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Growth in vitro of arrested embryos from lethal mutants of *Arabidopsis thaliana*

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Summary. Seventeen embryo-lethal mutants of *Arabidopsis thaliana* with lethal phases ranging from the globular to mature cotyledon stages of development were analyzed by culturing arrested embryos on nutrient media designed to promote either callus formation or the completion of embryo development and the recovery of homozygous mutant plants. Enriched media supplemented with vitamins, amino acids, and nucleosides were used to identify potential auxotrophic mutants. Wild-type embryos produced extensive callus on basal and enriched media supplemented with 2,4-D and kinetin. Numerous roots developed when wild-type callus was grown in the presence of NAA and kinetin. Mutant embryos arrested prior to the heart stage of development formed only a slight amount of callus on basal and enriched media. Arrested embryos from mutants 122G-E and 112A-2A reached a later stage of development and gave the most interesting responses in culture. 122G-E mutant embryos failed to grow on basal media but produced extensive callus and homozygous mutant plants on enriched media. The specific nutrient required for growth of this mutant remains to be determined. Arrested embryos from mutant 112A-2A developed into abnormal plants without roots when placed in culture. Mutant callus also failed to form roots on a variety of root-inducing media. Expression of this mutant gene therefore disrupts development of the root apical meristem during both embryogenesis in vivo and organogenesis in vitro.

Key words: *Arabidopsis thaliana* – Auxotrophs – Embryo-lethal mutants – Organogenesis – Root development – Tissue culture

Introduction

Arabidopsis thaliana (Cruciferae) has been used as a model system for numerous studies in plant genetics (Rédei 1975a; Koornneef et al. 1983; Somerville 1984) and molecular biology (Leutwiler et al. 1984; Meyerowitz and Pruitt 1985).

The developmental and molecular genetics of embryogenesis in *Arabidopsis* has been examined in part through the isolation and characterization of embryo-lethal mutants (Müller 1963; Meinke and Sussex 1979a; Meinke 1986). Several different approaches have been taken in the analysis of these developmental mutants (Meinke 1982; Meinke et al. 1985; Marsden and Meinke 1985; Heath et al. 1986). The purpose of the present study was to examine how the growth response of mutant embryos in culture might be used to analyze the underlying causes of lethality in mutants with different patterns of abnormal development.

Previous studies on the growth of *Arabidopsis* cells in culture have dealt with conditions for cell proliferation (Negrutiu et al. 1975; Negrutiu and Jacobs 1977; Xuan and Menczel 1980), in vitro morphogenesis (Negrutiu and Jacobs 1978a, b; Negrutiu et al. 1978a, b; Gotto 1979; Huang and Yeoman 1984), anther culture (Keathley and Scholl 1983), and interspecific protoplast fusion (Gleba and Hoffmann 1980). The response in culture of other crucifers has also been examined in detail (Monnier 1976; Keller 1984; Zee and Johnson 1984).

We describe in this report how plant tissue culture can be used in the analysis of embryo-lethal mutants to recover homozygous mutant plants, identify potential auxotrophic mutants, and study the expression of mutant genes at later stages of development.

Materials and methods

Maintenance of plants

Wild-type and heterozygous mutant plants of *Arabidopsis thaliana* (L.) Heynh. strain 'Columbia' were grown in pots at

23 °C ± 3 °C under 16 h/8 h light/dark cycles (Meinke 1985; Heath et al. 1986). Mutants were isolated following EMS seed mutagenesis and were known from previous studies to be recessive embryonic lethals with characteristic patterns of abnormal development (Meinke and Sussex 1979 b; Meinke 1985; Meinke et al. 1985). Mutant lines were maintained as heterozygotes that were phenotypically normal except for the presence of 25% aborted seeds following self-pollination.

Culture media

Basal media (B) contained the inorganic salts of Murashige and Skoog (1962), 3% glucose, 0.8% (w/v) purified Difco agar, 0.55 mM inositol, and 5 μM thiamine hydrochloride. Basal media used for callus formation (BC) contained 0.5 mg/l kinetin and 2 mg/l of either 2,4-D (BC-2D.5K) or NAA (BC-2N.5K). Modified media used in the analysis of mutant 112A-2A contained either 5 mg/l NAA and 1 mg/l kinetin (BC-5N 1K) or 10 mg/l NAA and 0.5 mg/l kinetin (BC-10N.5K). All media were adjusted to pH 5.8 with NaOH, autoclaved for 15–20 min at 122 °C and 18 psi, and poured in 10 ml aliquots into 60 × 15 mm plastic Petri plates.

