Theor Appl Genet (1985) 69:543–552

Embryo-lethal mutants of *Arabidopsis thaliana*: analysis of mutants with a wide range of lethal phases

D.W. Meinke

Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078, USA

Received July 10, 1984; Accepted November 17, 1984
Communicated by R. Hagemann

Summary. Embryo-lethal mutants of *Arabidopsis thaliana* were isolated by treating mature seeds with an aqueous solution of ethyl methanesulfonate (EMS), screening the resulting M-1 plants for siliques containing 25% aborted seeds following self-pollination, and verifying the presence of induced mutations in subsequent generations. Thirty-two recessive lethals with a Mendelian pattern of inheritance were examined in detail. Developmental arrest of mutant embryos ranged from the zygotic stage of embryogenesis in mutant 53D-4A to the linear and curled cotyledon stages of development in mutants 112A-2A and 130B-A-2. These lethal phases did not change significantly when plants were grown at 18 °C rather than at 24 °C. Differences between mutant lines were found in the color of arrested embryos and aborted seeds, the percentage and distribution of aborted seeds in heterozygous siliques, the size of arrested embryos, and the extent of abnormal development. Unusual mutant phenotypes included the presence of unusually large suspensors, distorted and fused cotyledons, reduced hypocotyls, and arrested embryos without distinct cotyledons or hypocotyl tissue. The isolation of eight new mutants with a non-random distribution of aborted seeds in heterozygous siliques provides further evidence that many of the genes that control early stages of embryogenesis in plants are also expressed prior to fertilization.

Key words: *Arabidopsis* – Embryo-lethal mutants – Pollen-tube growth – Seed development – Suspensor

Introduction

Arabidopsis thaliana (Cruciferae) has been described previously as a model system for the isolation and characterization of embryo-lethal mutants (Müller 1963; Meinke and Sussex 1979a, b). The patterns of abnormal development observed in these recessive lethals may be used to study the genetic control of embryo development and the regulation of gene expression in higher plants.

The six mutants originally described by Meinke and Sussex (1979b) were chosen because developmental arrest of the mutant embryos occurred at specific stages of early embryo development. Subsequent studies have shown that gametophytic expression of these mutant genes can be determined by examining the distribution of aborted seeds in heterozygous siliques (Meinke 1982). Two mutants (79A and 124D) with a non-random distribution of aborted seeds have been identified in which the mutant genes are expressed not only during early embryogenesis but also at some point prior to fertilization (Meinke 1982). Developmental interactions between the embryo proper and suspensor have also been examined in aborted seeds from one mutant (50B) with an abnormal suspensor (Marsden and Meinke 1985). The results of histological studies with this mutant have provided further evidence for the hypothesis that continued growth of the suspensor during normal development is inhibited by the developing embryo proper.

The purpose of the present study was to isolate additional embryo-lethal mutants with a wider range of lethal phases and a greater diversity of embryonic abnormalities. The mutants described in this report differ with respect to the stage of developmental arrest, the color of arrested embryos and aborted seeds, the percentage and distribution of aborted seeds in heterozygous siliques, and the extent of abnormal development. The diversity of mutant phenotypes observed suggests that many additional aspects of embryo development in plants may now be amenable to mutant analysis.

Materials and methods

Embryo-lethal mutants of *Arabidopsis thaliana* (L.) Heynh. strain 'Columbia' were isolated as described previously by

Meinke and Sussex (1979b). Approximately 1,500 wild-type seeds were soaked for 7 h in a glass vial containing 5.0 ml of a 0.2% (v/v) aqueous solution of ethyl methanesulfonate (EMS) purchased from Sigma Chemical Co. (St. Louis, Mo., USA). A small magnetic stirring bar was used to gently agitate the seeds throughout the mutagenesis period. All EMS solutions were prepared in a well-ventilated hood in order to minimize the chance of accidental human exposure to this potent mutagen. Glassware and waste EMS solutions were decontaminated with mercaptoacetic acid before disposal (Lewis and Bacher 1968). Mutagenized seeds were washed with water for 12 h and then planted in 3-inch pots containing a 12 : 1 : 3 mixture of vermiculite, sand, and potting soil. Half of the M-1 plants were grown in a constant-temperature growth chamber at 32 °C ± 0.5 °C in 16 h/8 h light/dark cycles. The remaining M-1 plants were grown at 24 °C ± 2 °C in 16 h/8 h light/dark cycles. All plants were watered from below with a nutrient solution containing 4 g/gal of 7-6-19 All Purpose Hyponex (Hyponex Co., Copley, Ohio, USA) and 0.25 g/gal of 15-16-17 Peat-Lite Special (Peters Co., Allentown, Pa., USA).

Embryo-lethal mutants were identified by screening immature siliques of M-1 plants for the presence of aborted seeds following self-pollination (Meinke and Sussex 1979a; Meinke 1982). Many of the mutant lines were recovered by saving the immature siliques used for screening, transferring the phenotypically normal seeds present in these siliques to petri plates, germinating these green seeds on pieces of filter paper moistened with distilled water, and later transplanting the young seedlings to soil. In this way it was possible to recover mutant lines without returning to the M-1 plant and identifying the mutant sector as described by Meinke and Sussex (1979a). The original method of harvesting dry siliques from the mutant sector was still required when frozen siliques were used for screening or when the phenotypically normal seeds present in screened siliques did not germinate.

Segregation ratios were determined by counting the number of normal and aborted seeds in heterozygous siliques following self-pollination. The lethal phase for each mutant was established by examining several hundred aborted seeds and arrested embryos under a dissecting microscope (Meinke and Sussex 1979a, b). Mutant embryos arrested at early stages of development were analyzed by squashing aborted seeds in a drop of stain and examining the debris under a compound light microscope (Meinke and Sussex 1979b). The distribution of aborted seeds in heterozygous siliques was determined by mapping the positions of normal and aborted seeds on a piece of graph paper as described previously (Meinke 1982). Development of mutant embryos at a low temperature was studied by transferring heterozygous plants to a growth chamber maintained at 18 °C ± 1 °C with 16 h/8 h light/dark cycles.

