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# In vitro morphogenesis of arrested embryos from lethal mutants of *Arabidopsis thaliana*

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**Summary.** Arrested embryos from lethal (*emb*) mutants of *Arabidopsis thaliana* were rescued on a nutrient medium designed to promote plant regeneration from immature wild-type cotyledons. The best response was observed with mutant embryos arrested at the heart to cotyledon stages of development. Embryos arrested at a globular stage produced callus but failed to turn green or form normal shoots in culture. Many of the mutant plants produced in culture were unusually pale with abnormal leaves, rosettes, and patterns of reproductive development. Other plants were phenotypically normal except for the presence of siliques containing 100% aborted seeds following self-pollination. These results demonstrate that genes with essential functions during plant embryo development differ in their pattern of expression at later stages of the life cycle. Most of the 15 genes examined in this study were essential for embryogenesis but were required again for subsequent stages of development. Only EMB24 appeared to be limited in function to embryo development. These differences in the response of mutant embryos in culture may facilitate the classification of embryonic lethals and the identification of genes with developmental rather than housekeeping functions.

**Key words:** *Arabidopsis thaliana* – Developmental mutants – Embryo culture – Embryonic lethals – Morphogenesis

## Introdution

Embryo development in higher plants has been approached in part through the isolation and characterization of embryo-lethal mutants (Meinke 1986). The most extensive studies have dealt with defective kernel mutants of maize (Mangelsdorf 1926; Sheridan and Neuffer 1982; Sheridan and Clark 1987) and embryo-lethal mutants of *Arabidopsis* (Müller 1963; Meinke and Sussex 1979a; Meinke et al. 1988). In previous studies, we have shown that arrested embryos from lethal mutants of *Arabidopsis* differ with respect to their size and color (Meinke 1985), pattern of abnormal development (Marsden and Meinke 1985), expression of mutant genes prior to fertilization (Meinke 1982), ultrastructure (Patton 1986), extent of cellular differentiation (Meinke et al. 1985), and accumulation of seed storage proteins (Heath et al. 1986). Mutant genes with particularly interesting patterns of abnormal development have been mapped in preparation for molecular isolation through chromosome walking (Meinke et al. 1988). Additional mutants are currently being isolated to identify specific genes that merit detailed analysis at the molecular level.

Several years ago we began to study the response of arrested embryos cultured on media designed to promote either callus formation or the completion of embryogenesis and the growth of homozygous mutant plants (Baus et al. 1986). Our objective was to compare the morphogenetic response of embryos arrested at different stages of development, produce homozygous mutant plants with interesting phenotypes, screen for auxotrophs, determine which genes perform essential functions at later stages of the life cycle, generate additional tissue for biochemical and molecular studies, and facilitate the classification of embryonic lethals and the identification of genes with important developmental functions. Although a wide range of mutant embryos produced callus in culture, the only plants recovered in our initial study were from two mutant lines with relatively late lethal phases (Baus et al. 1986). Subsequent studies have shown that one of these mutants is a biotin auxotroph (Schneider et al. 1989).

In this report we describe the formation of homozygous mutant plants from a wide range of arrested embryos cultured on a medium designed to promote plant regeneration from wild-type leaves (Lloyd et al. 1986) and immature cotyledons (Patton and Meinke 1988) of *Arabidopsis*. Every mutant examined with a lethal phase extending through the cotyledon stage of development produced shoots on this medium. Some shoots developed into relatively normal plants that flowered and produced 100% aborted seeds following self-pollination. Other shoots formed plants that were consistently abnormal and failed to bolt or produce fertile inflorescences. These results demonstrate that arrested embryos from a wide range of lethal mutants are capable of continued growth and morphogenesis in culture. The presence of developmental abnormalities among mutant plants rescued in culture suggests that many genes required for embryo development are expressed again at later stages of the life cycle. The response of mutant plants in culture may therefore be used to identify genes with important developmental functions restricted to embryogenesis. The availability of fertile plants produced from arrested embryos should also facilitate genetic and molecular analysis of embryonic lethals in *Arabidopsis*.