Enriched media (E) were supplemented with 0.2 mM each of 20 L-amino acids; 0.1 mM each of 5 nucleosides; 0.5 μM each of 7 vitamins (p-aminobenzoic acid, biotin, folic acid, nicotinamide, Ca-pantothenate, pyridoxine HCl, and riboflavin-5′-phosphate); 50 μM choline chloride; and 0.5 mM each of DL-malic and citric acids. Thiamine-HCl was included in both basal and enriched media because the thiamine auxotrophs of *Arabidopsis* are seedling lethals (Li and Rédei 1969) and were not expected to be included among embryonic lethals. Enriched media used for callus formation (EC) contained 0.5 mg/l kinetin and 2 mg/l of either 2,4-D (EC-2D.5K) or NAA (EC-2N.5K). Modified media with different combinations of amino acids, vitamins, and nucleosides were used in the analysis of mutant 122G-E. Organic supplements were sterilized with a 0.2 μm Gelman filter and then added to autoclaved media. Chemicals used in media preparation were obtained from Fisher and Sigma Chemical Companies.

Culture conditions

Intact seeds and isolated embryos were used to determine the growth response of mutant and wild-type embryos in culture. Leaves, stamens, and stem segments were also used to generate wild-type callus. Immature siliques were surface sterilized by soaking for 30 sec in 95% ethanol followed by 4 min in 20% Clorox (1% sodium hypochlorite) containing a small amount of detergent. Siliques were then rinsed in distilled water, transferred to Petri plates, and dissected under a stereomicroscope in a laminar flow hood. Mature seeds were surface sterilized by a 30 sec exposure to 95% ethanol and a 6 min treatment with 50% Clorox.

Aborted seeds from 17 mutants with a wide range of lethal phases were cultured on basal and enriched media designed to promote callus formation. Heterozygous siliques used for these studies were at a mature green stage of development (Meinke and Sussex 1979 a) and contained aborted seeds that had not yet degenerated. Optimal growth of mutant embryos occurred when aborted seeds were placed with the site of funiculus attachment in contact with the agar surface. Leaves formed callus most readily when cut pieces were placed with the abaxial surface in contact with the medium. Culture plates were sealed with parafilm, placed under fluorescent lights, and maintained at room temperature.

Results

Wild-type embryos

Wild-type embryos, leaves, stamens, and stem segments all formed friable callus without roots on basal (BC-2D.5K) and enriched (EC-2D.5K) media containing 2,4-D and kinetin. Growth was not inhibited by the organic supplements present in the enriched media. Immature seeds cultured at a globular to linear cotyledon stage of development completed the remaining stages of embryo development in vitro before the embryo started to form callus. Initiation of callus occurred from both the hypocotyl and cotyledons of wild-type embryos but not from the seed coat or endosperm tissue. Isolated embryos formed callus when cultured between the linear and mature cotyledon stages of development. Wild-type embryos cultured on a basal medium containing NAA and kinetin (BC-2N.5K) produced a greener and more nodular callus with numerous roots. Mature embryos often germinated and formed relatively little callus when placed on an enriched NAA medium (EC-2N.5K), but callus subcultured to this medium grew well and produced numerous roots.

Mutant embryos

Aborted seeds from 10 mutants with lethal phases prior to the heart stage of development formed only a slight amount of callus on basal and enriched media (Table 1). This mutant callus was produced by the arrested embryo, reached a maximal diameter of 3–5 mm after several months in culture, did not enlarge further when subcultured to fresh media, and failed to differentiate roots in the presence of NAA. Similar responses were observed on basal and enriched media. Many of the aborted seeds from these mutants failed to produce even a limited amount of callus tissue. This poor response may have been caused by culture conditions that were not suitable for the growth of embryos arrested at early stages of development.

Arrested embryos from mutant 115D-4A were a normal green color but lacked a defined hypocotyl and cotyledons (Meinke 1985). Aborted seeds from this mutant occasionally produced a small amount of callus in culture. Mutant embryos turned pale and elongated slightly as if they were beginning to germinate, but no sustained growth was observed in the presence of either NAA or 2,4-D. This response in culture indicates that 115D-4A mutant embryos are defective not only in morphogenesis but also in cellular differentiation because they fail to produce the extensive callus characteristic of mature wild-type embryos. The same conclusion has been drawn from recent studies on the

Table 1. Response of arrested embryos and aborted seeds from 17 embryo-lethal mutants of *Arabidopsis thaliana* cultured on basal and enriched media designed to promote callus formation[a]