Results

Isolation of mutants

The frequency of recessive embryonic lethals recovered following EMS seed mutagenesis was significantly higher than expected based on the results of previous mutagenesis studies (Meinke and Sussex 1979b). Approximately 99% of the mutagenized seeds germinated within 5 days of planting, and more than 50% of the resulting M-1 plants appeared to be segregating for an embryo-lethal mutation. Many of the M-1 plants appeared to have several different mutant sectors located within the main stem (Meinke and Sussex 1979a). The higher frequency of induced mutations observed in the current study was probably the result of differences in either the age of the EMS used to prepare stock solutions, the amount of time required for the onset of germination, or the efficiency of uptake of the EMS during the mutagenesis period.

Despite the presence of numerous multiple mutants, it was still possible to identify a significant number of M-1 plants that appeared to be segregating for a single mutant gene with a characteristic lethal phase. Each of these suspected mutants was then labeled according to its pot number (1–133) and position within the pot (A–E for pots 1–108 and A–T for pots 109–133). A variety of mutants with different lethal phases were chosen for more detailed studies in subsequent generations.

Segregation ratios in heterozygous siliques

Heterozygous siliques from most of the 32 mutant lines isolated following EMS seed mutagenesis (Table 1) contained approximately 25% aborted seeds following self-pollination. The unusually low segregation ratios observed in mutants 127AX–A, 113J–4A, 95A–2B, 109F–5D, 112G–1A, 115D–4A, and 126E–B can be explained by gametophytic expression of the mutant genes (Tables 3 and 4). The unusually high ratios observed in mutants 113K–1B, 117N–1B, 129AX2–A, and 111B–5B are more difficult to explain. Numerous mutant lines examined in the M-2 generation were discarded because they were clearly multiple mutants with segregation ratios approaching 50% aborted seeds following self-pollination. The slightly high ratios observed in the four mutants noted above may be the result of a competitive advantage of mutant pollen grains, the presence of two closely-linked mutations with similar phenotypes, random variation in a relatively small sample size, or some other undetermined factor. The general conclusion from the data in Table 1, however, is that most if not all of these new mutants appear to be segregating for a single recessive gene.

One of the initial objectives of this study was to screen for temperature-sensitive mutants by growing M-1 plants at a high temperature (32 °C or 24 °C) and then examining subsequent generations for the presence of aborted seeds in plants grown at a lower temperature (18 °C). Two different high temperatures were chosen: 32 °C ± 1 °C for plants in pots Nos. 1–108, and 24 °C ± 2 °C for plants in pots Nos. 109–133. Most of the mutants included in Table 1 were recovered from M-1 plants grown at 24 °C because in this particular experiment, the plants grown at 32 °C had unusually poor seed set. All mutant lines were then tested for the

presence of aborted seeds in heterozygous plants grown at 18 °C. No temperature-sensitive mutants were found because heterozygous siliques still contained approximately 25% aborted seeds, and the mutant embryos were phenotypically indistinguishable from those found at the higher temperature. The percentage and distribution of aborted seeds in mutants 95A–2B and 126E–B were clearly influenced by temperature, but these differences appear to be caused by changes in the rates of pollen-tube growth rather than the presence of an altered gene product that fails to function properly at either the high or low temperature.

Stages of developmental arrest

Many different stages of developmental arrest are included among the 32 mutants (Fig. 1). Some mutants such as 109A–1B and 112A–2A have lethal phases that cover a very narrow range of developmental stages. In these cases it is clear that the mutant gene is required for the completion of a specific stage of embryogenesis. Other mutants such as 126E–B, 63A–1A, and 122G–E have much broader lethal phases that extend over several developmental stages. These mutants are more difficult to interpret because it is not clear whether: (1) expression of the mutant gene is required throughout the lethal phase, and the variable phenotypes of arrested embryos result from the presence of a leaky mutation; (2) expression of the mutant gene is limited to the beginning of the lethal phase, but the mutation is not immediately lethal and permits a variable amount of continued development; or (3) the mutation directly affects development of the endosperm tissue and only indirectly results in embryonic lethality. In several of the mutants with arrested embryos ranging from the globular to the curled cotyledon stages of embryogenesis, arrested embryos at the earliest stages of devel-

Table 1. Segregation ratios for embryo-lethal mutants of *Arabidopsis* grown at 24 °C and 18 °C[a]

Mutant	Segregation ratios at 24 °C			Segregation ratios at 18 °C		
	Seeds screened	% aborted seeds	Chi-square	Seeds screened	% aborted seeds	Chi-square
53D-4A	1,327	25.5	0.13	504	22.2	1.93
127AX-A	2,265	23.4	3.18	1,917	22.2	7.96**
113K-1B	1,152	28.0	5.20*	1,129	27.5	3.51
112E-2A	1,076	26.1	0.83	1,546	25.5	0.17
113J-4A	2,299	21.0	19.75***	1,887	20.8	17.75***
112E-1B	3,299	25.8	1.07	572	25.9	0.19
111H-2B-1	2,579	24.1	1.12	505	25.7	0.11
130B-A-1	927	27.6	3.32	569	25.3	0.02
111B-5E	1,516	23.1	2.86	527	27.1	1.17
95A-2B	3,683	23.1	6.01*	3,439	21.5	22.43***
57B-4C	1,370	26.3	1.13	502	23.1	0.86
109A-1B	1,697	25.3	0.06	563	25.9	0.17
109F-5D	2,789	20.3	29.77***	1,824	23.6	1.90
112G-1A	2,323	21.5	14.79***	1,798	24.0	0.96
117N-1B	2,791	26.7	4.36*	520	21.7	2.79
129AX2-A	2,027	29.8	15.10***	632	38.5	60.25***
115D-4A	2,662	18.7	53.76***	2,013	19.0	38.63***
126E-B	2,232	25.2	0.03	2,042	12.2	177.92***
109F-1C	1,250	25.8	0.35	808	29.5	8.32**
115J-4A	1,390	25.6	0.25	509	28.7	3.49
122G-E	2,526	26.5	3.05	989	24.4	0.18
63A-1A	2,365	25.0	0.00	525	25.3	0.02
111B-5B	1,049	28.7	7.44**	510	24.1	0.17
115H-1A	1,165	25.8	0.39	523	28.5	3.21
115C-1C	1,133	26.7	1.74	551	25.8	0.14
112A-2A	1,107	26.1	0.67	522	26.4	0.50
130B-A-2	2,672	23.3	3.43	2,851	24.1	0.98
66C-3A	1,314	25.3	0.07	532	22.9	1.11
112C-1A	1,357	25.0	0.00	512	26.5	0.59
114D-1A	1,334	23.9	0.78	525	28.4	3.03
21C-2D	1,214	23.5	1.42	518	22.2	2.02
111H-2B-2	1,680	24.6	0.11	524	24.6	0.01