## Materials and methods

Mutant lines of *Arabidopsis thaliana* (L.) Heynh. strain Columbia were grown in pots at 23±3°C beneath 40 W fluorescent lights maintained on daily 16 h light/8 h dark cycles (Meinke 1985; Heath et al. 1986). Plants heterozygous for recessive lethal mutations isolated following EMS seed mutagenesis (Meinke and Sussex 1979b; Meinke 1985) were identified by the presence of siliques containing 25% aborted seeds following self-pollination. Each mutant was assigned a number with a common *emb* prefix (Table 1) to be consistent with nomenclature adopted at the Third International *Arabidopsis* Meetings in East Lansing (April 1987). Fourteen additional mutants (*emb32* to *emb45*) isolated from M-2 populations following EMS seed mutagenesis (Meinke et al. 1988) were not included in this study. Allelism tests were performed between mutants with similar phenotypes to establish complementation groups and to determine whether mutants with similar responses in culture were defective in the same gene. Crosses were performed as described previously (Meinke and Sussex 1979b) by emasculating the female parent and saturating the stigma surface with pollen prior to self-pollination. The resulting siliques were screened prior to desiccation for the presence of normal and aborted seeds.

Aborted seeds and arrested embryos were cultured on a nutrient medium containing the inorganic salts of Murashige and Skoog (1962), B5 vitamins (Gamborg et al. 1968), 3% sucrose, 0.8% agar, 1.0 mg/l 6-benzylaminopurine (BAP), and 0.1 mg/l 1-naphthaleneacetic acid (NAA). This medium was chosen because it was known to promote a high frequency of plant regeneration from immature wild-type cotyledons (Patton and Meinke 1988). Media with different phytohormones (2 mg/l NAA or IAA and 0.5 mg/l kinetin) were used in some cases to promote rooting of regenerated plants. All media were adjusted to pH 5.7 with NaOH, autoclaved for 15 min at 122°C and 18 psi, and poured into 60 × 20 mm sterile Petri plates. Chemicals used in media preparation were obtained from Fisher and Sigma Chemical Companies.

**Table 1.** Revised nomenclature and overview of embryonic lethals. Mutants preceded by (*) were examined in this study. Lethal phases followed by (S) included abnormal suspensors. Seeds and embryos were either creamy white (1), very pale yellow-green (2), pale green (3), or normal green (4). Some mutants (+) exhibited a tip prevalence of aborted seeds in heterozygous siliques caused by gametophytic expression of the mutant gene

| Mutant | Original | Lethal phase | Embryo | Seed | Tip |
|---|---|---|---|---|---|
| *emb1* | 53D-4A | Early preglobular | – | 1 | |
| *emb2* | 124D | Preglobular | – | 1 | + |
| *emb3* | 71E | Preglobular | – | 1 | |
| *emb4* | 50B | Preglobular (S) | – | 1 | |
| *emb5* | 127AX-A | Preglobular | 1 | 1 | + |
| *emb6* | 113K-1B | Preglobular | 1 | 1 | |
| *emb7* | 112E-2A | Preglobular | 1 | 1 | |
| *emb8* | 113J-4A | Preglobular | 1 | 1 | + |
| **emb9* | 79A | Early globular | 1 | 1 | + |
| *emb10* | 112E-1B | Early globular | 1 | 1-2 | |
| *emb11* | 111H-2B1 | Early globular | 1 | 1-2 | |
| *emb12* | 130BA-1 | Early globular | 1 | 1-2 | |
| *emb13* | 111B-5E | Early globular | 1-2 | 1-2 | |
| *emb14* | 95A-2B | Globular | 1-2 | 2 | + |
| **emb15* | 57B-4C | Globular | 1 | 1 | |
| **emb16* | 123B | Globular | 1 | 1 | |
| **emb17* | 109A-1B | Globular | 1 | 1 | |
| **emb18* | 109F-5D | Globular-heart (S) | 1 | 1-2 | + |
| *emb19* | 112G-1A | Globular-heart (S) | 1-2 | 1-2 | + |
| **emb20-1* | 117N-1B | Globular-heart | 1 | 1 | |
| **emb20-2* | 87A | Globular-heart | 1 | 1 | |
| *emb21* | 129AX2-A | Globular-heart | 1 | 1 | |
| **emb22* | 115D-4A | Green blimp | 3-4 | 3-4 | + |
| *emb23* | 126E-B | Globular-linear | 1-4 | 1-3 | + |
| **emb24* | 109F-1C | Globular-cotyledon | 1-3 | 2-3 | |
| **emb25* | 115J-4A | Globular-cotyledon | 1 | 1 | |
| **bio1* | 122GE | Globular-cotyledon | 1-2 | 1-3 | |
| **emb26* | 63A-1A | Globular-cotyledon | 1-2 | 1-2 | |
| **emb27* | 111B-5B | Globular-cotyledon | 1 | 1 | |
| **emb28* | 115H-1A | Globular-cotyledon | 1 | 1 | |
| **emb29* | 115C-1C | Globular-cotyledon | 1 | 1 | |
| *emb30* | 112A-2A | Cotyledon rootless | 4 | 3-4 | |
| *emb31* | 130BA-2 | Reduced cotyledon | 3-4 | 3-4 | + |

