and color (Meinke and Sussex 1979a). The position of aborted seeds along the length of heterozygous siliques was noted by drawing an outline of each silique on a piece of graph paper, assigning a single square to each seed, and placing a mark in squares corresponding to aborted seeds. In this way it was possible to map the position of normal and aborted seeds in a large number of siliques. Each row of seeds was then divided into upper and lower halves; the number of normal and aborted seeds in each half was noted, and the percentage of aborted seeds positioned in the top half of the silique (the region closest to the stigma) was determined. Data from 20 or more siliques were combined to make the results statistically more significant. Segregation ratios (percent aborted seeds) were also calculated for different regions of the silique.

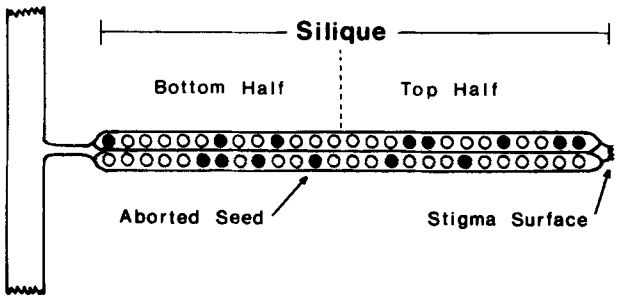

Fig. 2. Drawing of a silique from a plant heterozygous for a recessive embryo-lethal mutation. The top and bottom halves of this silique contain an equal number of aborted seeds

Results

The results of studies on the distribution of aborted seeds in heterozygous siliques are summarized in Table 1. In four of the six mutants studied (87A, 123B, 50B, and 71E), aborted seeds were distributed equally between the top and bottom halves of the silique, and segregation ratios were close to the expected value of 25% aborted seeds. In the remaining two mutants (79A and 124D), approximately 60% of the aborted seeds were located in the top half of the silique, and this deviation from a random distribution was highly significant ($P < 0.001$). Segregation ratios for these two mutants were slightly low, but they were not significantly different (at $P = 0.05$) from 25% aborted seeds. Similar results were obtained when heterozygous plants were grown at 18 °C rather than at 25 °C.

Additional evidence for a non-random distribution of aborted seeds in mutants 79A and 124D was obtained when segregation ratios were calculated for the top and bottom halves of the silique. As shown in Table 2, segregation ratios for these two mutants were significantly higher than 25% aborted seeds in the top half of the silique, and significantly lower than 25% aborted seeds in the bottom half of the silique. The overall segregation ratio (Table 1) was slightly low

Table 1. Distribution of aborted seeds in heterozygous siliques from six embryo-lethal mutants of *Arabidopsis*

Mutant	Siliques scored	Segregation ratio[a]	Chi-square	Aborted seeds	Percent top half[b]	Chi-square
87A	40	24.3	0.6	544	50.7	0.1
123B	40	24.5	0.3	487	47.8	0.8
79A	98	23.9	3.3	1,269	59.9	48.5***
50B	136	25.1	0.04	1,784	51.5	1.6
71E	40	24.9	0.006	524	53.2	2.1
124D	100	23.9	3.4	1,365	61.5	71.3***

*** Significantly different from 50.0% at P = 0.001
[a] Percent aborted seeds throughout the silique
[b] Percentage of total aborted seeds positioned in the top half of the silique. This should equal 50.0% if aborted seeds are distributed randomly along the length of the silique

Table 2. Segregation ratios in the top and bottom halves of heterozygous siliques from mutants 79A, 124D, and 50B

Mutant	Total seeds	Segregation ratios[a]			
		Top half of silique	Chi-square	Bottom half of silique	Chi-square
79A	5,306	28.6	18.3***	19.2	46.9***
124D	5,703	29.4	29.5***	18.4	65.0***
50B	7,104	25.9	1.4	24.4	0.8

*** Significantly different from 25.0% at P = 0.001
[a] Percent aborted seeds

because in both mutants the most significant change was a reduction in the number of aborted seeds in the bottom half of the silique.