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

[a] Heterozygous siliques were expected to contain approximately 25% aborted seeds following self-pollination

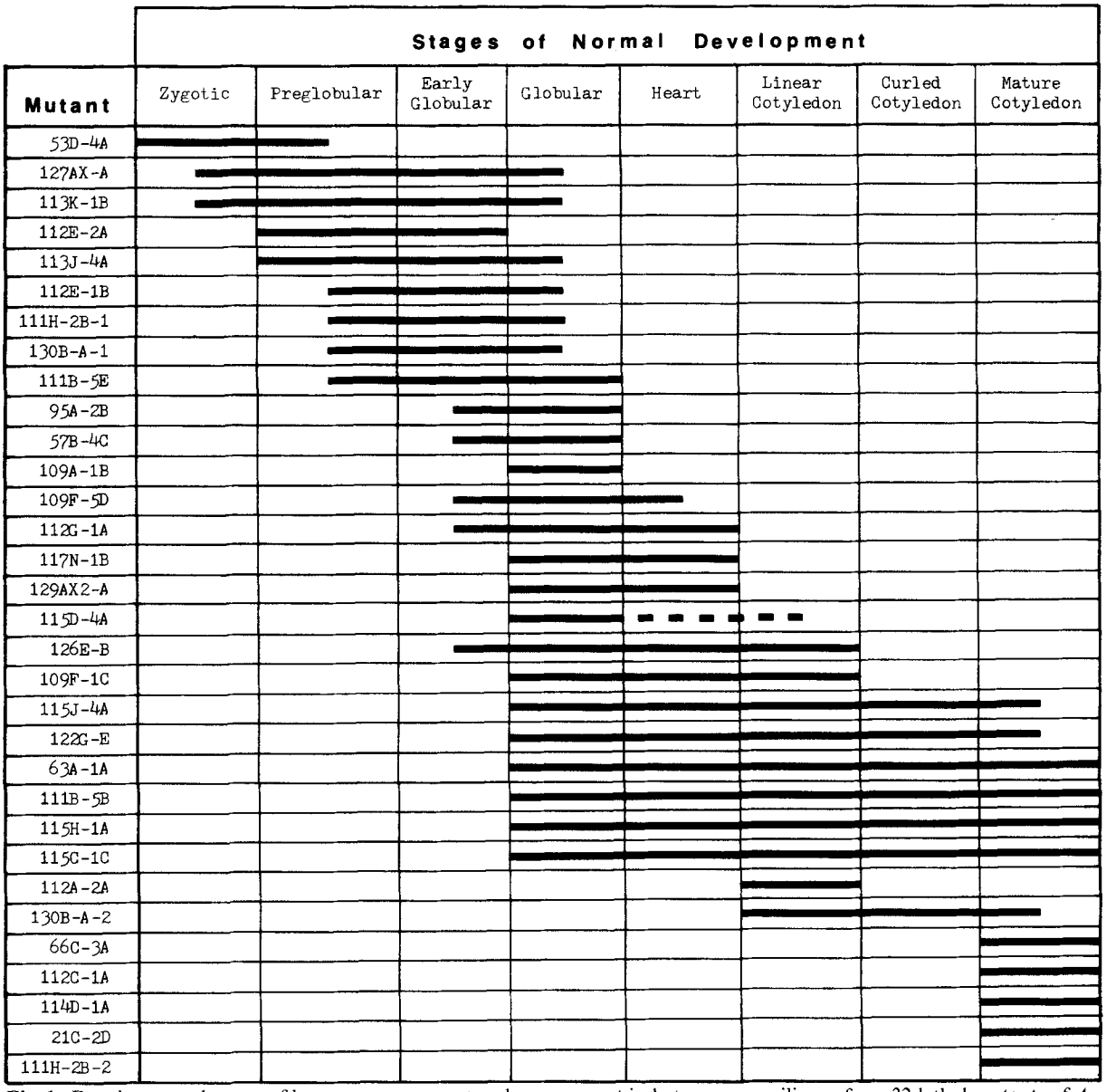

Fig. 1. Developmental arrest of homozygous mutant embryos present in heterozygous siliques from 32 lethal mutants of *Arabidopsis thaliana*. The stages of embryo development reached by arrested embryos are represented by a solid line beneath the corresponding stages of normal development. Stages that are partially covered by a solid line (e.g. globular stage in 127AX-A) are only occasionally reached by mutant embryos. The *dashed line* in mutant 115D-4A represents an abnormal pattern of development that cannot be classified on the basis of external morphology. The lethal phase for mutants 66C-3A, 112C-1A, 114D-1A, 21C-2D, and 111H-2B-2 does not occur until the seedling stage of development. *Solid lines* have been drawn at the mature cotyledon stage of development because homozygous mutant embryos complete all of the normal stages of embryogenesis

opment were more often found in relatively young siliques, whereas arrested embryos at later stages of development were more common in older siliques. This suggests that the broad lethal phases observed in some mutants may be the result of mutant genes that are not immediately lethal to the developing embryo.