Immature siliques from heterozygous plants were surface-sterilized (Baus et al. 1986) and dissected under a stereomicroscope in a laminar flow hood. Aborted seeds were removed from heterozygous siliques and placed in culture with the site of funiculus attachment in contact with the agar surface. Arrested embryos were removed from aborted seeds with Dumont #4 forceps and transferred immediately to the agar surface. The smallest embryos cultured were at a globular stage of development and 80 µm in diameter. Culture plates were sealed with parafilm, placed under fluorescent lights, and maintained at room temperature. Mutant tissue was subcultured to fresh media and transferred to Magenta boxes as required for continued development. Regenerated plants with established roots were transferred to pots containing vermiculite and soil, kept moist for several days in a beaker covered with plastic, and then placed in a growth room.

## Results

### *Complementation tests*

The results of complementation tests between mutants with similar phenotypes are summarized in Table 2. Pairwise crosses between heterozygotes defective in different genes were expected to produce siliques containing 100% phenotypically normal seeds. Two mutants (*emb20-1* and *emb20-2*) with similar responses in culture failed to complement, and produced siliques containing 25% aborted seeds. These mutants are probably allelic although we cannot eliminate the possibility that they were accidentally collected from the same parent. The remaining mutants appear to be defective in different genes. The low frequency of aborted seeds in these siliques was the result of accidental self-pollination. Allelism was considered unlikely among this small group of mutants because mutagenesis studies have established the high frequency of induced embryonic lethals and the large number of genes required for embryo development in *Arabidopsis*. Additional crosses between mutants with different lethal phases will be completed as more efficient methods of genetic analysis become established.

**Table 2.** Complementation tests between mutants with similar phenotypes. Number of phenotypically normal seeds produced by crosses between plants heterozygous for different lethal mutations. The numbers of aborted seeds produced with the expected mutant phenotype are noted in parentheses. Crosses often resulted in a low frequency of spontaneous abortants arrested early in development. These seeds resulted from injury to the silique and were excluded from the totals above

Early lethal phases

| Mutants | *emb15* | *emb16* | *emb17* | *emb18* | *emb20-1* |
|---|---|---|---|---|---|
| *emb20-2* | 87 (1) | 59 (1) | 92 (0) | 58 (0) | 195 (70) |
| *emb20-1* | 85 (1) | 97 (0) | 83 (0) | 51 (2) | |
| *emb18* | 71 (1) | 73 (3) | 69 (0) | | |
| *emb17* | 83 (3) | 113 (0) | | | |
| *emb16* | 64 (0) | | | | |

Late lethal phases

| Mutants | *bio1* | *emb24* | *emb25* | *emb26* | *emb27* | *emb28* |
|---|---|---|---|---|---|---|
| *emb29* | 42 (0) | 71 (0) | 71 (0) | 37 (1) | 62 (1) | 83 (0) |
| *emb28* | 49 (1) | 115 (0) | 56 (0) | 49 (0) | 73 (0) | |
| *emb27* | 61 (1) | 49 (0) | 40 (0) | 73 (0) | | |
| *emb26* | 63 (3) | 112 (0) | 95 (4) | | | |
| *emb25* | 40 (0) | 62 (1) | | | | |
| *emb24* | 119 (2) | | | | | |

### *Mutants with early lethal phases*

Arrested embryos exhibited a wide range of responses in culture (Table 3). Aborted seeds from one preglobular mutant (*emb9*) were examined in detail but failed to grow on any medium tested. The poor response of this mutant may reflect unusual phytohormone requirements of embryos arrested early in development or fundamental defects in cellular processes that are difficult to correct in culture. Subsequent studies dealt with embryos that had reached at least a globular stage of development. Preglobular mutants with arrested embryos that resume development in culture remain to be identified.