Although the distribution of aborted seeds in individual siliques varied considerably, more than 80% of the siliques from mutants 79A and 124D contained a greater number of aborted seeds in the top half of the silique (Fig. 3). In contrast, approximately half of the siliques from mutants 87A, 123B, 50B, and 71E contained a greater number of aborted seeds in the top half. The aberrant segregation ratios in mutants 79A and 124D were therefore not caused by a few highly unusual siliques, but rather by a shift in the frequency

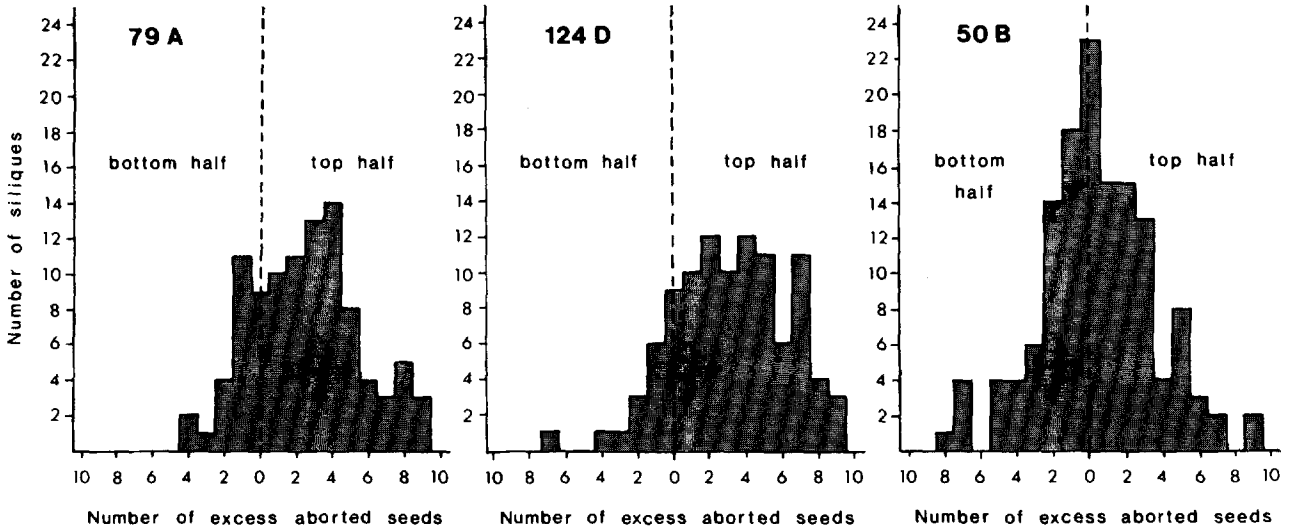

Fig. 3. Frequency of siliques from mutants 79A, 124D, and 50B that contain different numbers of excess aborted seeds in either the top or bottom half of the silique. Siliques at X = 0 contain an equal number of aborted seeds in the top and bottom halves. Most of the siliques from mutants 79A and 124D contain a greater number of aborted seeds in the top half of the silique

Table 3. Segregation ratios in specific regions of heterozygous siliques from mutants 79A, 124D, and 50B

Seed position in silique[b]	Segregation ratios[a]					
	Mutant 79A	Chi-square	Mutant 124D	Chi-square	Mutant 50B	Chi-square
1 – 5 from base	17.9	25.3***	10.3	113.2***	22.9	3.0
6 – 10 from base	18.6	20.3***	19.9	13.5***	24.8	0.03
11 – 15 from base	23.5	1.0	26.8	1.6	25.5	0.1
1 – 5 from tip	30.2	13.6***	30.7	16.9***	26.7	1.8
6 – 10 from tip	28.5	6.2*	29.4	10.0**	24.9	0.001
11 – 15 from tip	24.6	0.07	28.3	5.5*	25.7	0.3

* Significantly different from 25.0% at P = 0.05; ** at P = 0.01; *** at P = 0.001

[a] Percent aborted seeds

[b] The average number of seeds per row (± S.D.) was 27.2 ± 2.3 for 79A; 28.6 ± 2.8 for 124D; and 26.6 ± 2.9 for 50B. Siliques with fewer than 20 seeds per row were not included in this table

of siliques containing more aborted seeds in the top half of the silique.