Mutant	Nutrient medium		Number cultured		Lethal phase
	Basal	Enriched	Basal	Enriched	
111B-5E	–	–	75	105	Early globular
95A-2B	○	○	200	170	Globular
123B	–	–	125	75	Globular
57B-4C	–	–	25	70	Globular
109A-1B	–	–	95	75	Globular
109F-5D	○	–	115	90	Globular
112G-1A	–	–	30	15	Globular-Heart
117N-1B	–	–	265	235	Globular-Heart
87A	–	–	160	80	Globular-Heart
129AX2-A	–	–	50	55	Globular-Heart
115D-4A	–	–	280	265	Green blimp
126E-B	+	+	230	290	Globular-Linear
109F-1C	+	+	85	50	Globular-Linear
115J-4A	+	+	20	20	Globular-Mature
122G-E	–	+	615	1,585	Globular-Mature
115H-1A	+	+	20	20	Globular-Mature
112A-2A	+	+	250	110	Fused cotyledon

[a] The response of mutant embryos in culture was classified as either no growth (○), slight callus (–), or extensive callus (+). Results from two different basal (BC-2D.5K and BC-2N.5K) and enriched (EC-2D.5K and EC-2N.5K) media are combined

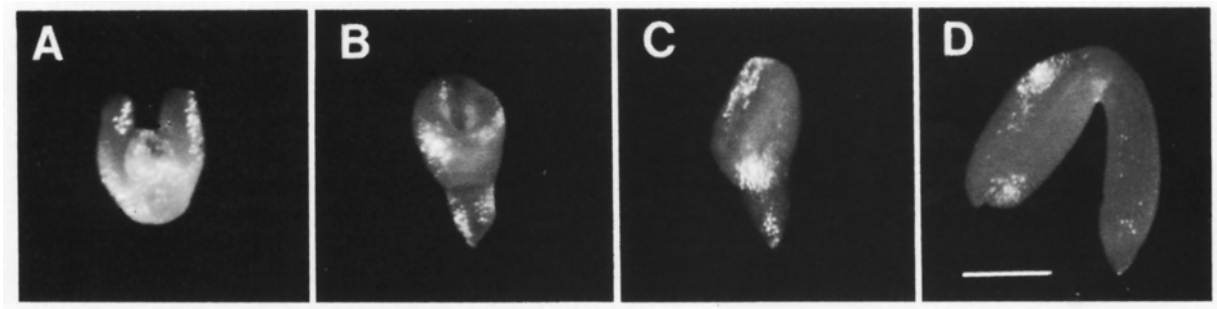

Fig. 1 A–D. Embryos from normal and aborted seeds of *Arabidopsis thaliana*. Arrested embryos from mutants 122G-E (**A**) and 112A-2A (**B, C**) were removed from heterozygous siliques containing normal embryos (**D**) at a mature green stage of development. All embryos are at the same magnification; scale bar = 200 μm

accumulation of storage proteins in normal and aborted seeds (Heath et al. 1986).

Aborted seeds from six other mutants with lethal phases extending through the linear and mature cotyledon stages of development produced extensive callus that continued to grow when subcultured to fresh media (Table 1). This mutant callus was in several cases noticeably greener than the arrested embryo. The loss of chlorophyll during early stages of embryo development may therefore be reversed through subsequent growth in culture. Callus from mutants 126E-B, 109F-1C, 115J-4A, 122G-E, and 115H-1A produced roots in the presence of NAA and kinetin. The formation of a root apical meristem through in vitro organogenesis was therefore not blocked by the presence of these mutant alleles. In contrast, expression of the 112A-2A mutant allele consistently prevented the formation of roots in culture. Many of these mutants had wide lethal phases that covered several stages of development (Meinke 1985). Callus formed most readily from embryos that were arrested relatively late in development. Mutants 122G-E and 112A-2A (Fig. 1) were examined in most detail because they exhibited particularly interesting responses in culture.

122G-E mutant embryos

The lethal phase for mutant 122G-E extended from the globular to mature cotyledon stages of embryo development (Meinke 1985). Arrested embryos were white or very pale green and had distorted cotyledons with a variety of abnormal shapes (Fig. 2). Aborted seeds

Table 2. Lengths and developmental stages of 122G-E arrested embryos removed from heterozygous siliques at different stages of development

Stage of normal embryos present in same silique	No. (%) of arrested embryos observed at the following stages			No. (%) of arrested embryos observed with the following lengths			
	Globular-Heart	Torpedo-Linear	Cotyledon-Mature	100–190 μm	200–290 μm	300–390 μm	> 400 μm
Linear-curled	14 (20)	48 (71)	6 (9)	27 (40)	29 (43)	9 (13)	3 (4)
Early mature	20 (18)	82 (71)	13 (11)	14 (13)	35 (31)	48 (43)	15 (13)
Late mature	12 (17)	48 (68)	11 (15)	4 (6)	10 (14)	37 (52)	20 (28)

Table 3. Response of 122G-E arrested embryos and aborted seeds cultured on basal (BC) and enriched (EC) media