Six mutants with embryos arrested at the globular to heart stages of development produced callus but failed to develop normal shoots in culture. Mutant callus grew more slowly than wild-type callus and reached a maximal diameter of 10 mm after several months in culture. Callus produced by aborted seeds originated from the arrested

**Table 3.** Response of mutant embryos in culture. Embryos were arrested at the preglobular (PG), globular (G), globular-heart (GH), blimp (B), and globular-cotyledon (GC) stages of development. The color of mutant callus and shoots was either creamy white (1), very pale yellow-green (2), pale green (3), or green (4). Responses noted in parentheses were rarely observed. Roots and root hairs were included in the same category. The response noted for *bio1* was in the presence of biotin (Schneider et al. 1989)

| Mutant | Embryo | Callus | Color | Roots | Shoots | Leaves | Trichomes | Flowers | Seeds |
|---|---|---|---|---|---|---|---|---|---|
| *emb9* | PG | – | – | – | – | – | – | – | – |
| *emb15* | G | + | 1 | (+) | – | – | – | – | – |
| *emb16* | G | + | 1 | – | – | – | – | – | – |
| *emb17* | G | + | 1 | (+) | – | – | – | – | – |
| *emb18* | GH | + | 1-3 | – | – | – | (+) | – | – |
| *emb20-1* | GH | + | 1 | + | Abnormal | – | – | – | – |
| *emb20-2* | GH | + | 1 | + | Abnormal | – | – | – | – |
| *emb22* | B | + | 3-4 | (+) | Abnormal | Abnormal | (+) | – | – |
| *emb24* | GC | + | 4 | + | Normal | Normal | + | Normal | Yes |
| *emb25* | GC | + | 1-3 | + | Present | Reduced | + | Abnormal | – |
| *bio11* | GC | + | 4 | + | Normal | Normal | + | Normal | Yes |
| *emb26* | GC | + | 2-4 | + | Normal | Normal | + | Pale | Yes |
| *emb27* | GC | + | 1-2 | + | Present | Reduced | + | (Abnormal) | – |
| *emb28* | GC | + | 1-3 | + | Present | Reduced | + | Abnormal | – |
| *emb29* | GC | + | 1 | + | Present | Reduced | + | – | – |