When segregation ratios were calculated for specific regions of heterozygous siliques, it became apparent that aborted seeds in mutants 79A and 124D were particularly rare at the base of the silique and most abundant at the tip. As shown in Table 3, segregation ratios in the first five seed positions from the base of the silique were 17.9% aborted seeds for mutant 79A and 10.3% aborted seeds for mutant 124D. The deficiency of aborted seeds was most pronounced in mutant 124D, where the segregation ratio in the first two seed positions dropped to only 6.6% aborted seeds. The highest segregation ratio in both mutants (31.3%) was found in the first two seed positions from the tip of the silique. The most striking abnormality in mutants 79A and 124D is therefore a reduction in the number of aborted seeds at the base of the silique, but there is also a significant increase in the number of aborted seeds at the tip.

Discussion

Three questions need to be addressed in the analysis of developmental mutants: (a) how does the mutation alter the normal pattern of development; (b) how is expression of the mutant gene regulated in different tissues; and (c) is the mutant gene expressed at more than one stage of the life cycle? The last question is particularly difficult to answer when working with recessive lethals because homozygotes die before they reach maturity. One solution to this problem has been to isolate temperature-sensitive, conditional-lethal mutants, and use temperature-shift experiments to identify stages of development that require the presence of the wild-type gene product. Temperature-sensitive lethals with more than one temperature-sensitive period have been described in *Drosophila melanogaster* (Suzuki et al. 1976; Shearn et al. 1978) and *Caenorhabditis elegans* (Herman and Horvitz 1980; Miwa et al. 1980), but very few genes have been identified that perform a critical function during both the haploid and diploid phases of development.

The results of the present study show that with embryo-lethal mutants of *Arabidopsis thaliana*, expression of a mutant gene prior to fertilization can be probed by examining the distribution of aborted seeds along the length of heterozygous siliques. Evidence for the expression of a mutant gene at more than one stage of the life cycle can therefore be obtained without the use of temperature-sensitive mutants. Any significant deviation from a random distribution of aborted seeds must be caused by gametophytic gene expression during pollen development, pollen germination, pollen-tube growth, or development of the egg.

It is unlikely that the non-random distribution of aborted seeds in mutants 79A and 124D is caused by a modification of either megasporogenesis (formation of the haploid megaspore) or megagametogenesis (formation of the mature embryo sac) because the distribution of mutant and wild-type ovules in heterozygous siliques is determined by the genotype of the functional megaspore within each ovule, and in most heterozygous plants, the two types of megaspores are produced in equal numbers. Segregation distorters and other factors affecting megagametogenesis are relatively rare in higher plants (Redei 1965; Harte 1975), and they appear to alter segregation ratios uniformly throughout the ovary. No known mutations cause the frequency of mutant ovules to increase in one region of the ovary and decrease in another.

The non-random distribution of aborted seeds in mutants 79A and 124D is more likely to be caused by a disruption of either pollen development, pollen germination, or pollen-tube growth. All three processes are known to be controlled in part by post-meiotic expres-

sion of the male haploid genome (Linskens 1974; Mulcahy 1975). Evidence for gametophytic expression has come from studies of gametophytic self-incompatibility (de Nettancourt 1977), pollen composition (Stanley and Linskens 1974), RNA synthesis (Mascarenhas 1975), isozyme patterns (Tanksley et al. 1981), gametophytic factors (Bianchi and Lorenzoni 1975), endosperm mutants and alcohol dehydrogenase mutants of corn (Pfahler 1974; Freeling 1976), differential rates of pollen-tube growth (Ottaviano et al. 1981), and electrophoretic patterns of proteins isolated from individual pollen grains (Mulcahy et al. 1979). Chromosomal translocations, deletions, and changes in chromosome number have also been shown to disrupt pollen development and pollen-tube growth (Avery et al. 1959; Pfahler 1975). It therefore appears that a large number of genes are expressed during development of the male gametophyte.

