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REVIEW ARTICLE

Perspectives on Genetic Analysis of Plant Embryogenesis

David W. Meinke

Department of Botany, Oklahoma State University, Stillwater, Oklahoma 74078

INTRODUCTION

Embryogenesis plays a central role in the life cycle of flowering plants. Ever since the process of double fertilization was documented at the turn of the century, plant embryologists have attempted to characterize the cellular and biochemical changes that occur within developing seeds (Maheshwari, 1950; Raghavan, 1976). For many years, the emphasis was on comparative morphology and the analysis of cell division patterns during early stages of embryo development (Johansen, 1950; Wardlaw, 1955). Attention then shifted to experimental studies of somatic embryogenesis, embryo culture, and haploid embryos derived from microspores (Raghavan, 1986). Manipulation of zygotic embryos was limited by the presence of surrounding maternal tissues. Electron microscopy provided additional details on cellular changes associated with embryogenesis but generally failed to identify the mechanisms responsible. Plant embryologists believed that genetic factors played an important role in morphogenesis, but mutants with altered patterns of embryo development were largely ignored (Meinke, 1986).

Recent advances in molecular biology have led to a renewed interest in plant embryology and the underlying patterns of gene expression that occur throughout seed development (Goldberg et al., 1989). Many genes transcribed during embryonic maturation have now been examined at the molecular level. Genetics provides a complementary approach to the study of plant embryo development by allowing the identification of genes with essential functions during this critical stage of the life cycle (Meinke, 1986). The purpose of this review will be to explore the potential benefits and limitations of mutant analysis in relation to plant embryo development. Additional information can be obtained through recent reviews on plant development (Steeves and Sussex, 1989; Lyndon, 1990), experimental plant embryogenesis (Johri, 1984; Raghavan, 1986; Williams and Maheswaran, 1986), and plant developmental genetics (Sheridan, 1988; Meinke, 1991a, 1991b).

OVERVIEW OF PLANT EMBRYOGENESIS

Plant embryologists originally attempted to explain characteristic patterns of cell division observed early in development by establishing fundamental laws of embryogeny (Johansen, 1950). These laws of parsimony, numbers, origin, disposition, and destination were thought to reflect the fundamental organization of embryos from different species. This view has gradually been replaced by the realization that cell division patterns are determined more by genetic and biophysical factors than by laws of embryogeny. Examination of developmental pathways in different angiosperms has nevertheless revealed a number of common features. The most critical events appear to be the formation of apical meristems, the establishment of basic patterns of symmetry and cellular organization, and the transition from a heterotrophic zygote dependent upon nutrient reserves of surrounding maternal tissues to an autotrophic embryo capable of surviving desiccation and producing a viable seedling after germination. Early stages of plant embryo development are characterized by cell division and morphogenesis. This is followed by a period of cell specialization and embryonic maturation in preparation for dormancy and germination.

Several features distinguish embryogenesis in angiosperms from related pathways in animal systems: (1) the process of double fertilization and subsequent interactions between embryo and endosperm tissues, (2) the apparently minor role played by maternal mRNAs stored in unfertilized eggs, (3) the absence of cellular migration during embryo development, (4) the absence of a germ line established early in development, (5) the activation of large numbers of zygotic genes during very early stages of embryogenesis, (6) the presence of a male gametophyte active in transcription that leads to elimination of many deleterious alleles before fertilization, (7) the establishment of relatively few specialized cell types, (8) the formation of apical meristems that ultimately produce the adult plant, and (9) the small size of the zygote and its location deep within maternal tissues. The zygote in flowering plants is also not unique; a wide range of somatic cells can be

induced to produce embryos either as part of the normal life cycle or through experimental manipulation. Genetic studies of embryogenesis in Drosophila and C. elegans may therefore not be directly applicable to higher plants.

Capsella (Cruciferae) has served for many years as a model for embryo development in dicots (Maheshwari, 1950; Schulz and Jensen, 1968). A similar pattern of development has been found in Arabidopsis (Mansfield and Briarty, 1991; Webb and Gunning, 1991). Early stages of embryogenesis are summarized in Figure 1. The egg at fertilization is located within a multicellular megagametophyte that contains eight haploid nuclei derived from a single functional megaspore within the developing ovule. Formation of the megagametophyte is outlined in Figure 2. Two sperm from a single pollen tube enter the megagametophyte and participate in fertilization. One fuses with the egg to produce the zygote. The other fuses with two polar nuclei to produce the primary endosperm nucleus. Molecular signals responsible for double fertilization remain to be identified, but recent studies have shown that in many plants the two sperm cells involved are dimorphic (Russell, 1991).

The zygote produces an embryo composed of two parts: the embryo proper and the suspensor. Initial patterns of

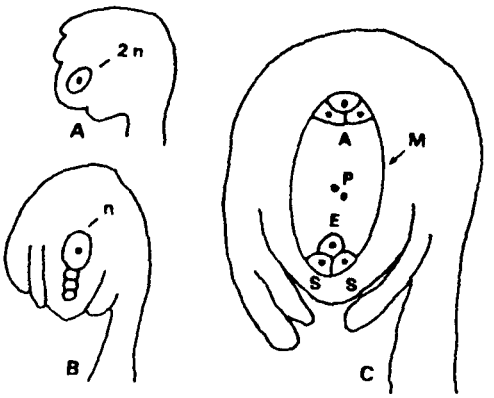

Figure 2. Common Pathway of Megagametophyte Development in Angiosperms.

(A) Immature ovule containing the megasporocyte (2n) before meiosis.
(B) Immature ovule after meiosis. The surviving megaspore (n) undergoes three postmeiotic mitoses to form the megagametophyte.
(C) Ovule with a mature megagametophyte (M) containing the egg (E), two synergids (S), three antipodals (A), and two polar nuclei (P).
Figure adapted from Foster and Gifford (1974).

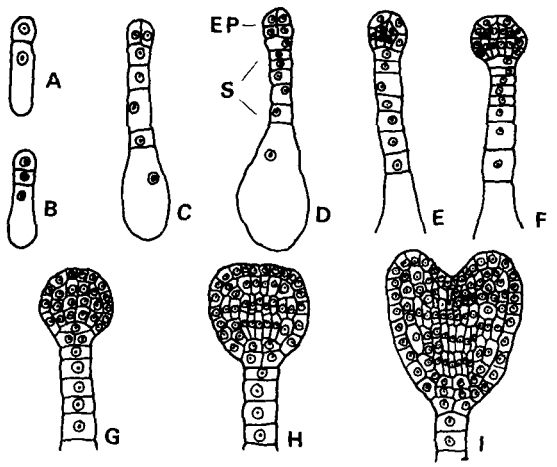

Figure 1. Early Stages of Embryogenesis in Capsella and Arabidopsis.