Mutant embryos also differed in their extent of abnormal development (Fig. 2). Examples of clearly abnormal development included the presence of an unusually large suspensor in mutants 109F–5D and 112G–1A (Fig. 2E), fused cotyledons and the absence of a normal hypocotyl in mutant 112A–2A (Fig. 2M–N), an elongated ("blimp") embryo without a distinct hypocotyl or cotyledons in mutant 115D–4A (Fig. 2F), and arrested embryos with abnormally-shaped cotyledons in many of the mutants with lethal

phases extending through the heart and cotyledon stages of development (Fig. 2G–P). Many of the arrested embryos were also significantly larger than the corresponding wild-type embryos (Fig. 2). The largest arrested embryos were most often found in the oldest siliques.

Homozygous mutant embryos from mutants 66C–3A, 112C–1A, 114D–1A, 21C–2D, and 111H–2B–2 reached a mature cotyledon stage of development, were phenotypically normal except for their pale color, and germinated to produce pale seedlings that soon died if not grown on a defined nutrient medium. These mutants were included in this study even though they are actually seedling lethals rather than embryonic lethals because the mutant embryos were clearly distinguishable in heterozygous siliques, and both segregation ratios and gametophytic expression of the mutant genes could be readily determined.

Color of aborted seeds and arrested embryos

Aborted seeds and arrested embryos from each mutant exhibited a characteristic range of colors prior to desiccation (Fig. 3). Mutants with lethal phases prior to the globular stage of embryogenesis generally contained

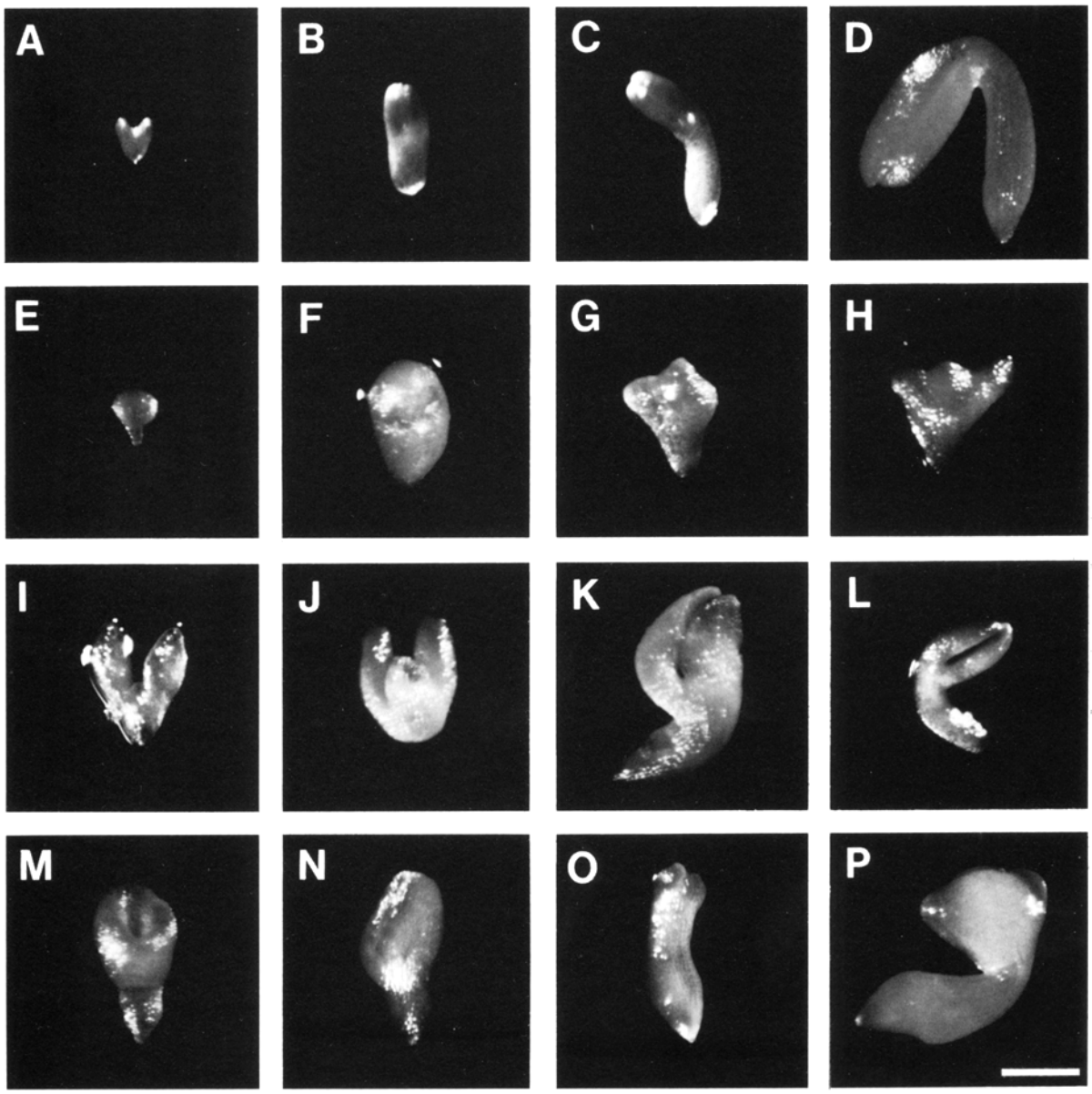

Fig. 2. Development of wild-type and mutant embryos of *Arabidopsis thaliana*. *A–D* wild-type embryos at the heart (*A*), linear cotyledon (*B*), early curled cotyledon (*C*), and mature cotyledon (*D*) stages of development. *E–P* arrested embryos from mutants 109F-5D (*E*), 115D-4A (*F*), 122G-E (*G–K*), 63A-1A (*L*), 112A-2A (*M–N*), and 130B-A-2 (*O–P*). All embryos are at the same magnification; scale bar in Fig. *2P* = 200 μm