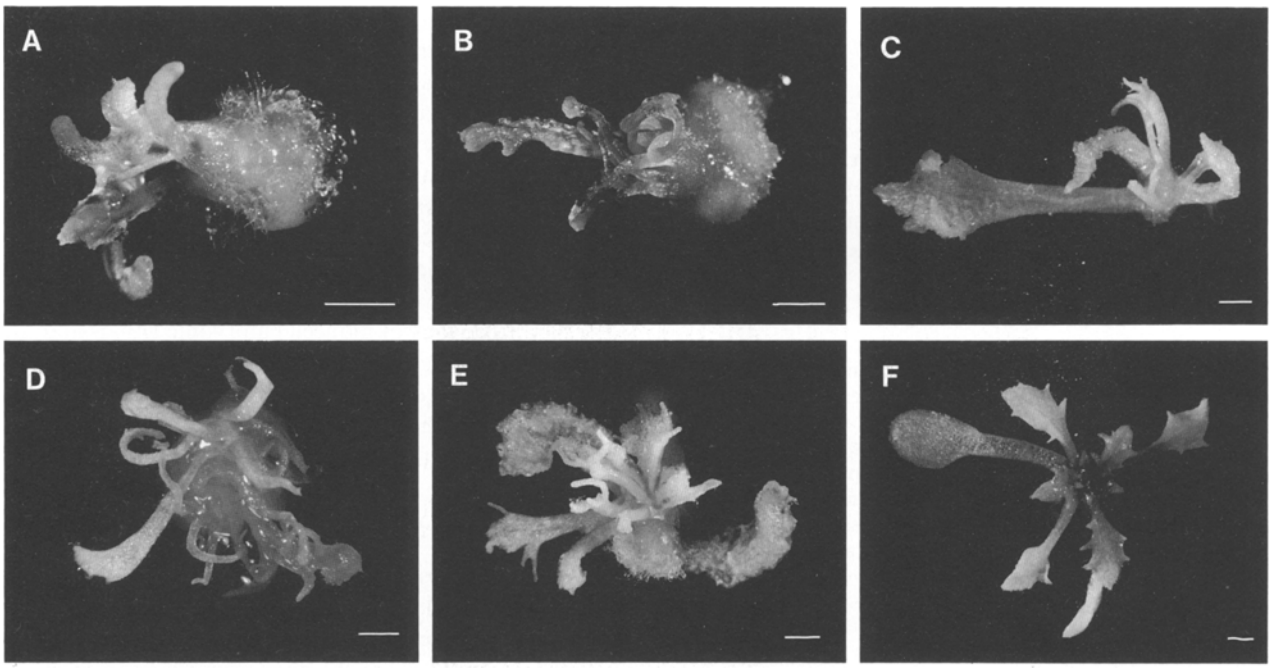


**Fig. 1 A–F.** Shoots produced by arrested embryos from early mutants **A** *emb20-2* and **B** *emb20-1* and late mutants **C** *emb27*, **D** *emb25*, **E** *emb29* and **F** *emb28* after 3–6 weeks in culture. Most shoots were creamy white; only **F** *emb28* was pale yellow-green. Callus, root hairs, branched trichomes and a variety of abnormal shoots are visible. *Scale bar* = 1 mm

embryo and not the seed coat or endosperm tissue. The best response was often obtained from isolated embryos placed in direct contact with the medium. Mutant callus grew more rapidly than in previous studies (Baus et al. 1986) but still failed to survive more than a few months in culture.

Mutant embryos exhibited different patterns of morphogenesis and cellular differentiation in culture. Arrested embryos from three globular mutants produced creamy white callus with a few root hairs but no evidence of shoots or trichomes. Globular-heart embryos from *emb20* produced a variety of abnormal shoots (Fig. 1 A, B) that remained white throughout the culture period. Mutant callus was often polarized with clusters of adventitious shoots and root hairs at opposite ends. Shoots did not resemble normal leaves or stems, generally lacked trichomes, and failed to develop further when subcultured to fresh media. Other embryos arrested at the globular-heart stage (*emb18*) produced a mixture of white and pale green callus with a few branched trichomes but no shoots or leaves. The failure of these mutants to form normal shoots was not related to culture conditions because globular and heart embryos from other mutants with broader lethal phases occasionally produced normal leaves and trichomes in culture. The six globular-heart mutants examined in this study therefore appeared to have fundamental defects that interfered with both embryogenesis in vivo and organogenesis in vitro.

*Green blimp mutant*

Arrested embryos from *emb22* produced extensive callus and numerous abnormal shoots in culture (Fig. 2 A, B). The lethal phase for this mutant has been difficult to establish because arrested embryos have an elongated shape that does not resemble any stage of normal development. The green color is indicative of an embryo arrested late in development, whereas the absence of cotyledons and protein bodies is consistent with an early stage of developmental arrest (Meinke et al. 1985). The initial response of mutant embryos in culture was similar to that of mature wild-type cotyledons. Both explants expanded rapidly and produced green callus within the first ten days of culture. Subsequent patterns of morphogenesis clearly differed. Mutant callus produced 5–20 pale-green shoots with whorls of tubular leaves that occasionally unfolded to form thick and distorted leaf blades. A few branched trichomes were found on the leaf surface. Similar patterns of abnormal development were observed on media with different levels of phytohormones. Callus from a single embryo often reached 20 mm in diameter and continued to grow when subcultured to fresh media. Mutant shoots did not develop further when subcultured to fresh media. These results provide further evidence that *emb22* arrested embryos are not simply mature embryos that have failed to form cotyledons. This mutant gene allows growth and cellular differentiation in culture but disrupts the formation of normal shoots.