(A) to **(C)** Early development of the proembryo.
(D) Octant stage; four cells of the embryo proper (EP) are visible. The filamentous suspensor (S) terminates with an enlarged basal cell.
(E) Initiation of the epidermal layer (dermatogen; protoderm) at the 16-cell stage.
(F) to **(H)** Globular stages of embryogenesis.
(I) Heart stage of embryogenesis.
The globular embryo of Arabidopsis is approximately 40 μm in diameter. Figure adapted from Maheshwari (1950).

cell division in the suspensor and early embryo proper are highly reproducible (Figure 1). The periclinal division that produces the 16-cell embryo proper is particularly significant because it establishes an epidermal layer known as the dermatogen. Subsequent planes of cell division in the embryo proper are more irregular. The suspensor attaches the embryo proper to surrounding maternal tissues and functions early in development to provide nutrients to the embryo proper (Yeung, 1980). The distal portion of the suspensor in contact with the embryo proper is known as the hypophysis and gives rise to both the root apex and root cap.

Several critical events occur during the transition from globular to heart stages of embryogenesis in Capsella: (1) the embryo proper establishes bilateral symmetry with the initiation of cotyledons, (2) cellular differentiation becomes apparent with the formation of incipient vascular tissue, (3) the embryo proper begins to turn green and becomes visible through the translucent seed coat, and (4) the triploid endosperm tissue that was present as a syncytium of free nuclei during early stages of embryogenesis starts to become cellular. Both the suspensor and cellular endosperm begin to degenerate during later stages of development and are not present at maturity, as seen in Figure 3. Further expansion of the cotyledons and hypocotyl occurs as the embryo passes through the torpedo, early curled, and mature cotyledon stages of development. The shoot apex forms a cluster of small cells between the

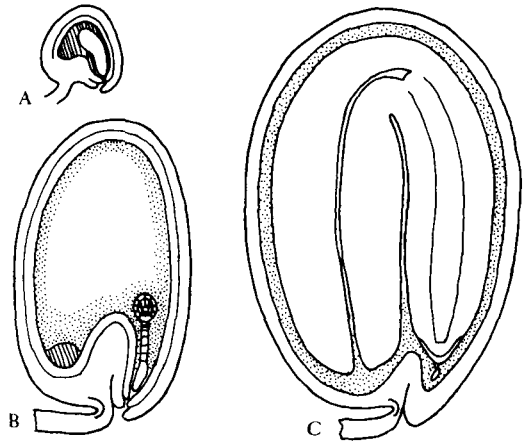

Figure 3. Three Stages of Seed Development in Arabidopsis.

(A) Ovule at the time of fertilization. The megagametophyte (clear) is located within maternal nucellar tissue (hatched).
(B) Immature seed at a globular stage of embryogenesis. The nuclear endosperm tissue (stippled) and chalazal nucellar tissue (hatched) are in contact with the inner integument (seed coat).
(C) Mature seed (0.5 mm in length) with a hypocotyl (right) and two cotyledons (left) visible.
Figure adapted from Müller (1963).

expanding cotyledons but remains quiescent until the initial stages of germination.

Although the pattern of development observed in Capsella can serve as a model for plant embryogenesis, it cannot reflect the diversity of embryonic pathways observed throughout the angiosperms (Wardlaw, 1955). Development of the megagametophyte itself is highly variable (Maheshwari, 1950). Some plants lack synergids, whereas others differ in the number and placement of antipodals and polar nuclei. The endosperm tissue is not always triploid; it can range from 2n to 15n depending on the number of polar nuclei produced during megagametogenesis. Two or more megaspores may remain functional and contribute to the mature megagametophyte. This can result in genotypic differences among the haploid nuclei present at the time of fertilization.

The initial division of the zygote is often transverse, but subsequent division patterns clearly differ between taxonomic groups. Particular emphasis has been placed on division of the terminal cell at the two-cell stage and the relative contributions of the basal cell and its descendants to the embryo proper (Johansen, 1950; Maheshwari, 1950). Many angiosperms can be classified according to predictable patterns of cell division during early stages of embryogenesis. Other species exhibit irregular patterns even at the earliest stages of development. Gymnosperm embryos differ in a more dramatic fashion because they often progress through a free nuclear phase before the globular stage of development. As shown in Figure 4, the size and appearance of the suspensor also differ greatly among angiosperms. Suspensors range from a single cell to a massive column of several hundred cells. Some contain polytene chromosomes, others are polyploid or multinucleate, and a few produce large haustoria that invade surrounding maternal tissues and apparently facilitate the transfer of nutrients to the developing embryo proper. The extent of differentiation at the time of seed maturation also varies. In a few cases, the "mature" seed contains an undifferentiated globular embryo that completes the remaining stages of development after being shed from the plant. The shoot apex is quiescent and barely visible in some embryos at maturity, whereas in others it produces several leaves before germination. Most embryos prepare for extended periods of desiccation and dormancy following completion of embryogenesis, but in viviparous mangroves,

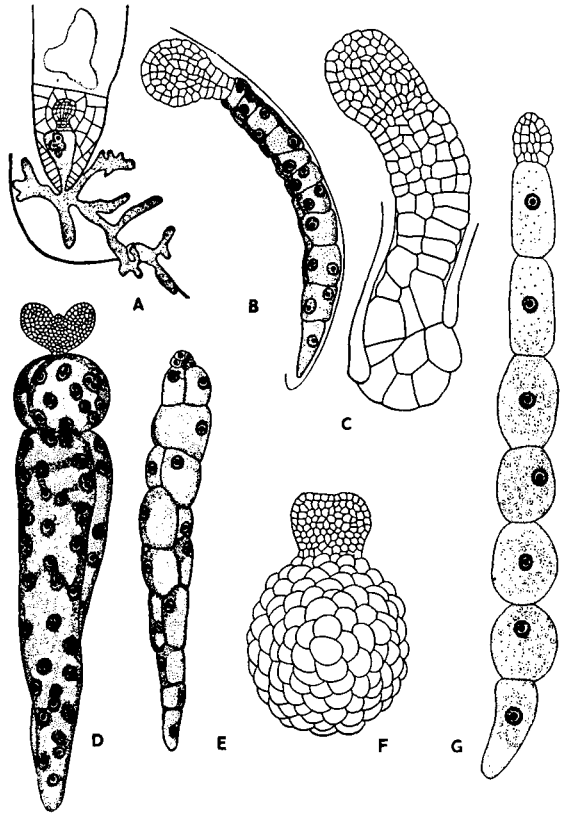

Figure 4. Variations in Development of the Suspensor in Angiosperms.