white aborted seeds. This was not surprising because normal seeds remain white throughout the preglobular stages of development (Meinke and Sussex 1979a). Differences in the colors of aborted seeds and arrested embryos became apparent in mutants with later lethal phases. For example, aborted seeds from mutants 57B–4C, 109F–5D, and 112G–1A all contained mutant embryos arrested at a globular stage of development. Aborted seeds from mutant 57B–4C were white, whereas aborted seeds from the other two mutants were different shades of pale green. In mutants 115J–4A and 122G–E, the lethal phase for both mutants extended from the globular to the curled cotyledon stages of development. Arrested embryos and aborted seeds from mutant 115J–4A were generally white, whereas arrested embryos from mutant 122G–E were usually a very pale green color, and the aborted seeds from this mutant were even darker green. Individual plants from other mutants such as 126E–B and 109F–1C contained aborted seeds and arrested embryos that exhibited a wide range of colors. The darkest embryos in these mutants were often those that had reached the latest stage of development.

Mutant	Seed Color[a]				Embryo Color[b]				
	1	2	3	4	1	2	3	4	5
53D-4A	●				-				
127AX-A	●				●				
113K-1B	●				●				
112E-2A	●				●				
113J-4A	●				●				
112E-1B	●	●			●				
111H-2B-1	●	●			●				
130B-A-1	●	o			●				
111B-5E	o	●			●	●			
95A-2B		●			●	o			
57B-4C	●				●				
109A-1B	●				●				
109F-5D	●	●			●				
112G-1A	o	●	o		●	o	●		
117N-1B	●				●				
129AX2-A	●				●				
115D-4A		●	●	o			●	●	●
126E-B	●	●	●		●	●	●	●	
109F-1C		●	●		●	●	●	●	
115J-4A	●				●		o		
122G-E		●	●		●	●	●		
63A-1A	●	o			●	o			
111B-5B	●				●				
115H-1A	●				●				
115C-1C	●				●				
112A-2A			o	●				●	●
130B-A-2			●	●			●	●	●
66C-3A		●				●	●		
112C-1A		●				●	●		
114D-1A		●				●	●		
21C-2D		●				●	●		
111H-2B-2		●				●	●		

[a] Seed Colors:
1 - White or cream with no sign of green
2 - Very pale yellow-green
3 - Pale green or yellow-green
4 - Green

[b] Embryo Colors:
1 - White or cream with no sign of green
2 - Very pale yellow-green
3 - Initially as 1 or 2 above but turns a darker color on drying
4 - Pale green or yellow-green
5 - Green

Fig. 3. Colors of aborted seeds and arrested embryos prior to desiccation. *Open circles* represent colors that were only occasionally found. No arrested embryos were found in aborted seeds from mutant 53D-4A. Some of the seeds and embryos classified as very pale yellow-green appeared white against a black backgrond but faintly yellow-green against a white background

Gametophytic expression of the mutant genes

Gametophytic expression of the mutant genes was studied by examining the distribution of aborted seeds along the length of heterozygous siliques (Meinke 1982). In many of the mutants shown in Fig. 1, aborted seeds were distributed randomly between the top and bottom halves of the silique (Table 2). In the remaining eight mutants (127AX–A, 113J–4A, 95A–2B, 109F–5D, 112G–1A, 115D–4A, 126E–B, and 130B–A–2), gametophytic expression of the mutant genes resulted in a non-random distribution of aborted seeds (Table 3). Most of these mutants had lethal phases during early stages of embryogenesis. The distribution of aborted seeds in many of these mutants became even more non-random when heterozygous plants were grown at 18 °C rather than at 24 °C. Siliques from mutant 95A–2B were unusual in having a base prevalence of aborted seeds when plants were grown at 24 °C but not at 18 °C. One possible explanation for this result is that the mutant gene may have a positive effect on the growth of mutant pollen tubes that becomes noticeable only when plants are grown at a relatively high temperature. In the remaining seven mutants, significantly more than 50% of the aborted seeds were located in the top half of heterozygous siliques. All of these mutants had unusually low segregation ratios in the basal five seed positions of the silique (Table 4). Segregation ratios in the top five seed positions ranged from approximately 25% aborted seeds in some mutants to significantly greater than 25% aborted seeds in other mutants. These results suggest that embryo-lethal mutations differ in their effect on pollen development and pollen-tube growth. Additional studies designed to test the effect of the mutant genes on gametogenesis are in progress.

Abnormal development of the suspensor

Abnormally large suspensors were consistently found in aborted seeds from mutants 109F–5D and 112G–1A (Table 5). Many of these suspensors were sufficiently sturdy to remain intact when arrested embryos were removed from aborted seeds and examined under a

Table 2. Random distribution of aborted seeds in heterozygous siliques from 24 mutant lines of *Arabidopsis*

Mutant	Siliques screened	Aborted seeds	% top half[a]	Chi-square
53D-4A	20	191	51.8	0.19
113K-1B	40	491	52.1	0.81
112E-2A	20	160	49.4	0.01
112E-1B	20	231	53.3	0.85
111H-2B-1	22	263	52.9	0.75
130B-A-1	20	219	52.1	0.29
111B-5E	20	225	52.9	0.64
57B-4C	20	240	47.9	0.34
109A-1B	28	306	51.0	0.08
117N-1B	21	278	50.7	0.03
129AX2-A	30	258	48.1	0.31
109F-1C	21	234	53.0	0.72
115J-4A	20	233	50.2	< 0.01
122G-E	44	556	50.2	< 0.01
63A-1A	40	490	52.0	0.74
111B-5B	20	230	47.8	0.35
115H-1A	20	207	49.3	0.02
115C-1C	20	261	51.3	0.14
112A-2A	21	265	47.9	0.38
66C-3A	20	276	50.0	< 0.01
112C-1A	20	241	52.3	0.41
114D-1A	20	244	50.8	0.04
21C-2D	20	238	53.0	0.71
111H-2B-2	49	507	50.7	0.07