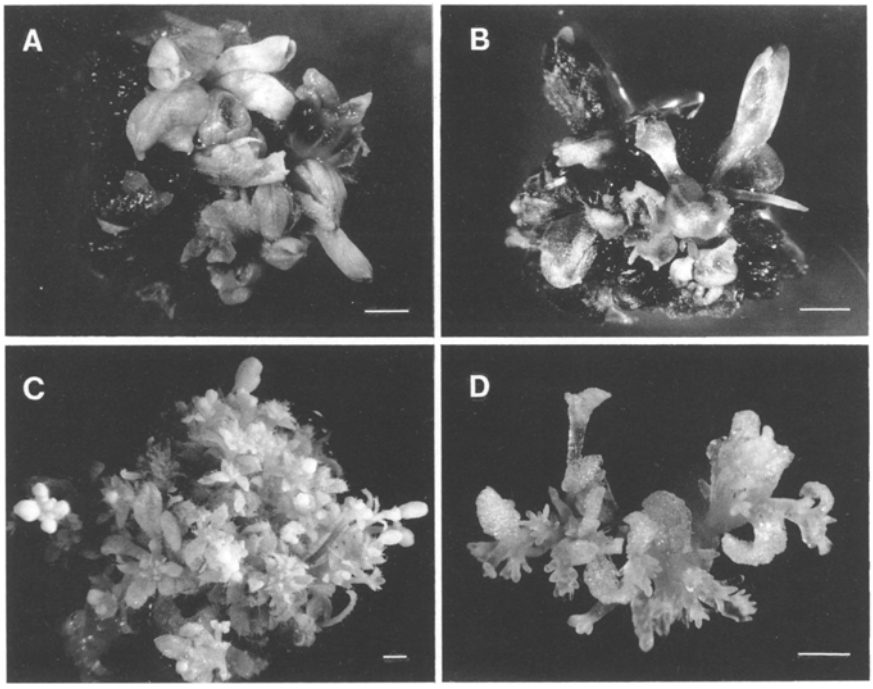


**Fig. 2 A–D.** Shoots produced by arrested embryos from the green blimp **A, B** *emb22* and late mutants **C** *emb25* and **D** *emb29* after several months in culture. Note the presence of tubular leaves on *emb22* and floral buds on *emb25*. Stems produced by *emb29* often terminated in a cluster of distorted leaves and small protrusions **D** not found in other mutants. Tissues ranged in color from **A, B** green to **C** pale yellow-green and **D** white. *Scale bar* = 1 mm

*Mutants with late lethal phases*

Arrested embryos from mutants with globular to cotyledon lethal phases produced a wide range of shoots in culture (Fig. 1 C–F). Some of the mutant plants were phenotypically normal except for their pale color. Others had distorted leaves, trichomes, rosettes, and inflorescences. Some abnormalities (e.g. serrated leaves and distorted trichomes) were attributed in part to variations induced in culture because they were also found in regenerated wild-type plants. Other patterns of abnormal development were characteristic of specific mutants. Emphasis was placed on the identification of plants with normal phenotypes. Consistent abnormalities were expected among plants defective in genes required for later stages of development.

Arrested embryos did not immediately complete the remaining stages of embryogenesis in culture. Instead, they enlarged to form distorted embryos which then produced leaves from a single apex. Adventitious shoots developed occasionally after several months in culture. This pattern of shoot development clearly differed from that seen in the blimp and globular-heart mutants. The frequency of embryos responding in culture varied considerably and appeared to be determined more by culture conditions than by genotype. Globular embryos did not respond as well as cotyledon embryos but still occasionally developed into plants. Mutant plants produced from aborted seeds and isolated embryos often differed slightly in phenotype. This variation was probably caused by differential uptake of nutrients and not by the presence of maternal tissue. Plants regenerated from globular embryos were phenotypically similar to those regenerated from cotyledon embryos.

Trichomes on the surface of mutant plants varied considerably in shape and distribution. The most common abnormalities were increased branching and reduced density. Mutant plants appeared to have a higher frequency of abnormal trichomes than regenerated wild-type plants. The specific effects of mutant genes on trichome development were difficult to determine because abnormalities were not observed consistently within mutant lines. Leaves produced by regenerated plants also varied considerably in size and shape. In some cases, the initial leaves were more abnormal than those produced after 2–3 months in culture (Fig. 1 D and 2 C). Other mutants produced leaves that were phenotypically identical to those present on wild-type plants (Fig. 1 F).