(A) Basal portion of the ovule of *Sedum acre* showing the suspensor with branched haustoria.
(B) to **(G)** Immature embryos from various Leguminosae. Each embryo is oriented with the suspensor below the embryo proper.
Figure reprinted from Wardlaw (1955).

precocious germination occurs within the fruit. Endosperm development among angiosperms also differs in the timing and extent of cellularization, the presence of specialized haustoria, and the amount of tissue present at maturity. Models of gene function in relation to plant embryo development must therefore account for both the common features and interesting variations observed among angiosperm embryos.

GENETIC ANALYSIS OF PLANT EMBRYOGENESIS

Mutants have been used in several ways to study plant development (Meinke, 1991a). One approach has been to analyze cell division patterns by genetically marking single cells or cell layers and noting the distribution of resulting clonal sectors (Tilney-Bassett, 1986; Poethig, 1989). Three major questions have been addressed through clonal analysis: (1) how many cells within the meristem contribute to the formation of a particular plant structure? (2) when do meristematic cells become restricted to predictable developmental fates? and (3) how variable are the lineages and division patterns of meristematic cells at different stages of development? Clonal analysis has provided extensive information on the origin and developmental fates of cells within the embryonic shoot apex of maize (Poethig et al., 1986; McDaniel and Poethig, 1988). The conclusion from these studies has been that meristematic cells become restricted in their developmental fates but do not always divide in the same manner or contribute to precisely the same portion of the adult plant. Similar studies with other plants have supported the conclusion that cell lineages play a minor role in plant development (Jegla and Sussex, 1989). Clonal analysis has not been widely used to study cell lineages during early stages of embryogenesis (Christianson, 1986). One problem has been the identification of cell-autonomous genetic markers that can be readily scored throughout embryonic development. Another limitation has been marking specific cells at precisely the same stage of development. It should nevertheless be possible to apply the principles of clonal analysis to embryogenesis in Arabidopsis and to characterize in detail the nature of cell lineages during early stages of embryo development.

Mutants can also be used to identify genes with important developmental functions. The field of plant developmental genetics has expanded dramatically in recent years with the identification of mutants defective in almost every aspect of development (Meinke, 1991a). With floral mutants of Arabidopsis (Bowman et al., 1991) and Antirrhinum (Schwarz-Sommer et al., 1990), considerable progress has been made toward identifying genes with direct roles in the regulation of morphogenesis (Coen, 1991). Emphasis in this case was placed on a small number of mutants with homoeotic changes in floral structure. Embryogenesis presents a greater challenge with respect to mutant analysis because a large number of genes are first expressed at this stage of the life cycle. Jürgens et al. (1991) have followed the Drosophila approach and focused on a small number of pattern mutants with defects in both embryo development and the establishment of apical-basal pattern elements in adult plants. My laboratory has taken the approach that a wide range of mutants with different phenotypes must be examined to understand the relationship between gene function and embryo development.

Many embryonic mutants are likely to be defective in essential housekeeping functions (Haughn and Somerville, 1988). Several strategies have been proposed to facilitate the identification of genes involved in the regulation of morphogenesis (Meinke, 1991a). These include focusing on mutants with phenotypes that appear to result from changes in cellular identity, mutants defective in genes with embryo-specific functions, mutants with defective embryos that are green rather than white, and mutants that consistently block at a critical stage of morphogenesis. Mutations in housekeeping genes expressed during both gametogenesis and embryogenesis may be eliminated before fertilization (Meinke, 1982; Ottaviano and Mulcahy, 1989). The long style of maize provides a greater selective barrier to mutant pollen tubes than the short style of Arabidopsis. Housekeeping defects that result in embryonic lethality may still provide valuable information on the genetic control of cellular functions. For example, a biotin auxotroph of Arabidopsis that becomes lethal during embryogenesis has provided the most definitive information to date on biotin synthesis in plants (Schneider et al., 1989; Shellhammer and Meinke, 1990). Other mutants should facilitate the identification of genes that function in cell division and metabolism. Many laboratories are utilizing molecular approaches to identify housekeeping genes with important functions. Analysis of embryonic mutants will enable a complementary genetic approach to be pursued.

Some embryonic lethals may be defective in genes with important developmental functions. Early stages of embryo development are characterized by patterns of morphogenesis that differ from subsequent iterative events associated with the shoot apex (Poethig, 1990). Defects in genes regulating early embryogenesis may result in lethality, whereas defects in genes regulating the shoot apex may result in homoeosis or heterochrony. Some transcription factors required for completion of embryogenesis may also serve global functions throughout growth and development. Loss of a single factor through mutation may consequently disrupt a wide range of essential functions and result in lethality during embryogenesis. Although pattern mutants are clearly interesting and should provide valuable insights into the genetic control of plant embryogenesis, there are several reasons why research with other embryonic mutants should also be continued. Perhaps the most compelling reason is that polarity and pattern formation

are not the only questions that merit analysis in relation to plant embryogenesis. Many genes are likely to play a critical role in the complex interactions that occur between different parts of a developing seed. Understanding the nature of interactions between the embryo proper, suspensor, endosperm, seed coat, nucellus, and maternal plant is as important from a developmental perspective as understanding how meristems become positioned in the proper location. Other genes may play important roles in determining cell division patterns in the early embryo, cellularization of endosperm tissues, nuclear changes in the suspensor, establishment of the dermatogen, initiation of cotyledons, and differentiation of provascular tissues. Identifying these genes may be difficult, but defining their functions during embryogenesis will be required to answer fundamental questions in plant development. Embryonic lethals, defectives, and pattern mutants should therefore play complementary roles in the genetic analysis of plant embryo development.