[a] 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

dissecting microscope (Fig. 2E). The pattern of abnormal development in these mutants was slightly different than in mutant 50B as described by Marsden and Meinke (1985). Developmental arrest of the embryo proper in mutants 109F–5D and 112G–1A occurred at a later stage of development than in mutant 50B, and the abnormal suspensor contained a relatively small number of unusually large cells arranged in one or two files rather than the multiple files of small cells characteristic of the abnormal suspensor in mutant 50B. The abnormal suspensor was also significantly larger in mutant 109F–5D than in 112G–1A (Table 5). The isolation of two new mutants with abnormal suspensors provides further evidence that certain mutations cause abnormal growth of the suspensor by interfering with continued development of the embryo proper, thus removing an inhibitory effect that the embryo proper normally exerts on continued development of the suspensor (Marsden and Meinke 1985).

Unusual mutant phenotypes

Many examples of unusual mutant phenotypes were found among mutants with later stages of developmental arrest. One of the most consistently abnormal embryos was found in mutant 115D–4A. These mutant embryos were referred to as green blimps because they lacked any defined hypocotyl or cotyledons (Fig. 2F). Arrested embryos with similar shapes were occasionally

Table 3. Non-random distribution of aborted seeds in heterozygous siliques from 8 mutant lines of *Arabidopsis*

Mutant	Temperature	Siliques screened	Total seeds	% aborted seeds	% top half[a]	Chi-square
127AX-A	24 °C	41	1,993	23.6	58.9	14.66***
	18 °C	40	1,971	22.2	62.3	26.14***
113J-4A	24 °C	43	1,919	21.3	64.5	33.55***
	18 °C	44	1,887	20.8	70.9	67.78***
95A-2B	24 °C	57	2,290	24.5	40.6	19.62***
	18 °C	78	3,206	22.0	51.6	0.63
109F-5D	24 °C	60	2,644	21.0	62.8	35.89***
	18 °C	42	1,824	23.6	56.6	7.28**
112G-1A	24 °C	60	3,306	20.7	57.7	13.94***
	18 °C	40	1,798	24.0	50.3	0.01
115D-4A	24 °C	40	1,896	18.8	57.9	8.50**
	18 °C	40	1,836	19.1	61.5	18.23***
126E-B	24 °C	40	2,056	23.8	65.9	49.03***
	18 °C	40	2,029	12.3	84.0	114.24***
130B-A-2	24 °C	41	2,067	24.1	58.1	12.88***
	18 °C	64	2,851	24.1	57.7	16.07***

** Significantly different from 50.0% at $P=0.01$; *** at $P=0.001$

[a] 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

found in other mutants (e.g. 109F–1C) with lethal phases that extended beyond the globular stage of development, but in these cases the mutant embryos were usually white or pale green and not much larger than a normal torpedo-stage embryo. Some of the arrested embryos from mutant 115D–4A reached a length of 300 μm and were approximately half the size of a mature wild-type embryo.

The pattern of abnormal development observed in mutant 115D–4A clearly illustrates one problem encountered when assigning lethal phases based on normal stages of development. External morphology alone cannot be used to determine whether these blimp embryos most closely resemble an elongated globular embryo, an early cotyledon embryo without distinct cotyledons, or a more mature embryo. This question could be answered in the future by combining histological and biochemical studies on arrested embryos. Ultrastructural features such as protein bodies or lipid bodies might provide information on the types of cells present, and biochemical markers such as seed storage proteins could be used to determine whether the mutant embryos are accumulating specialized gene products that are normally found only at later stages of seed development. A similar approach has recently been taken in the comparison of zygotic and somatic embryos of *Brassica napus* (Crouch 1982).

Mutants 112A–2A and 130B–A–2 provide an interesting comparison of abnormal development during later stages of embryogenesis. All of the arrested embryos from mutant 112A–2A were green and con-

Table 4. Segregation ratios in the top and bottom 5 seed positions of heterozygous siliques from mutants with a non-random distribution of aborted seeds[a]

Mutant	Temp.	Total nos. of seeds screened	Segregation ratio: seed positions nos. 1–5 from tip	Chi-square	Segregation ratio: seed positions nos. 1–5 from base	Chi-square
127AX-A	24 °C	1,994	24.9	< 0.01	12.7	32.52***
	18 °C	1,971	23.8	0.27	13.5	27.60***
113J-4A	24 °C	1,919	31.6	9.72**	13.0	32.27***
	18 °C	1,887	33.6	17.04***	10.0	52.00***
95A-2B	24 °C	2,290	21.6	3.55	34.7	27.73***
	18 °C	3,206	22.1	5.69*	22.2	5.31*
109F-5D	24 °C	2,644	28.7	4.11*	12.2	52.03***
	18 °C	1,824	26.7	0.54	17.4	12.50***
112G-1A	24 °C	3,306	22.7	1.75	17.2	20.42***
	18 °C	1,798	20.3	4.43*	21.8	1.97
115D-4A	24 °C	1,896	21.0	3.20	12.8	31.36***
	18 °C	1,841	28.0	1.76	11.8	70.08***
126E-B	24 °C	2,066	28.8	2.80	12.8	31.36***
	18 °C	2,042	23.3	0.56	0.8	124.16***
130B-A-2	24 °C	2,067	30.5	6.29*	17.3	12.51***
	18 °C	2,851	28.9	5.00*	15.9	27.55***