Material cultured	Medium	No. cultured	Response in culture
Arrested embryo[a]	BC-2D.5K	345	Slight callus forms
	BC-2N.5K	245	Slight callus forms
Arrested embryo[a]	EC-2D.5K	390	Extensive callus forms
	EC-2N.5K	1,130	Green plantlets form
Mutant callus[b]	BC-2D.5K	40	Continued callus growth
	EC-2D.5K	40	Continued callus growth
Mutant leaves[c]	BC-2D.5K	10	Extensive callus forms
	EC-2D.5K	10	Extensive callus forms

[a] Includes both aborted seeds and isolated embryos
[b] Callus subcultured from an enriched medium
[c] Leaves from homozygous mutant plants

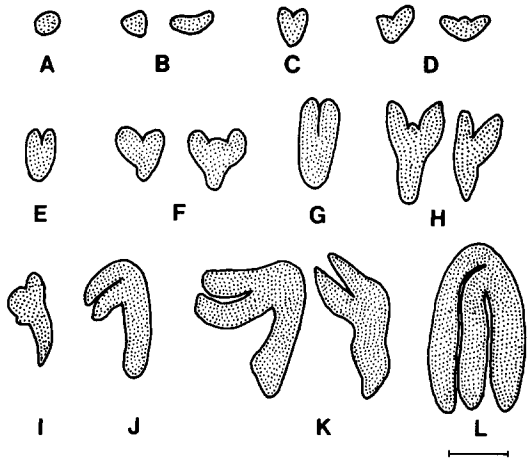

Fig. 2. Examples of 122G-E mutant phenotypes. Arrested embryos were found at the globular (*A*), irregular globular (*B*), heart (*C*), irregular heart (*D*), torpedo (*E*), irregular torpedo (*F*), linear cotyledon (*G*), irregular linear cotyledon (*H, I*), curled cotyledon (*J*), irregular curled cotyledon (*K*), and mature cotyledon (*L*) stages of development. The frequencies of these mutant phenotypes in a random sample of 254 arrested embryos was 10.3% A–B; 10.7% C–D; 39.5% E–F; 17.4% G–I; 10.2% J–K; 0.4% L; and 11.5% not readily classified. Scale bar = 100 μm

were pale green but were similar in size and shape to mature wild-type seeds. Mutant embryos isolated from relatively mature siliques were on the average slightly larger but not more advanced morphologically than embryos isolated from younger siliques (Table 2). Arrested embryos from mutant 122G-E were therefore able to enlarge during their period of arrested development, but they did not usually progress to a later stage of embryogenesis.

The response of aborted seeds from mutant 122G-E on basal and enriched media is summarized in Table 3. Approximately 30% of the aborted seeds responded in culture; optimal growth occurred with embryos arrested late in development. Mutant embryos grew only slightly on a basal medium but produced extensive callus on an enriched 2,4-D medium and homozygous mutant plants on an enriched NAA medium. The pale callus that formed on a basal medium reached a maximal diameter of 2 mm after several months in culture and did not continue to grow when subcultured to fresh basal media. Even embryos arrested late in embryogenesis failed to produce a significant amount of callus on a basal medium. Mutant callus produced on an enriched 2,4-D medium closely resembled wild-type callus in morphology and rate of growth. Homozygous mutant seedlings formed following the completion of embryo development on an enriched NAA medium were also phenotypically normal.

This differential response of aborted seeds on basal and enriched media initially suggested that mutant 122G-E might be an auxotroph. To test this hypothesis, mutant callus initiated on an enriched 2,4-D medium was subcultured to basal and enriched media. The results as summarized in Table 3 demonstrated that mutant callus grew equally well on both types of media. This sustained growth of mutant cells on a basal medium was not caused by nutrients carried over from the enriched medium because the callus continued to grow when subcultured several additional times to fresh basal media. Leaves from homozygous mutant plants also produced callus on both basal and enriched media. Expression of the 122G-E wild-type allele there-