DIVERSITY OF EMBRYONIC MUTANTS

Embryonic mutations have been identified in a wide range of flowering plants including Arabidopsis (Müller, 1963; Franzmann et al., 1989; Jürgens et al., 1991; Meinke, 1991b), maize (Sheridan and Neuffer, 1982; Dolfini and Sparvoli, 1988; Clark and Sheridan, 1991), barley (Bosnes et al., 1987; Felker et al., 1987), carrot (Lo Schiavo et al., 1988; Schnall et al., 1988), rice (Nagato et al., 1989), and pea (Bhattacharyya et al., 1990). Many of these mutations have been induced with chemical mutagens (Neuffer and Sheridan, 1980; Meinke, 1985; Jürgens et al., 1991), X-irradiation (Müller, 1963), transposable elements (Clark and Sheridan, 1991), or T-DNA from Agrobacterium (Errampalli et al., 1991). Important features of maize and Arabidopsis as model systems for genetic analysis of plant embryogenesis are summarized in Table 1. Early stages of embryogenesis in maize are shown in Figure 5.

Various strategies have been employed to identify mutants defective in embryo development: (1) screening ears of maize produced by M_1 plants after pollen mutagenesis (Neuffer and Sheridan, 1980), (2) screening immature siliques of Arabidopsis produced by chimeric M_1 plants after seed mutagenesis (Müller, 1963; Meinke and Sussex, 1979a, 1979b), and (3) screening M_2 seedlings of Arabidopsis germinated on agar plates (Jürgens et al., 1991). Variant lines of carrot defective in somatic embryogenesis have also been identified in culture (Lo Schiavo et al., 1988; Schnall et al., 1988). Recessive embryonic lethals are generally maintained as heterozygotes that produce 25% aborted seeds after self-pollination. Heterozygous and wild-type plants are distinguished at maturity by screening for the presence of defective seeds.

Table 1. Maize and Arabidopsis: Model Systems for Genetic Analysis of Plant Embryogenesis

General Feature	Arabidopsis	Maize
Chromosome number	$n = 5$	$n = 10$
Haploid genome size	7×10^4 kb	3×10^6 kb
Generation time	40 days	120 days
Fruits per plant	Many	Few
Seeds per fruit	30–60	400–600
Seed length	0.5 mm	8 mm
Seed weight	30 μg	200 mg
Shoot apex (seed)	Small	Large
Leaf primordia (seed)	Absent	Present
Endosperm at maturity	Absent	Present
Target of mutagenesis	Seeds	Pollen
Insertional mutagenesis	Available	Available
Embryo mutants	Numerous	Numerous
Endosperm mutants	Uncertain	Numerous
Chromosome walking	Practical	Impractical
Plant transformation	Established	Limited
B-A translocations	Absent	Available
Somatic embryogenesis	Difficult	Difficult
Clonal analysis	Possible	Established
Relevance of model	Dicots	Monocots

The high frequency of mutants defective in embryogenesis is consistent with a large number of target genes performing essential functions at this stage of the life cycle. Molecular studies of RNAs present in developing seeds indicate that 20,000 genes may be expressed during embryogenesis (Goldberg et al., 1989). Many of these genes may perform nonessential functions, be duplicated in the genome, or be required during gametogenesis. The precise number of genes identified through loss-of-function mutations is therefore difficult to predict. Jürgens et al. (1991) estimate that Arabidopsis contains approximately 4000 genes essential for embryogenesis. This number is based on the relative frequencies of embryonic lethals and pigment mutants recovered following seed mutagenesis. The actual number of essential genes may be significantly lower because spontaneous embryo abortion in large populations of M_2 plants may lead to overestimates of the frequency of embryo-lethal mutations. We estimate that only 500 genes may be identified through embryonic lethals and defectives in Arabidopsis. Existing collections of embryonic mutants have therefore not approached saturation.

Most embryonic mutants are defective in either (1) early stages of cell division and morphogenesis, (2) accumulation of pigments and storage materials during embryonic maturation, or (3) preparation for dormancy and germination. Included in the first class are many defective-kernel (*dek*) and germless (*emb*) mutants of maize (Sheridan, 1988; Clark and Sheridan, 1991) and embryonic (*emb*) mutants of Arabidopsis (Meinke, 1991b). The second class includes a wide range of mutants defective in endosperm

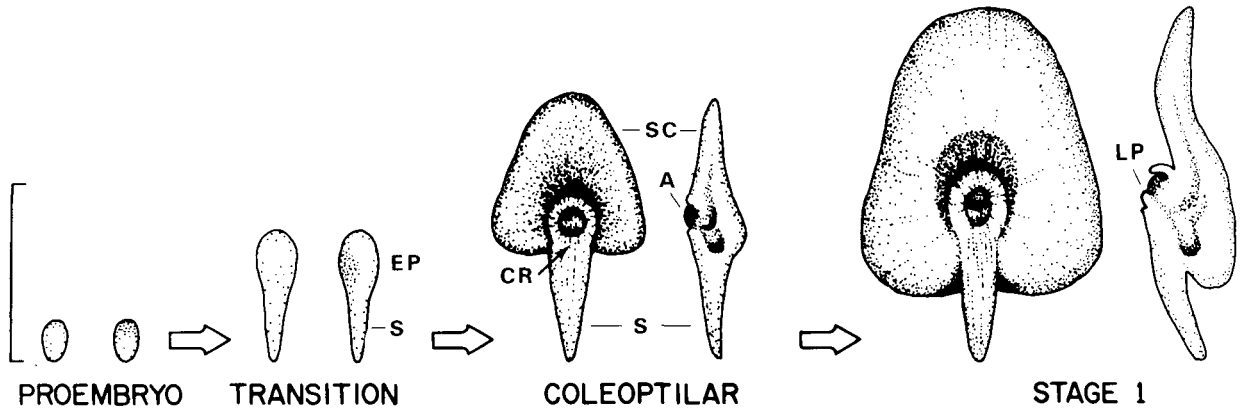

Figure 5. Early Stages of Embryogenesis in Maize.