There is also evidence for extensive overlap between sporophytic and gametophytic gene expression in higher plants (Mulcahy 1979). For example, Tanksley et al. (1981) examined isozyme profiles for nine enzyme systems in *Lycopersicon esculentum* and found that many of the structural genes coding for these enzymes were expressed in both the sporophyte and the male gametophyte. Recent studies with inbred lines of corn (Ottaviano et al. 1981) have also shown that genotypes with a positive effect on the rate of pollen-tube growth generally have a positive effect on the quality of the sporophytic generation. Many genes of interest to the plant breeder are therefore expressed not only during sporophytic growth and development, but also prior to fertilization. The results of the present study suggest that many additional genes that function during both the haploid and diploid phases of plant development may be identified by analyzing embryo-lethal mutants of *Arabidopsis*.

The deficiency of aborted seeds in the bottom half of heterozygous siliques from mutants 79A and 124D is probably caused by a slight reduction in the growth rate of mutant pollen tubes. This hypothesis is consistent with the observation that the greatest deficiency of aborted seeds was found at the extreme base of the silique. An alternative explanation is that mutant pollen grains may germinate more slowly than wild-type pollen grains, and may therefore be less likely to fertilize ovules at the base of the silique. Similar mechanisms have been proposed to explain the non-random distribution of mutant kernels on ears segregating for the waxy, sugary, and certain defective-kernel mutations of corn (Mangelsdorf and Jones 1926; Jones 1928; Pfahler 1975). Competition between male gametophytes may also have been responsible for many of the aberrant segregation ratios found by Müller (1963) in his analysis of embryo-lethal mutants of *Arabidopsis*. The deleterious effect of the mutant allele was particularly severe in mutant No. 1420 isolated by Müller (1963); the overall segregation ratio in this mutant was reduced to 7.25% aborted seeds, and 96.6% of the aborted seeds were located in the top half of the silique. Embryo-lethal mutations may therefore differ with respect to the severity of their effect on development of the male gametophyte.

The observed excess of aborted seeds in the top half of siliques from mutants 79A and 124D is more difficult to explain. One possibility is that ovules at the tip of the silique may mature slightly after those at the base, and may therefore be preferentially fertilized by the more slowly-growing mutant pollen tubes. Additional studies with modified pollinations will be needed to verify this hypothesis. It is also possible that the non-random distribution of aborted seeds in mutants 79A and 124D may be caused by closely-linked gametophytic factors induced during seed mutagenesis rather than by gametophytic expression of the mutant alleles. This alternative explanation is considered unlikely because multiple mutants should be more common among plants grown from seeds exposed to a high concentration of the chemical mutagen ethyl methanesulfonate (EMS), and in this particular case, mutants 87A, 123A, 79A, and 124D were isolated from seeds exposed to 0.1% (w/v) EMS, whereas mutants 50B and 71E were isolated from seeds exposed to 0.5% (w/v) EMS (Meinke and Sussex 1979b).

Evidence of gametophytic expression may also be useful in the classification of embryo-lethal mutants as cellular, nutritional, or developmental lethals (Meinke and Sussex 1979a). For example, mutants 79A and 124D cannot be developmental lethals because expression of the mutant genes is not limited to a single stage of development. These two mutants are more likely to be examples of nutritional lethals in which the synthesis of a diffusible nutrient such as an amino acid or vitamin is blocked. Auxotrophic embryo-lethal mutants of *Arabidopsis* may therefore be most common among mutants with a non-random distribution of aborted seeds in heterozygous siliques. *Arabidopsis* is an excellent system for recovering mutations with a deleterious effect on pollen-tube growth because the style is extremely short, and the ovary at the time of pollination is only 1 mm in length. Pollen tubes lacking an essential nutrient may be unable to grow as quickly as wild-type pollen tubes, but they may still reach the megagametophyte and participate in fertilization by utilizing nutrient reserves in the surrounding maternal tissue (Linskens and Pfahler 1977). Mutations with a deleterious effect on pollen-tube growth are less likely to be recovered in corn because the style is much longer than in *Arabidopsis*. This may be one reason why auxotrophs have been so difficult to find among the defective-kernel mutants of corn (Sheridan and Neuffer 1980). Additional studies on the growth in vitro of arrested embryos from mutants 79A and 124D are planned to test the hypothesis that developmental arrest of mutant embryos and the non-random distribution of aborted seeds are both caused by a defect in the synthesis of an essential amino acid, vitamin, or other diffusible nutrient.

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