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

[a] Segregation ratios for the top and bottom 5 seed positions in heterozygous siliques should be 25% aborted seeds if there is no gametophytic expression of the mutant genes (Meinke 1982). All siliques used in this table contained at least 15 seeds per row

Table 5. Abnormal suspensors in aborted seeds from mutants 109F-5D and 112G-1A

	Mutant 109F-5D	Mutant 112G-1A
No. of seeds examined	50	44
Width of suspensor[a]		
Mean ± SD	50± 14 μm	27± 9 μm
Range	30 – 110 μm	20 – 50 μm
Diameter of embryo proper[b]		
Mean ± SD	108± 22 μm	108± 25 μm
Range	70 – 170 μm	80 – 150 μm

[a] Wild-type suspensors at a globular stage of embryo development are 12–15 μm wide

[b] The wild-type embryo proper at a globular stage of development is 40–60 μm in diameter

tained an unusually small hypocotyl. The cotyledons were slightly smaller than normal and were either thick but still distinct, fused along one side to form a folded structure (Fig. 2N), or fused along both sides to form a conical structure that was open only at the top (Fig. 2M). Developmental arrest in mutant 112A–2A is therefore characterized by reduced growth of the hypocotyl and a unique pattern of morphogenesis in the cotyledons. Aborted seeds from this mutant were not easily identified because they were nearly the same color and shape as wild-type seeds.

Arrested embryos from mutant 130B–A–2 contained a relatively normal hypocotyl but clearly abnormal cotyledons. Many of the aborted seeds present in immature siliques contained arrested embryos with reduced cotyledons as shown in Fig. 2 ("O"). These unusually small cotyledons were often noticeably paler than the corresponding hypocotyl tissue. Arrested embryos present in older siliques generally contained larger cotyledons as shown in Fig. 2 ("P"). Some of the mutant embryos in these older siliques differed only slightly in morphology from normal embryos. These observations suggest that both the pattern of morphogenesis and the timing of development of the cotyledons are altered in arrested embryos from mutant 130B–A–2. In contrast, development of the hypocotyl appears to proceed normally.

Discussion

Embryogenesis in higher plants has been approached in part through the isolation and characterization of lethal mutants. The most extensive studies have dealt with defective kernel mutants of corn (Mangelsdorf 1926; Sheridan and Neuffer 1980, 1981, 1982), embryo-lethal mutants of *Arabidopsis* (Müller 1963; Usmanov and Müller 1970; Meinke and Sussex 1979a, b; Meinke 1982), and variant cell lines of carrot unable to complete somatic embryogenesis in vitro (Breton and Sung 1982). The viviparous mutants of corn, which in some cases do not survive beyond the seedling stage of development, have also been studied in detail (Robertson 1955; Robichaud et al. 1980). Each of these systems has provided valuable information on the genetic control of plant embryo development.

Procedures for the isolation and characterization of embryo-lethal mutants of *Arabidopsis thaliana* were originally described by Müller (1963). Segregation ratios and stages of developmental arrest were determined for 72 mutant lines isolated following X-irradiation of mature seeds (Müller 1963; Usmanov and Müller 1970). The primary purpose of this work and subsequent studies with embryo-lethal mutants of *Arabidopsis* (Rédei 1970; Usmanov and Sokhibnazarov 1974) was to examine the mutagenic effects of ionizing radiation and various chemical treatments on mature seeds.

The six mutants described by Meinke and Sussex (1979a, b) were isolated for experimental studies on the genetic control of embryogenesis in plants (Meinke 1982; Marsden and Meinke 1985). Emphasis was placed in these studies on mutants with lethal phases that covered early stages of embryo development. The 32 additional mutants described in this report expand the range of lethal phases and abnormal phenotypes that may now be used in the analysis of plant embryo development. Some of these new mutants appear to be similar to those described previously by Müller (1963).

Eight new mutants (127AX–A, 113J–4A, 95A–2B, 109F–5D, 112G–1A, 115D–4A, 126E–B, and 130B–A–2) with a non-random distribution of aborted seeds in heterozygous siliques have now been identified. The isolation and characterization of these mutants, when combined with the results of previous studies with mutants 79A and 124D (Meinke 1982), demonstrate conclusively that many of the genes that perform critical functions during early stages of embryogenesis in plants are also expressed prior to fertilization. Gametophytic expression of these mutant genes appears to alter the ability of mutant pollen tubes to fertilize ovules at the base of the silique (Meinke 1982). The distribution of lethal genes in plant populations may therefore be determined in part by the ability of pollen tubes carrying the mutant allele to participate in fertilization (Mulcahy 1979). The results of studies with embryo-lethal mutants of *Arabidopsis* suggest that gametophytic expression may be more common among genes that regulate early rather than late stages of embryogenesis. Additional mutants with a non-random distribution of aborted seeds will need to be identified before this conclusion can be established. The analysis of segregation ratios in radiation-induced mutants of *Arabidopsis* (Dellaert 1980) suggests that many other mutant genes are expressed during both the haploid and diploid stages of development.

There may be several reasons why no temperature-sensitive ("ts") lethals were found among the 32 mutants examined in this study. It is possible that the restrictive (24 °C) and permissive (18 °C) temperatures may not have been sufficiently different to allow the identification of "ts" mutants. Similar temperatures have been used to isolate "ts" mutants of *Caenorhabditis elegans* (Herman and Horvitz 1980; Isnenghi et al. 1983) and *Drosophila melanogster* (Suzuki et al. 1976), but it may be necessary in future studies with *Arabidopsis* to use a higher restrictive temperature even though this often results in reduced seed set. Temperature-sensitive embryo-lethal mutants of *Arabidopsis* may also have unusually low segregation ratios or highly variable phenotypes when grown at the restrictive temperature and may therefore only rarely be chosen for subsequent studies. There is no obvious physiological reason why the frequency of temperature-sensitive lethals in higher plants should be significantly lower than in animal systems. Several temperature-sensitive seedling lethals have even been described in *Arabidopsis* (Langridge 1965; Li and Rédei 1968). It is therefore likely that temperature-sensitive embryonic lethals of *Arabidopsis* are present following EMS seed mutagenesis and are simply difficult to identify among M-1 plants.