Each stage is represented by a face view (left) and a longitudinal section (right) with the embryo face at left. The transition embryo contains an embryo proper (EP) and suspensor (S). The scutellum (SC), shoot apex (A), suspensor (S), and coleoptilar ring (CR) are visible at the coleoptilar stage. The first leaf primordium (LP) appears at the base of the shoot apex at stage 1. Bar = 0.5 mm. Figure adapted from Clark and Sheridan (1986). See also Sheridan and Clark (1987).

development in maize (Coe et al., 1988) and barley (Bosnes et al., 1987). Tester lines of maize carrying B-A chromosomal translocations have been particularly useful in demonstrating which mutants are defective only in endosperm development (Neuffer and Sheridan, 1980). A wrinkled seed mutant of pea defective in starch-branching enzyme (Bhattacharyya et al., 1990) and mutants of soybean with altered levels of abundant seed proteins (Goldberg et al., 1989) have also been described. Albino and pale-green mutants identified following seed mutagenesis in Arabidopsis (Müller, 1963; Feldmann, 1991; Jürgens et al., 1991) can be viewed as embryonic mutants because wild-type embryos remain green before maturation. Fusca mutants that accumulate anthocyanin during embryogenesis and become lethal at germination have also been described in Arabidopsis (Weiland and Müller, 1972). The final class of mutants defective in seed dormancy includes viviparous mutants of maize (McCarty et al., 1989) and hormone mutants of Arabidopsis (Finkelstein et al., 1988).

A wide range of interesting phenotypes has been identified among Arabidopsis mutants defective in embryo development. Jürgens et al. (1991) have divided their pattern mutants into four classes: (1) pattern deletions that lack specific regions of the mature embryo, (2) deletions associated with duplications of embryonic structures, (3) multiplication of cotyledons, and (4) transformation of cotyledons into shoots. The first class is further divided into phenotypic groups that differ with respect to deletion pattern. Included among this class is the *gnom* mutant, which results in deletion of the root apical meristem. The second class includes a particularly interesting mutant known as *doppelwurzel*, in which the apical portion of the embryo, including the shoot meristem, is deleted and replaced with a mirror image of the basal portion. The resulting seedling is well differentiated but produces roots from both apices. Genetic studies indicate that after screening 44,000 M_2 families on plates and identifying 250 pattern mutants representing perhaps 40 to 50 different genes, Jürgens et al. (1991) may have saturated for putative pattern mutants of Arabidopsis. Further analysis of these pattern mutants should provide dramatic insights into genetic control of plant development.

Interesting phenotypes have also been identified among embryonic lethals and defectives of Arabidopsis (Meinke, 1991b). These include the presence of fused cotyledons, twin embryos, abnormally large suspensors, green blimps, distorted epidermal layers, single cotyledons, enlarged shoot apices, split hypocotyls, embryos with altered patterns of symmetry, bloated embryos with giant vacuolated cells, reduced hypocotyls that fail to produce roots, and embryos that protrude through the seed coat late in maturation. A female gametophytic factor that results in 50% aborted seeds regardless of pollen genotype has also been identified. These mutants may be used to study the establishment and maintenance of symmetry during embryo development, the relationship between cell size and embryogenesis, the genetic control of polyembryony, the fusion of plant tissues during development, the regulation of cell division patterns in the dermatogen, the control of apical development during embryogenesis, the relationship between embryo development and seed maturation, and the nature of interactions between the developing embryo proper and suspensor.

Two approaches to mutant isolation are likely to become more widespread in the future: transposon tagging in maize (Chandlee, 1991) and T-DNA tagging in Arabidopsis

(Feldmann, 1991). Over 50 *emb* mutants of maize have recently been isolated from lines containing Robertson's *Mutator* (Clark and Sheridan, 1991). Other collections of putatively tagged *dek* mutants are being analyzed by Donald Robertson (Iowa State), Mary Tierney (Ohio State), and Carol Rivin (Oregon State). Screening of 6000 transgenic Arabidopsis families produced at Du Pont after Agrobacterium-mediated seed transformation has yielded at least 100 additional mutants defective in embryo development. Analysis of these mutants is being performed in my laboratory at Oklahoma State University and in the laboratories of Robert Goldberg, Robert Fischer, and John Harada at the University of California. Populations of transgenic Arabidopsis plants generated through other methods (Koncz et al., 1989, 1990; Van Lijsebettens et al., 1991) may also yield tagged embryonic mutants. Mutagenesis with diepoxybutane and molecular cloning of deletion mutants through genomic subtraction represents an approach to gene isolation that may become more widespread in the future (Straus and Ausubel, 1990).

Two questions will need to be addressed as embryonic mutants become more abundant: (1) which mutants should be examined in detail? and (2) which mutants are defective in the same gene? The first question may be addressed from opposite directions. Some tagged mutants will be chosen simply because their pattern of insertion facilitates gene cloning. Others will be selected on the basis of interesting phenotypes, sequence similarities with known genes, or existence of multiple alleles. The second question is not trivial in cases where large numbers of target genes are involved. Over 10,000 pairwise crosses would be required to complete allelism tests among the 150 embryonic mutants currently maintained in my laboratory. This number could be reduced if crosses were limited to mutants with similar phenotypes, but alleles with different patterns of abnormal development would be missed. The alternative approach is to map the chromosomal locations of mutant genes and limit complementation tests to genes that map to similar regions. Sixteen genes essential for embryo development in Arabidopsis have already been mapped relative to visible markers (Patton et al., 1991). Another 75 lines including T-DNA insertional mutants are currently being mapped in my laboratory. Many *dek* and *emb* loci of maize have also been localized to chromosome arms (Neuffer and Sheridan, 1980; Clark and Sheridan, 1991; Scanlon et al., 1991).

MOLECULAR ANALYSIS OF EMBRYONIC MUTANTS

Detailed analysis of embryonic mutants will require molecular isolation of the defective genes. The most promising approach with maize involves transposon tagging (Sheridan, 1988). This approach has already been used to isolate genes with important functions (Chandlee, 1991) and should be applicable to the analysis of embryonic mutants (Clark and Sheridan, 1991). Several methods of gene isolation are currently being employed with Arabidopsis: (1) chromosome walking from adjacent restriction fragment length polymorphism markers, (2) insertional mutagenesis with T-DNA or maize transposons followed by recovery of flanking plant sequences, and (3) deletion mutagenesis and subsequent gene isolation through genomic subtraction. T-DNA insertional mutagenesis offers several advantages for the near future. Numerous mutants have already been isolated following Agrobacterium-mediated seed transformation (Errampalli et al., 1991; Feldmann, 1991; R. B. Goldberg, personal communication), and additional families are being generated that should contain new mutations. Flanking plant sequences can be isolated through either plasmid rescue (Yanofsky et al., 1990), inverse polymerase chain reaction (Earp et al., 1990), or screening of genomic libraries. The primary disadvantages of seed transformation are that approximately half of the embryonic mutants identified to date are not tagged, and most have duplications and rearrangements of T-DNA that complicate molecular analysis. Transposon tagging could become a valuable alternative to seed transformation if maize elements transpose at high frequency to linked chromosomal sites in Arabidopsis. Chromosome walking is likely to remain tedious for several years but should become the method of choice once a physical map of the genome is completed.