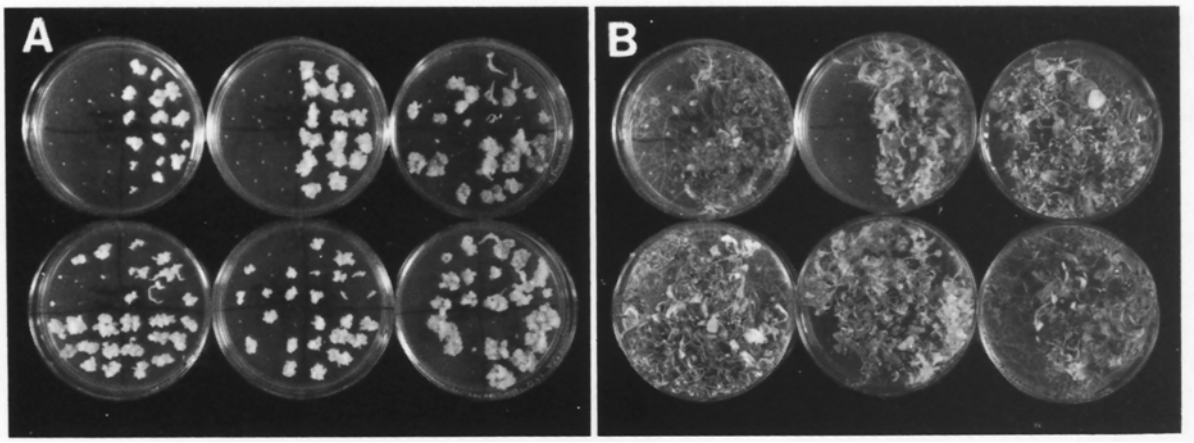

Fig. 3 A, B. Response of mutant 122G-E on modified enriched media. Each plate was constructed by placing aborted seeds in the *top left* quadrant, arrested embryos in the *lower left* quadrant, wild-type seeds in the *top right* quadrant, and wild-type embryos in the *lower right* quadrant. Media were designed to promote either callus formation (**A**) or the development of homozygous mutant plants (**B**). **A** Composition of plates clockwise from *upper left:* (a) BC-2D.5K; (b) BC-2D.5K with 4.0 mM glutamine; (c) EC-2D.5K; (d) EC-2D.5K without vitamins; (e) EC-2D.5K without nucleosides and organic acids; and (f) EC-2D.5K without amino acids. **B** Composition of plates clockwise from *upper left:* (a) BC-2N.5K; (b) BC-2N.5K with 4.0 mM glutamine; (c) EC-2N.5K; (d) EC-2N.5K without vitamins; (e) EC-2N.5K without nucleosides and organic acids; and (f) EC-2N.5K without amino acids

fore appears to be required for the completion of embryo development but not for continued growth of undifferentiated cells in culture.

The response of aborted seeds on modified basal and enriched media was then studied in an attempt to identify the specific nutrient required for growth. The results as shown in Fig. 3 were not those expected for a typical auxotrophic mutant. Aborted seeds cultured on modified enriched media lacking either vitamins, amino acids, or nucleosides grew just as well as on the original enriched medium, and seeds cultured on modified basal media supplemented with either vitamins, amino acids, or nucleosides responded no better than on the standard basal medium. The beneficial effect of the enriched medium did not appear to be caused simply by an increase in reduced nitrogen because aborted seeds cultured on a basal medium supplemented with 4.0 mM glutamine also failed to produce callus (Fig. 3A). Aborted seeds cultured adjacent to wild-type seeds on a basal NAA medium initially formed only a small amount of pale callus, but this mutant callus enlarged slightly and turned greener as it came into contact with roots from the surrounding wild-type plants (Fig. 3B). These results suggest that the defect in arrested embryos from mutant 122G-E can be at least partially corrected in vitro by a diffusible factor present in wild-type roots.

122G-E mutant plants

Homozygous mutant seedlings initiated on an enriched NAA medium (Fig. 4) developed into phenotypically normal plants even when transferred to pots without organic supplements. Ninety percent (16/18) of the transplanted seedlings survived to form small rosettes with normal leaves, stems, and trichomes. Mutant plants consistently became abnormal at the onset of flowering, when many of the rosette and cauline leaves turned pale green and then white. Chlorosis in these leaves appeared first in areas between the major veins. Inflorescences turned white shortly after bolting and produced only a few flowers before senescing. Many of

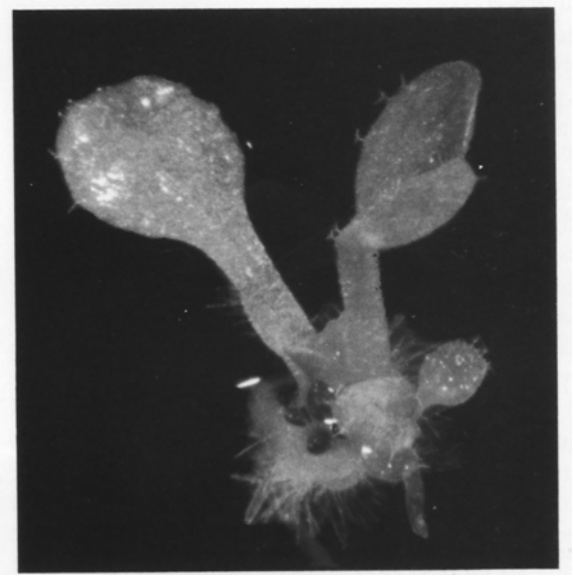

Fig. 4. Phenotypically normal 122G-E mutant seedling produced following germination of an arrested embryo on an enriched NAA medium

these flowers were phenotypically abnormal and produced little pollen. No evidence was found of fertilization or silique elongation in any of these homozygous mutant plants.