Mutant plants regenerated in culture also differed in color. Arrested embryos from *emb24* and *bio1* (+biotin) developed into green plants that were indistinguishable in color from wild-type plants. Other mutants produced leaves that were slightly paler than normal (*emb26*) or very pale yellow-green (*emb27* and *emb28*). One mutant (*emb29*) produced plants that remained white throughout the culture period. These differences appear to result from indirect effects of the mutant genes on chloroplast function during vegetative development. Abnormal pig-

mentation therefore provides additional evidence for expression of mutant genes at later stages of the life cycle. Embryos defective in the biosynthesis of photosynthetic pigments complete embryogenesis and become arrested at the seedling stage.

*Flowering of regenerated plants*

Regenerated plants from six mutant lines produced flowers in culture. The most normal flowers were produced by *bio1* plants initiated in culture and transplanted to soil supplemented with biotin (Schneider et al. 1989). Other mutants (*emb24* and *emb26*) produced a mixture of normal and abnormal flowers in culture and primarily normal flowers in soil. Siliques produced following self-pollination of *emb24* flowers were phenotypically normal except for the presence of 100% aborted seeds. The number of seeds per silique was similar to that found in wild-type plants. Siliques produced by self-pollination of *emb26* flowers were pale yellow with an unusually long style and a pale green pedicel. These siliques also contained 100% aborted seeds although the total number of seeds per silique was reduced. Mutant leaves and inflorescences produced by *emb26* plants also became pale at the onset of flowering. Siliques from both mutants completed maturation despite the absence of phenotypically normal seeds. Silique development in *Arabidopsis* can therefore occur in the absence of normal seed development.

Aborted seeds and arrested embryos produced following self-pollination of *emb24* and *emb26* homozygotes were indistinguishable from those produced by the corresponding heterozygotes. The lethal phase for these mutants is therefore not altered by the presence of the mutant gene in both maternal and embryonic tissue. This suggests that the defective gene product is not a diffusible substance supplied jointly by embryonic and maternal tissues. Crosses between wild-type pollen and homozygous *emb24* and *emb26* flowers resulted in the formation of 100% phenotypically normal seeds that completed embryogenesis and germinated to produce heterozygous plants with the expected 25% aborted seeds. This provides further evidence that the presence of the mutant gene in maternal tissue does not disrupt normal embryo development.

Other mutants occasionally produced inflorescences after several months in culture. Some of the *emb28* flowers appeared normal except for their pale color, slightly distorted shape, and failure to produce mature pollen. Small buds produced by *emb25* were unusually pale and remained closed at the surface of bushy plants with miniature leaves (Fig. 2C). A single *emb27* inflorescence with pale sepals and distorted internal organs was observed in culture. Stems produced by *emb29* often terminated with clusters of leaves and small protrusions in place of flowers (Fig. 2D). These structures were white and clearly differed from those produced by other mutants. None of the plants described above produced seeds or survived when transferred to soil.

## Discussion

Embryonic lethals have been used in a variety of animal systems to identify genes with essential developmental functions (Wilkins 1986). Analysis of these mutants has been limited by the failure of homozygotes to complete later stages of the life cycle. The most thorough studies of embryonic lethals have therefore involved the use of genetic mosaics (Bryant and Zornetzer 1973; Girton and Bryant 1980) and temperature-sensitive mutations (Suzuki et al. 1976). We describe in this report an alternative method of producing homozygotes from lethal mutants defective in plant embryo development. This method of plant regeneration in culture is applicable to a wide range of mutants with embryos arrested at the globular to cotyledon stages of development. Embryo culture provides an approach not available in animal systems to examine the developmental expression of essential genes and produce homozygotes for genetic and molecular studies.

We have shown in previous studies that expression of mutant genes prior to fertilization can result in a nonrandom distribution of aborted seeds along the length of heterozygous siliques (Meinke 1982). Ten loci have been identified that perform essential functions during both embryogenesis and gametogenesis (Meinke 1985). Genes with similar patterns of expression have been identified among defective-kernel mutants of maize (Clark and Sheridan 1988; Ottaviano et al. 1988). In this report, we demonstrate that the response of arrested embryos in culture can provide information on the expression of mutant genes at later stages of the life cycle. Our results suggest that many embryo-lethal mutants are defective in genes required for both embryogenesis and vegetative development. This overlap in gene expression is consistent with molecular studies of mRNAs present at different stages of plant development (Goldberg 1988). Many genes required for embryo development in *Drosophila* (Perrimon et al. 1984; Reynolds and O'Donnell 1987) and *Caenorhabditis* (Miwa et al. 1980; Isnenghi et al. 1983) are also expressed at more than one stage of the life cycle.