Molecular complementation of embryonic lethals presents a special challenge because homozygous mutant tissues are not readily available. This problem may be circumvented if multiple alleles are isolated and shown to differ in nucleotide sequence. An alternative approach might be to transform heterozygotes and screen for a distortion of segregation ratios in the next generation. The disadvantage in this case might be the limited number of seeds produced by primary transformants. The best approach may be to introduce the putative wild-type allele into wild-type plants through transformation, cross F_1 plants carrying this inserted gene with plants heterozygous for the embryonic mutation, and test for a reduction in the percent mutant seeds produced after self-pollination. The frequency of defective seeds in rescued transformants should be reduced from 25% to 6% if the introduced wild-type gene is unlinked to the mutant locus and restores normal function to homozygous mutant embryos. Another advantage to this strategy is that any wild-type ecotype can be chosen for transformation.

FUTURE DIRECTIONS

Research with mutants defective in plant embryogenesis has reached a critical transition point. Previous studies with maize and Arabidopsis have clearly demonstrated

that a wide range of mutants can be isolated. The challenge now is to demonstrate the value of these mutants to plant biology. The most significant long-term contribution will be the identification and molecular isolation of a large number of essential genes in higher plants. These genes are likely to play critical roles in cell division, metabolism, and differentiation.

The immediate concern is how specific mutants can provide information relevant to plant embryo development. The presence of abnormal suspensors in embryonic mutants of Arabidopsis illustrates how lethality can be informative. The suspensor is known to play an active role in regulating development of the embryo proper during early stages of plant embryogenesis (Yeung, 1980). Much less attention has been placed on the role of the embryo proper in regulating development of the suspensor. Several studies have shown that destruction of the embryo proper at early stages of development is often followed by abnormal growth of the suspensor (Raghavan, 1976). One model proposed to explain this phenomenon states that continued growth of the suspensor is normally inhibited by the embryo proper, and that the developmental potential of cells in the suspensor is revealed when this inhibitory effect is removed through a mutation lethal only to the embryo proper (Marsden and Meinke, 1985). This model is consistent with the relatively high frequency of mutants found with abnormal suspensors. It also suggests that variations in suspensor morphology found in angiosperms may be caused in part by different levels of inhibition by the embryo proper. This model can be tested at a molecular level now that several T-DNA insertional mutants of Arabidopsis have been shown to produce abnormal suspensors (Errampalli et al., 1991).

Embryonic mutants with a nonrandom distribution of aborted seeds in heterozygous siliques have been equally valuable in providing information on the extent of overlap between gametophytic and sporophytic gene expression in higher plants (Meinke, 1982). Similar patterns of gene expression have been described in maize (Clark and Sheridan, 1988; Ottaviano et al., 1988). The hypothesis that *dek* mutants represent null alleles of quantitative trait loci in maize (Lee et al., 1991) is being tested by comparing map locations of *Mutator*-induced *dek* mutants and known quantitative trait loci (Scanlon et al., 1991). Molecular isolation of embryonic genes from maize and Arabidopsis will provide access to a valuable collection of promoters active at different stages of embryo development. Suicide genes fused to these promoters could be used to examine the developmental consequences of selective cell death at different stages of embryogenesis. This represents a powerful new approach to experimental plant embryology. These promoters could also be used to change the timing of accumulation of major storage materials, introduce novel gene products into specialized cell types, or eliminate unwanted seeds during fruit production. Expression of essential genes during somatic and zygotic embryogenesis could be compared by inducing mutant cells to undergo embryogenesis in culture and determining whether defects occur at the same stage of development. Embryonic mutants might also be used to identify genes responsible for embryo abortion in interspecific hybrids (Brink and Cooper, 1947) and spontaneous embryo abortion in nature (Wiens et al., 1987, 1989).

Continued advances in related disciplines will be required to complement genetic approaches to plant embryogenesis. Recent studies on the microtubular cytoskeleton in Arabidopsis (Webb and Gunning, 1991) and double fertilization in a nonflowering seed plant (Friedman, 1990) serve as reminders that microscopy will continue to play an important role in plant embryology. The potential application of confocal light microscopy to this field remains to be explored. Experimental manipulation of somatic embryos (Schiavone and Racusen, 1990), in vitro fertilization of ovules (Zenkteler, 1990), and growth of immature seeds in culture (Raghavan, 1986) are methods that should complement genetic analysis of embryonic mutants. In situ localization of mRNAs (Fernandez et al., 1991) and β-glucuronidase fusions with *cis*-acting elements (Benfey et al., 1990) may provide additional information on patterns of cellular differentiation. With this combination of genetic, experimental, and molecular approaches, it should be possible over the next decade to make dramatic advances in our understanding of plant embryology.

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REFERENCES

Benfey, P.N., Ren, L., and Chua, N.-H. (1990). Tissue-specific expression from CaMV 35S enhancer subdomains in early stages of plant development. EMBO J. **9,** 1677–1684.

Bhattacharyya, M.K., Smith, A.M., Ellis, T.H.N., Hedley, C., and Martin, C. (1990). The wrinkled-seed character of pea described by Mendel is caused by a transposon-like insertion in a gene encoding starch-branching enzyme. Cell **60,** 115–122.

Bosnes, M., Harris, E., Aigeltinger, L., and Olsen, O.A. (1987). Morphology and ultrastructure of 11 barley shrunken endosperm mutants. Theor. Appl. Genet. **74,** 177–187.

Bowman, J.L., Smyth, D.R., and Meyerowitz, E.M. (1991). Genetic interactions among floral homeotic genes of *Arabidopsis*. Development **112,** 1–20.

Brink, R.A., and Cooper, D.C. (1947). The endosperm in seed development. Bot. Rev. **13,** 423–541.

Chandlee, J.M. (1991). Analysis of developmentally interesting genes cloned from higher plants by insertional mutagenesis. Dev. Genet. **12,** 261–271.

Christianson, M.L. (1986). Fate map of the organizing shoot apex in *Gossypium*. Am. J. Bot. **73,** 947–958.

Clark, J.K., and Sheridan, W.F. (1986). Developmental profiles of the maize embryo-lethal mutants *dek22* and *dek23*. J. Hered. **77,** 83–92.