This pattern of development suggested that the 122G-E wild-type allele was expressed throughout reproductive development but not during vegetative growth of the rosette. The question then became whether the same enriched medium that rescued mutant embryos in culture could also be used to prevent the premature senescence of mutant plants grown in pots. Mutant and wild-type plants could not be maintained in pots watered with enriched media because the soil became contaminated with microorganisms. Mutant seedlings were therefore transplanted to sterile deep-dish containers, grown under aseptic conditions on various soil mixtures, and watered periodically with basal and enriched media. Wild-type seedlings grown under these conditions developed into normal plants that flowered but did not usually set seed. The reduced fertility of these plants appeared to be caused by excessive moisture inside the sterile containers.

Homozygous mutant seedlings watered with an enriched medium developed into phenotypically normal plants that flowered and remained green for several months but did not produce any seeds. In contrast, plants watered with a liquid basal medium produced leaves and buds that began to turn pale shortly after the onset of flowering. A few of the cauline leaves on plants watered with the enriched medium turned pale after several weeks, but the response was not as striking as that found with the basal medium. A similar response was observed when seedlings were transplanted to agar media in deep-dish containers. Once again, mutant plants grown on an enriched medium remained green much longer than plants grown on a basal medium. Some factor present in the enriched medium therefore allows not only continued development of arrested embryos but also continued viability of homozygous mutant plants after the onset of flowering.

112A-2A mutant embryos

Aborted seeds from mutant 112A-2A contained green embryos arrested at a late cotyledon stage of development (Meinke 1985). Mutant embryos had a reduced hypocotyl and abnormal cotyledons that were either distinct or fused along one or both sides to form a folded or conical structure (Fig. 1). The relative frequency of these mutant phenotypes varied between plants. Embryos with partially fused cotyledons (Fig. 1 C) were generally more common than those with either completely fused cotyledons (Fig. 1 B) or distinct but unusually thick cotyledons. Mutant embryos lacked a root apical meristem but formed normal protein and lipid bodies (Meinke et al. 1985) and accumulated normal levels of seed storage proteins (Heath et al. 1986).

Aborted seeds from mutant 112A-2A produced extensive callus and homozygous mutant plants on both basal and enriched media containing the appropriate phytohormones (Table 4). No further development of these aborted seeds was observed in soil. Aborted seeds removed from mature green and brown siliques gave similar responses in culture. Arrested embryos present in these aborted seeds enlarged and broke through the seed coat when placed on a basal medium without phytohormones (Table 4). These enlarged embryos reached a maximal diameter of 6–8 mm in culture, retained their characteristic mutant phenotype, did not exhibit the hypocotyl elongation or root formation normally associated with germination, and remained green for several weeks before senescing. Mutant embryos did not continue to accumulate storage proteins during this period of growth in culture and instead appeared to follow the normal pathway of protein degradation associated with germination (Heath et al. 1986). Some of the mutant embryos placed on a basal medium produced small plantlets that were similar to those produced in the presence of NAA. An exogenous supply of auxin and cytokinin was therefore not required for the development of homozygous mutant plants in vitro.

112A-2A mutant plants

Mutant embryos subcultured to a basal NAA medium developed into abnormal plantlets that survived for several months in culture without bolting or forming any roots (Table 4). These homozygous mutant plants produced 10–20 small leaves with short petioles and unusually thick leaf blades (Fig. 5 B). Mutant trichomes were reduced in number and appeared as single spikes without the normal branched structure. Callus was initially limited to the mutant hypocotyl and cotyledons but eventually covered the entire rosette. Similar results were obtained when mutant embryos were placed on an enriched NAA medium. Plants subcultured to a basal medium without hormones also failed to produce flowers or roots. Arrested embryos with completely fused cotyledons produced only a slight amount of callus on a basal NAA medium and only rarely developed into homozygous mutant plants (Fig. 5 A).