Many of the aborted seeds and arrested embryos described in this report were either pale green or white. The disruption of chlorophyll synthesis in these mutants must be an indirect result of the mutation and not the cause of developmental arrest because mutant embryos of *Arabidopsis* deficient in chlorophyll and carotenoid pigments are still able to complete all of the normal stages of embryogenesis (Rédei 1970; Koornneef et al. 1983). The question then becomes why embryo-lethal mutations differ in their effect on the accumulation of photosynthetic pigments. Relatively little is known about the relationship between morphogenesis, photosynthesis, and the accumulation of chlorophyll and carotenoid pigments in immature green seeds. It is apparent from the mutants described in this report that developmental arrest of mutant embryos is often accompanied by a disruption of chlorophyll synthesis in the arrested embryo, mutant endosperm tissue, and surrounding maternal tissues. It is particularly surprising that these mutations differed in their effect on cells of the seed coat because these cells are genotypically heterozygous for the recessive allele. Additional studies are planned to determine how embryo-lethal mutants of *Arabidopsis* may be used to study the developmental regulation of chlorophyll synthesis during embryogenesis in plants.

Acknowledgements. I wish to thank Linda Franzmann, Ann Baus, Chris Monnot, and David Patton for their technical assistance throughout this project. This research was supported by NSF Grant No. PCM82-15667 and by funds from the College of Arts and Sciences, Oklahoma State University.

References

Breton A, Sung ZR (1982) Temperature-sensitive carrot variants impaired in somatic embryogenesis. Dev Biol 89:58–66

Crouch ML (1982) Non-zygotic embryos of *Brassica napus* L. contain embryo-specific storage proteins. Planta 156: 520–524

Dellaert LMW (1980) Segregation frequencies of radiation-induced viable mutants in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 57:137–143

Herman RK, Horvitz HR (1980) Genetic analysis of *Caenorhabditis elegans.* In: Zuckerman BM (ed) Nematodes as biological models, vol 1. Academic Press, New York, pp 228–261

Isnenghi E, Cassada R, Smith K, Denich K, Radnia K, von Ehrenstein G (1983) Maternal effects and temperature-sensitive period of mutations affecting embryogenesis in *Caenorhabditis elegans.* Dev Biol 98:465–480

Koornneef M, van Eden J, Hanhart CJ, Stam P, Braaksma FJ, Feenstra WJ (1983) Linkage map of *Arabidopsis thaliana.* J Hered 74:265–272

Langridge J (1965) Temperature-sensitive, vitamin-requiring mutants of *Arabidopsis thaliana.* Aust J Biol Sci 18: 311–321

Lewis EB, Bacher F (1968) Method of feeding ethyl methane sulfonate (EMS) to *Drosophila* males. *Drosophila* Inf Serv 43:193

Li SL, Rédei GP (1968) Temperature-sensitive, thiamine-requiring mutants in *Arabidopsis. Arabidopsis* Inf Serv 5:28–29

Mangelsdorf PC (1926) The genetics and morphology of some endosperm characters in maize. Conn Agric Exp Stn Bull 279:513–620

Marsden MPF, Meinke DW (1985) Abnormal development of the suspensor in an embryo-lethal mutant of *Arabidopsis thaliana.* (submitted)

Meinke DW (1982) Embryo-lethal mutants of *Arabidopsis thaliana:* evidence for gametophytic expression of the mutant genes. Theor Appl Genet 63:381–386

Meinke DW, Sussex IM (1979a) Embryo-lethal mutants of *Arabidopsis thaliana*: a model system for genetic analysis of plant embryo development. Dev Biol 72:50–61

Meinke DW, Sussex IM (1979b) Isolation and characterization of six embryo-lethal mutants of *Arabidopsis thaliana.* Dev Biol 72:62–72

Mulcahy DL (1979) The rise of the angiosperms: a genecological factor. Science 206:20–23

Müller AJ (1963) Embryonentest zum Nachweis rezessiver Letalfaktoren bei *Arabidopsis thaliana.* Biol Zentralbl 82: 133–163

Rédei GP (1970) *Arabidopsis thaliana* (L.) Heynh. A review of the genetics and biology. Bibliogr Genet 20:1–151

Robertson DS (1955) The genetics of vivipary in maize. Genetics 40:745–760

Robichaud CS, Wong J, Sussex IM (1980) Control of in vitro growth of viviparous embryo mutants of maize by abscisic acid. Dev Genet 1:325–330

Sheridan WF, Neuffer MG (1980) Defective kernel mutants of maize. 2. Morphological and embryo culture studies. Genetics 95:945–960

Sheridan WF, Neuffer MG (1981) Maize mutants altered in embryo development. In: Subtelney S (ed) Levels of genetic control and development. Alan Liss, New York, pp 137–156

Sheridan WF, Neuffer MG (1982) Maize developmental mutants: Embryos unable to form leaf primordia. J Hered 73:318–329

Suzuki DT, Kaufman T, Falk D, UBC *Drosophila* Research Group (1976) Conditionally expressed mutations in *Drosophila melanogaster.* In: Ashburner M, Novitski E (eds). The genetics and biology of *Drosophila,* vol 1a. Academic Press, New York, pp 207–263

Usmanov PD, Müller AJ (1970) Use of the embryo test for the analysis of embryonic lethals induced by the irradiation of the pollen grains of *Arabidopsis thaliana* (L.) Heynh. Sov Genet 6:894–902

Usmanov PD, Sokhibnazarov Sh (1974) Genetic and somatic effects of N-nitrosomethylbiuret in *Arabidopsis thaliana* (L.) Heynh. Sov Genet 11:697–702