Clark, J.K., and Sheridan, W.F. (1988). Characterization of the two maize embryo-lethal defective kernel mutants rgh*-1210 and fl*-1253B: Effects on embryo and gametophyte development. Genetics **120,** 279–290.

Clark, J.K., and Sheridan, W.F. (1991). Isolation and characterization of 51 *embryo-specific* mutations of maize. Plant Cell **3,** 935–951.

Coe, E.H., Jr., Neuffer, M.G., and Hoisington, D.A. (1988). The genetics of corn. In Corn and Corn Improvement, 3rd ed., G.F. Sprague and J.W. Dudley, eds (Madison, WI: American Society of Agronomy), pp. 81–258.

Coen, E.S. (1991). The role of homeotic genes in flower development and evolution. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42,** 241–279.

Dolfini, S.F., and Sparvoli, F. (1988). Cytological characterization of the embryo-lethal mutant *dek-1* of maize. Protoplasma **144,** 142–148.

Earp, D.J., Lowe, B., and Baker, B. (1990). Amplification of genomic sequences flanking transposable elements in host and heterologous plants: A tool for transposon tagging and genome characterization. Nucl. Acids Res. **18,** 3271–3279.

Errampalli, D., Patton, D., Castle, L., Mickelson, L., Hansen, K., Schnall, J., Feldmann, K., and Meinke, D. (1991). Embryonic lethals and T-DNA insertional mutagenesis in *Arabidopsis*. Plant Cell **3,** 149–157.

Feldmann, K.A. (1991). T-DNA insertion mutagenesis in *Arabidopsis*: Mutational spectrum. Plant J. **1,** 71–82.

Felker, F.C., Peterson, D.M., and Nelson, O.E. (1987). Early grain development of the *seg2* maternal-effect shrunken endosperm mutant of barley. Can. J. Bot. **65,** 943–948.

Fernandez, D.E., Turner, F.R., and Crouch, M.L. (1991). In situ localization of storage protein mRNAs in developing meristems of *Brassica napus* embryos. Development **111,** 299–313.

Finkelstein, R., Estelle, M., Martinez-Zapater, J., and Somerville, C. (1988). *Arabidopsis* as a tool for the identification of genes involved in plant development. In Temporal and Spatial Regulation of Plant Genes, D.P.S. Verma and R.B. Goldberg, eds (New York: Springer-Verlag), pp 1–25.

Foster, A.S., and Gifford, E.M., Jr. (1974). Comparative Morphology of Vascular Plants, 2nd ed. (San Francisco: Freeman).

Franzmann, L., Patton, D.A., and Meinke, D.W. (1989). In vitro morphogenesis of arrested embryos from lethal mutants of *Arabidopsis thaliana*. Theor. Appl. Genet. **77,** 609–616.

Friedman, W.E. (1990). Double fertilization in *Ephedra*, a nonflowering seed plant: Its bearing on the origin of angiosperms. Science **247,** 951–954.

Goldberg, R.B., Barker, S.J., and Perez-Grau, L. (1989). Regulation of gene expression during plant embryogenesis. Cell **56,** 149–160.

Haughn, G.W., and Somerville, C.R. (1988). Genetic control of morphogenesis in *Arabidopsis*. Dev. Genet. **9,** 73–89.

Jegla, D.E., and Sussex, I.M. (1989). Cell lineage patterns in the shoot meristem of the sunflower embryo in the dry seed. Dev. Biol. **131,** 215–225.

Johansen, D.A. (1950). Plant Embryology. (Waltham, MA: Chronica Botanica).

Johri, B.M. (ed) (1984). Embryology of Angiosperms. (Berlin: Springer).

Jürgens, G., Mayer, U., Torres Ruiz, R.A., Berleth, T., and Miséra, S. (1991). Genetic analysis of pattern formation in the *Arabidopsis* embryo. In Molecular and Cellular Basis of Pattern Formation, K. Roberts, ed (Cambridge: Company of Biologists, Ltd.) pp. 27–38.

Koncz, C., Martini, N., Mayerhofer, R., Koncz-Kalman, Z., Korber, H., Redei, G.P., and Schell, J. (1989). High-frequency T-DNA mediated gene tagging in plants. Proc. Natl. Acad. Sci. USA **86,** 8467–8471.

Koncz, C., Mayerhofer, R., Koncz-Kalman, Z., Nawrath, C., Reiss, B., Redei, G.P., and Schell, J. (1990). Isolation of a gene encoding a novel chloroplast protein by T-DNA tagging in *Arabidopsis thaliana*. EMBO J. **9,** 1337–1346.

Lee, M., Veldboom, L., Scanlon, M., Myers, A., James, M., and Robertson, D. (1991). Mutator-tagged defective kernel mutants as putative QTL for kernel size. Maize Genet. Coop. Newsl. **65,** 10–11.

Lo Schiavo, F., Giuliano, G., and Sung, Z.R. (1988). Characterization of a temperature-sensitive carrot cell mutant impaired in somatic embryogenesis. Plant Sci. **54,** 157–164.

Lyndon, R.F. (1990). Plant Development. (London: Unwin Hyman).

Maheshwari, P. (1950). An Introduction to the Embryology of Angiosperms. (New York: McGraw-Hill).

Mansfield, S.G., and Briarty, L.G. (1991). Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. Can. J. Bot. **69,** 461–476.

Marsden, M.P.F., and Meinke, D.W. (1985). Abnormal development of the suspensor in an embryo-lethal mutant of *Arabidopsis thaliana*. Am. J. Bot. **72,** 1801–1812.

McCarty, D.R., Carson, C.B., Stinard, P.S., and Robertson, D.S. (1989). Molecular analysis of *viviparous-1*: An abscisic acid-insensitive mutant of maize. Plant Cell **1,** 523–532.

McDaniel, C.N., and Poethig, R.S. (1988). Cell-lineage patterns in the shoot apical meristem of the germinating maize embryo. Planta **175,** 13–22.

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

Meinke, D.W. (1985). Embryo-lethal mutants of *Arabidopsis thaliana*: Analysis of mutants with a wide range of lethal phases. Theor. Appl. Genet. **69,** 543–552.

Meinke, D.W. (1986). Embryo-lethal mutants and the study of plant embryo development. Oxford Surv. Plant Mol. Cell. Biol. **3,** 122–165.

Meinke, D.W. (1991a). Genetic analysis of plant development. In Plant Physiology: A Treatise, Vol. 10, Growth and Development, F.C. Steward and R.G.S. Bidwell, eds (New York: Academic Press), pp. 437–490.