Arrested embryos produced extensive callus when cultured on basal and enriched media containing 2,4-D and kinetin (Table 4). This mutant callus had the same appearance and growth rate as wild-type callus (Fig. 5 C), but it failed to form roots when transferred to

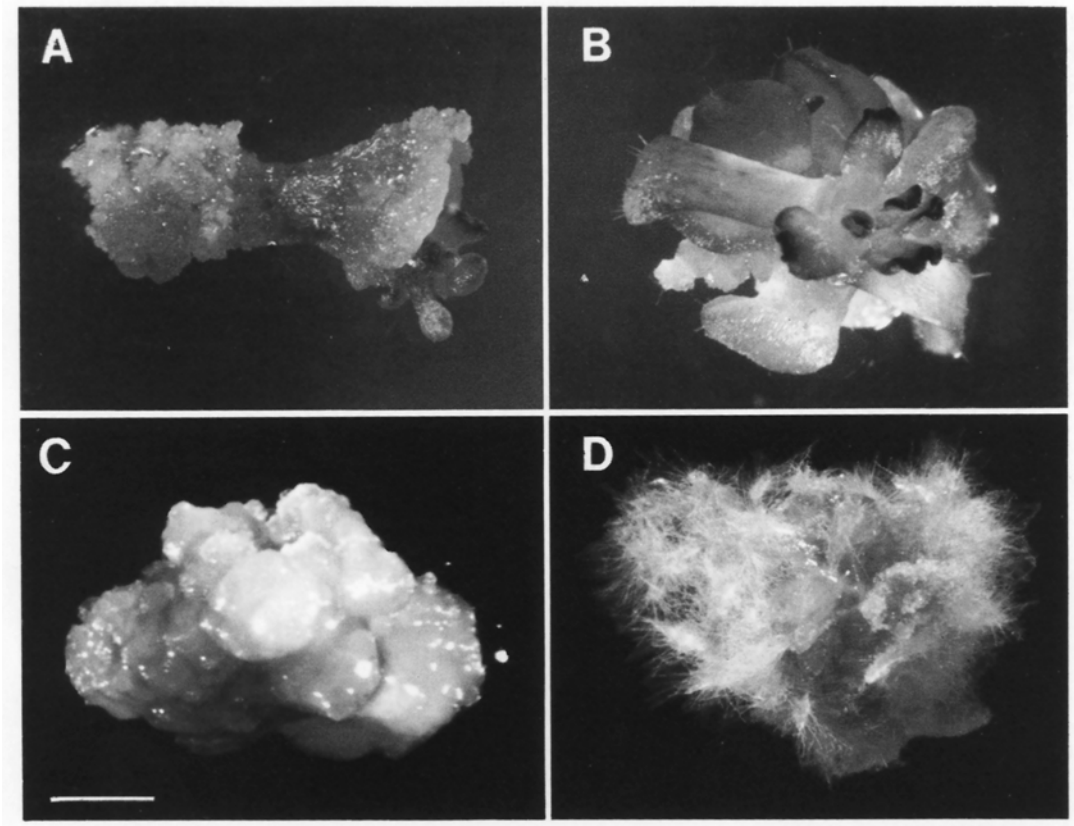

Fig. 5 A–D. Response of mutant 112A-2A in culture. Arrested embryos were placed initially on a basal medium without hormones and then transferred to either a BC-2N.5K medium to promote the development of homozygous mutant plants (**A, B**) or a BC-2D.5K medium to promote callus formation (**C**). Wild-type embryos cultured on a BC-2N.5K medium (**D**) produced extensive roots. Scale bar = 2 mm

Table 4. Response of 112A-2A arrested embryos and aborted seeds cultured on basal and enriched media

Material cultured	Medium	No. cultured	Response in culture
Aborted seed	Basal[a]	520	Embryo enlarges without forming roots
Enlarged embryo	BC-2N.5K EC-2N.5K	105 35	Abnormal plantlets form without roots or inflorescences
Enlarged embryo	BC-2D.5K EC-2D.5K	55 25	Extensive callus forms without roots
Mutant callus	BC-2N.5K BC-5N; 10N	220	Callus does not develop roots
Mutant leaves	BC-2N.5K BC-5N; 10N	40	Leaves form callus without roots
Enlarged embryo	BC-5N; 10N	25	Abnormal plantlets form without roots or inflorescences

[a] The basal medium used to promote "germination" of aborted seeds was similar to other basal media except that it contained no growth hormones. Modified media with higher concentrations of NAA (BC-5N 1K; BC-10N.5K) were used in an attempt to stimulate root formation

a root-inducing medium containing NAA and kinetin. Callus produced from the leaves of homozygous mutant plants also failed to form roots on media containing NAA and kinetin. Even modified media with different levels of auxins and cytokinins failed to stimulate the development of roots by mutant plants and callus tissue (Table 4). The 112A-2A mutant allele therefore appears to disrupt not only the development of a normal hypocotyl and cotyledons during embryogenesis in vivo, but also the formation of roots during vegetative growth in culture, the transition from a vegetative to floral state of development, and the differentiation of roots through in vitro organogenesis.