The response of mutant embryos in culture may also provide information on the cellular basis of abnormal development. There may be several reasons why arrested embryos resume growth and development in culture. Mutant embryos may be defective in genes expressed in the endosperm tissue and may reveal their potential for normal development only when removed from defective

seeds and grown in culture. Mutant embryos may also be defective in one member of a multigene family that serves an essential function throughout the life cycle. Growth of arrested embryos in culture may in this case activate a related gene that is normally expressed during later stages of development. Other mutant embryos may be defective in embryo-specific functions that are circumvented during the initial transition from embryogenesis to organogenesis in culture. And some mutations may result in a reduced level of gene activity that disrupts embryogenesis but not vegetative growth and development.

The failure of arrested embryos from globular mutants to produce shoots in culture is more difficult to explain. We had expected to find mutants that were unable to form cotyledons during embryogenesis but still produced adventitious shoots through in vitro organogenesis. This was based on the assumption that leaves and cotyledons utilize different developmental pathways requiring the activation of different genes. Instead we found that arrested embryos from mutants with early lethal phases grew slowly in culture and failed to produce normal shoots while similar embryos from mutants with later lethal phases developed into relatively normal plants. Arrested embryos from mutants with early lethal phases may, therefore, have fundamental defects in morphogenesis that are more difficult to rescue in culture.

The response of arrested embryos in culture may also facilitate the identification of mutants with defects in developmental rather than housekeeping functions. The biotin auxotroph described by Schneider et al. (1989) illustrates how the loss of a specific housekeeping function can result in embryonic lethality. Other mutants may have similar defects in cellular functions essential for growth but not for the regulation of development. The question then becomes how to identify genes with more interesting developmental functions. We have proposed that future studies might focus on mutants with particularly unusual patterns of development, genes that are expressed only during embryogenesis, lethal phases that correspond to a critical stage in morphogenesis, or arrested embryos that are green rather than white (Meinke et al. 1988).

In this report we describe one mutant (*emb24*) that appears to be defective in a gene required only for embryo development. The lethal phase for this mutant extends from the globular to cotyledon stages of development, arrested embryos are white or pale green, aborted seeds are distributed randomly in heterozygous siliques, and regenerated plants are phenotypically normal except for the presence of siliques containing 100% aborted seeds. Does this pattern of development indicate that *emb24* is defective in a gene with an important developmental function? Recent studies have shown that although arrested embryos from this mutant produce normal plants on a basal medium, they respond more rapidly on an enriched medium containing amino acids and vitamins. The specific nutrients responsible for this enhanced growth remain to be determined. Other mutants with similar lethal phases do not respond better on enriched media. It therefore appears that EMB24 performs an essential housekeeping function that is limited to embryogenesis. Additional mutants are being tested to determine whether genes with important developmental functions can be identified among lethals defective only in embryogenesis. The discovery that a segmentation gene with a regulatory function during embryogenesis in *Drosophila* is expressed again in adult tissues (Doe et al. 1988) suggests that genes with regulatory functions during plant embryo development may also be expressed at other stages of the life cycle. Some of the mutants that produced abnormal plants in culture may therefore merit further analysis at the molecular level.

The response of mutant embryos in culture may also lead to the identification of interesting new phenotypes. Some mutations affecting vegetative and floral development may not be recovered from M-2 populations because they result in embryonic lethality. The availability of homozygous mutant plants should also simplify the analysis of embryonic lethals. Initial stages of developmental arrest can be observed more readily, the effects of mutant genes on maternal tissues can be determined, and genetic studies can be expanded to include the isolation of revertants and extragenic suppressors. The isolation of essential genes through chromosome walking (Meyerowitz 1987) may also require homozygous mutant tissue for transformation and molecular complementation. With additional modifications in culture conditions, it should be possible to increase the number of fertile plants produced and the diversity of lethal mutants rescued in culture.

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