Meinke, D.W. (1991b). Embryonic mutants of *Arabidopsis thaliana*. Dev. Genet., in press.

Meinke, D.W., and Sussex, I.M. (1979a). Embryo-lethal mutants of *Arabidopsis thaliana*: A model system for genetic analysis of plant embryo development. Dev. Biol. **72,** 50–61.

Meinke, D.W., and Sussex, I.M. (1979b). Isolation and characterization of six embryo-lethal mutants of *Arabidopsis thaliana*. Dev. Biol. **72,** 62–72.

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

Nagato, Y., Kitano, H., Kamijima, O., Kikuchi, S., and Satoh, H. (1989). Developmental mutants showing abnormal organ differentiation in rice embryos. Theor. Appl. Genet. **78,** 11–15.

Neuffer, M.G., and Sheridan, W.F. (1980). Defective kernel mutants of maize. I. Genetic and lethality studies. Genetics **95,** 929–944.

Ottaviano, E., and Mulcahy, D.L. (1989). Genetics of angiosperm pollen. Adv. Genet. **26,** 1–64.

Ottaviano, E., Petroni, D., and Pé, M.E. (1988). Gametophytic expression of genes controlling endosperm development in maize. Theor. Appl. Genet. **75,** 252–258.

Patton, D.A., Franzmann, L.H., and Meinke, D.W. (1991). Mapping genes essential for embryo development in *Arabidopsis thaliana*. Mol. Gen. Genet. **227,** 337–347.

Poethig, R.S. (1989). Genetic mosaics and cell lineage analysis in plants. Trends Genet. **5,** 273–277.

Poethig, R.S. (1990). Phase change and the regulation of shoot morphogenesis in plants. Science **250,** 923–930.

Poethig, R.S., Coe, E.H., Jr., and Johri, M.M. (1986). Cell lineage patterns in maize embryogenesis: A clonal analysis. Dev. Biol. **117,** 392–404.

Raghavan, V. (1976). Experimental Embryogenesis in Vascular Plants. (New York: Academic Press).

Raghavan, V. (1986). Embryogenesis in Angiosperms. (Cambridge: Cambridge University Press).

Russell, S.D. (1991). Isolation and characterization of sperm cells in flowering plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42,** 189–204.

Scanlon, M.J., James, M., Stinard, P.S., and Robertson, D.S. (1991). Genetic placement and mapping of Mutator-induced defective kernel mutants. Maize Genet. Coop. Newsl. **65,** 11.

Schiavone, F.M., and Racusen, R.H. (1990). Microsurgery reveals regional capabilities for pattern reestablishment in somatic carrot embryos. Dev. Biol. **141,** 211–219.

Schnall, J.A., Cooke, T.J., and Cress, D.E. (1988). Genetic analysis of somatic embryogenesis in carrot cell culture: Initial characterization of six classes of temperature-sensitive variants. Dev. Genet. **9,** 49–67.

Schneider, T., Dinkins, R., Robinson, K., Shellhammer, J., and Meinke, D.W. (1989). An embryo-lethal mutant of *Arabidopsis thaliana* is a biotin auxotroph. Dev. Biol. **131,** 161–167.

Schulz, R., and Jensen, W.A. (1968). *Capsella* embryogenesis: The egg, zygote, and young embryo. Am. J. Bot. **55,** 807–819.

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H., and Sommer, H. (1990). Genetic control of flower development by homeotic genes in *Antirrhinum majus*. Science **250,** 931–936.

Shellhammer, J., and Meinke, D. (1990). Arrested embryos from the *bio1* auxotroph of *Arabidopsis thaliana* contain reduced levels of biotin. Plant Physiol. **93,** 1162–1167.

Sheridan, W.F. (1988). Maize developmental genetics: Genes of morphogenesis. Annu. Rev. Genet. **22,** 353–385.

Sheridan, W.F., and Neuffer, M.G. (1982). Maize developmental mutants. J. Hered. **73,** 318–329.

Sheridan, W.F., and Clark, J.K. (1987). Maize embryogeny: A promising experimental system. Trends Genet. **3,** 3–6.

Steeves, T.A., and Sussex, I.M. (1989). Patterns in Plant Development, 2nd ed. (Cambridge: Cambridge University Press).

Straus, D., and Ausubel, F.M. (1990). Genomic subtraction for cloning DNA corresponding to deletion mutations. Proc. Natl. Acad. Sci. USA **87,** 1889–1893.

Tilney-Bassett, R.A.E. (1986). Plant Chimeras. (London: Arnold).

Van Lijsebettens, M., Vanderhaeghen, R., and Van Montagu, M. (1991). Insertional mutagenesis in *Arabidopsis thaliana*: Isolation of a T-DNA-linked mutation that alters leaf morphology. Theor. Appl. Genet. **81,** 277–284.

Wardlaw, C.W. (1955). Embryogenesis in Plants. (London: Methuen).

Webb, M.C., and Gunning, B.E.S. (1991). The microtubular cytoskeleton during development of the zygote, proembryo, and free-nuclear endosperm in *Arabidopsis thaliana* (L.) Heynh. Planta **184,** 187–195.

Weiland, U., and Müller, A.J. (1972). In-vitro-Kultur der Wurzeln von letalen *fusca*-Mutanten von *Arabidopsis thaliana*. Kulturpflanze **20,** 151–164.

Wiens, D., Calvin, C.L., Wilson, C.A., Davern, C.I., Frank, D., and Seavey, S.R. (1987). Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. Oecologia **71,** 501–509.

Wiens, D., Nickrent, D.L., Davern, C.I., Calvin, C.L., and Vivrette, N.J. (1989). Developmental failure and loss of reproductive capacity in the rare palaeoendemic shrub *Dedeckera eurekensis*. Nature **338,** 65–67.

Williams, E.G., and Maheswaran, G. (1986). Somatic embryogenesis: Factors influencing coordinated behaviour of cells as an embryogenic group. Ann. Bot. **57,** 443–462.

Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., and Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopsis* homeotic gene agamous resembles transcription factors. Nature **346,** 35–39.

Yeung, E.C. (1980). Embryogeny of *Phaseolus*: The role of the suspensor. Z. Pflanzenphysiol. **96,** 17–28.

Zenkteler, M. (1990). In-vitro fertilization and wide hybridization in higher plants. Crit. Rev. Plant Sci. **9,** 267–279.