Discussion

Developmental mutants have been used in a variety of plant and animal systems to study the molecular basis of morphogenesis and differentiation (Sang 1984; Meinke 1986). The basic approach has been to use developmental mutants to dissect morphogenetic pathways and identify genes with essential developmental functions. Analysis of lethal mutants has been particularly challenging because homozygous mutant individuals often die before they reach maturity. Expression of lethal genes at later stages of development has generally been studied through the isolation of temperature-sensitive mutants (Suzuki et al. 1976) and the construction of genetic mosaics composed of both mutant and wild-type cells (Girton and Bryant 1980). Gametophytic expression of lethal genes in plants has been demonstrated through studies on the non-random distribution of aborted seeds in heterozygous fruits (Meinke 1982; Meinke and Baus 1985).

Plant tissue culture provides an alternative approach to the analysis of embryo-lethal mutants without a temperature-sensitive phenotype. Even mutants that fail to complete embryogenesis in culture may be able to produce callus and form differentiated structures on a defined nutrient medium. Lethal mutants may also be used to study the similarity of zygotic embryogenesis in vivo and somatic embryogenesis in vitro (Meinke 1986).

Several seedling lethals of *Arabidopsis thaliana* have been examined previously for their response in culture (Weiland and Müller 1972; Avetisov et al. 1976). Arrested embryos from defective kernel mutants of maize have also been examined in detail (Sheridan and Neuffer 1980, 1981, 1982). One objective of this work with maize was to screen for auxotrophic mutants. The assumption as originally proposed by Langridge (1958) was that mutant embryos defective in the synthesis of an essential amino acid or vitamin might not be rescued by surrounding maternal tissues. The scarcity of auxotrophic mutants identified at the seedling level (Rédei 1975b) does not appear to be caused by gene duplication or alternative biochemical pathways because numerous auxotrophic cell lines have been isolated in culture (King 1984; Bright et al. 1985; Negrutiu et al. 1985). In contrast, the only auxotrophs identified among defective-kernel mutants of maize required proline and were allelic to a group of seedling lethals described previously by Gavazzi et al. (1975).

Our experiments with embryo-lethal mutants of *Arabidopsis* were designed to screen for auxotrophs and other mutants with interesting responses in culture. We felt that some nutritional mutants might be eliminated by gametophytic competition in maize and might be more readily recovered in plants such as *Arabidopsis* where pollen tubes travel short distances. We chose mutants with a wide range of lethal phases (Meinke 1985) and included mutant alleles known to be expressed prior to fertilization (Meinke and Baus 1985). The poor growth in culture of mutant embryos arrested at early stages of development complicated our search for auxotrophs and eliminated a large number of mutants from further analysis. This poor response of arrested globular embryos was surprising because wild-type seeds cultured at early stages of development routinely completed the remaining stages of embryogenesis and developed into phenotypically normal plants (Meinke 1979). Lethal mutants of *Arabidopsis* that arrest prior to the heart stage of development but also produce extensive callus in culture therefore remain to be identified.

The differential response of 122G-E mutant embryos and plants on basal and enriched media suggests that auxotrophs may be included among mutants with late lethal phases. The ability of mutant callus to grow when subcultured to a basal medium also suggests that the 122G-E wild-type allele is not required for the growth of undifferentiated cells in culture. It therefore appears that at least two different genes or biochemical pathways are involved in the synthesis of this missing gene product. One is active during embryo development and flowering while the other is active during vegetative growth in vivo and cell growth in vitro. Selection of variant cell lines in culture may therefore be the most efficient method of identifying auxotrophic mutants in plants, but other mutant alleles may escape detection because they are not expressed in undifferentiated cells.

The absence of roots in 112A-2A homozygous mutant plants illustrates how the growth of arrested embryos in culture can lead to the discovery of interesting new phenotypes. The only other rootless plants that have been described in detail were regenerated from mutagenized protoplasts of *Nicotiana tabacum* resistant to high concentrations of NAA (Muller et al. 1985). These mutants were not viable when grown in the greenhouse, failed to produce roots in culture, flowered only when grafted to wild-type plants, and transmitted both the NAA-tolerant and rootless traits to progeny produced from grafted plants. Infection of mutant plants with *Agrobacterium rhizogenes* also failed to produce the characteristic hairy-root phenotype (Tourneur et al. 1985). The failure of 112A-2A mutant callus tissue to differentiate roots in the presence of high levels of NAA suggests that a similar defect in the response of cells to auxin may be present in 112A-2A mutant plants.

The fused cotyledon phenotype of arrested embryos from mutant 112A-2A is also interesting because it resembles an abnormality often encountered in somatic embryos (Crouch 1982; Jelaska 1977; Lippmann and Lippmann 1984). Although the fused cotyledon and rootless phenotypes appear to be pleiotropic effects of a single gene, it is possible that mutant 112A-2A may be defective in two closely linked genes with different functions. The molecular basis of these mutant phenotypes remains to be determined.

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