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A *FUSCA* Gene of Arabidopsis Encodes a Novel Protein Essential for Plant Development

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Arabidopsis *fusca* mutants display striking purple coloration due to anthocyanin accumulation in their cotyledons. We describe six recessive *fusca* mutants isolated from Agrobacterium-transformed Arabidopsis families. These mutants first become defective during embryogenesis and exhibit limited seedling development. Double mutant constructs revealed that developmental defects were not simply a consequence of anthocyanin accumulation. *fusca* seedlings showed altered responses to several environmental and endogenous factors. Allelism tests established that three *fusca* loci are represented by mutants previously described as defective in light-regulated responses. To study the molecular basis of the *fusca* phenotype, we cloned the *FUS6* gene. *FUS6* encodes a novel protein that is hydrophilic, α-helical, and contains potential protein kinase C phosphorylation sites. The FUSCA proteins appear to act in a network of signal transduction pathways critical for plant development.

INTRODUCTION

Fusca is a Latin word that refers to a dark purple color. Arabidopsis *fusca* mutants are named for their purple seeds, which result from inappropriate accumulation of anthocyanin pigments in cotyledons of developing embryos. The *fusca* phenotype was first described by Müller (1963). Recessive *fusca* alleles have been recovered after chemical mutagenesis (Müller and Heidecker, 1968; Jürgens et al., 1991), x-irradiation (Müller, 1963), and Agrobacterium-mediated T-DNA insertional mutagenesis (Feldmann, 1991). Miséra (1993) has identified nine *fusca* (*fus*) complementation groups after screening for mutants at the seedling stage. A.J. Müller and U. Weiland (Fourth International Conference on Arabidopsis Research, June 2–5, 1990, Vienna, Austria) identified a total of 14 *fusca* loci by screening seeds in immature siliques. The *fusca* phenotype is therefore characteristic of a relatively small number of genes in Arabidopsis.

Anthocyanins are ubiquitous in the plant kingdom and are responsible for the red, blue, and purple colors of many plant organs (Harborne, 1988; Francis, 1989). The widespread occurrence of anthocyanins in nature indicates that they are not normally detrimental to plant development. Both environmental and developmental signals regulate anthocyanin production. Increased anthocyanin accumulation is often an indicator of nitrogen, phosphorus, or sulfur deficiency (Salisbury and Ross, 1992). Plant growth regulators such as gibberellic acid (GA) (Weiss et al., 1990), methyl jasmonate (Franceschi and Grimes, 1991), abscisic acid (ABA), and cytokinins can also enhance anthocyanin accumulation. Perhaps the best-characterized environmental signal that induces anthocyanin biosynthesis in plants is light (Mancinelli, 1985). Chalcone synthase (CHS) catalyzes the first committed step in the flavonoid biosynthetic pathway leading to anthocyanin. In many plant species, *CHS* is transcriptionally activated by UV-B and blue light (Kubasek et al., 1992), sucrose (Tsukaya et al., 1991), GA (Weiss et al., 1990), wounding, and fungal elicitors (Schmid et al., 1990; Wingender et al., 1990). In addition, *CHS* expression and subsequent anthocyanin biosynthesis occur in tissue- and developmental-specific patterns (Schmid et al., 1990; Fritze et al., 1991; Kubasek et al., 1992). Several of these factors can work together to effect anthocyanin biosynthesis.

Most *fusca* mutants complete embryogenesis and germinate but fail to develop into viable plants. The lethal nature of *fusca* mutations indicates that *FUSCA* genes are essential for critical developmental processes. The combination of anthocyanin overproduction and developmental arrest may be unique to Arabidopsis *fusca* mutants. Of the 250 embryo-defective mutants examined in our laboratory (Castle et al., 1993; Meinke, 1994), anthocyanin accumulation is characteristic only of *fusca* mutants and a homeotic *leafy-cotyledon* mutant with fundamental defects in embryo maturation (Meinke, 1992). Recessive mutations that lead to overproduction of anthocyanins in soybean seed coats (Nicholas et al., 1993) and intensification of pigment biosynthesis in maize (Franken et al., 1991) do not affect growth and development. The high pigment (*hp*) mutant of tomato has increased anthocyanin production and reduced height compared to the wild type, but it is fully viable (Adamse et al., 1989). Several of the constitutive photomorphogenic (*cop*) and deetiolated (*det*) mutants of Arabidopsis accumulate anthocyanin in leaves and grow as dwarfs with low fertility (Chory et al., 1989; Deng et al., 1991). The COP

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and DET proteins have been proposed to act as regulators of light-induced responses. Given the many factors that affect anthocyanin accumulation, it is likely that FUSCA proteins are involved in responses to several different signals, including light-regulated development.

We are interested in understanding genes with essential functions and regulatory roles in plant embryo development (Meinke, 1991; Castle and Meinke, 1993). *fusca* mutants are intriguing because defects that begin during embryogenesis affect development after germination. The effects of mutations at six *fusca* loci are described in this report. We showed that *det1*, *cop1*, and *cop9*, which have been described as defective in photomorphogenesis, are *fusca* mutants, as defined by Müller (1963). The embryonic phenotypes of these mutants have not been reported previously. We constructed double mutants to examine the relationship between anthocyanin biosynthesis and seedling lethality and performed germination tests to study the roles of environmental and endogenous factors in the *fusca* phenotype. Microscopy was used to evaluate morphological defects in mutant embryos. We cloned and sequenced the *FUS6* gene using two T-DNA–tagged alleles. *FUS6* encodes a novel polypeptide with no homology to known metabolic or regulatory proteins. Identification of a putative *FUS6* homolog in rice suggests that *fusca* genes may be conserved throughout the angiosperms. Our results are consistent with a model that FUSCA proteins function in a network of environmental and developmental signal transduction pathways that are established during embryogenesis and are essential for normal plant development.

RESULTS

Identification of *fusca* Mutants

We screened more than 5000 transgenic Arabidopsis families generated by Agrobacterium-mediated seed transformation (Feldmann and Marks, 1987; Feldmann, 1991) for embryo-defective mutants (Castle et al., 1993). Three families with *fusca* mutations were identified by the presence of 25% purple seeds in green siliques. Three additional *fusca* mutants were identified by K. Feldmann (University of Arizona, Tucson) during germination screens. These six mutants represent five *fusca* loci (*fus1*, *fus6*, *fus7*, *fus8*, and *fus9*). Complementation tests revealed that two alleles of *fus6* were included in our collection. Two *fusca* mutants described here (*fus1-1* and *fus7-1*) are identical to *cop1-5* (Deng et al., 1992) and *cop9-1* (Wei and Deng, 1992). The same seed stocks (Feldmann lines 5959 and 3612) were distributed to both laboratories. The *det1-1* mutant was obtained from J. Chory (The Salk Institute, San Diego, CA). Allelism tests conducted by S. Miséra (University of Munich, Germany) confirmed that *det1* is a member of the *fus2* complementation group (J. Chory, personal communication). The T-DNA–derived mutants were also placed into *fus* groups for us by S. Miséra. The different names given to the *fusca* mutants included in this study are summarized in Table 1. We have adopted the *fus* nomenclature but include other allele names for clarity and when referring to previously published results.

Table 1. Names Assigned to *fusca* Mutants Included in this Study

emb Nomenclature[a]	Map Position[b]	T-DNA Tagged	Feldmann Number[c]	Published Name[d]	*fus* Nomenclature[e]
emb78-1	3 (90)	yes	78	*emb78-1*	*fus6-1*
emb78-2	3 (90)	yes	4759	*emb78-2*	*fus6-2*
emb134	5	no	2817		*fus8-1*
emb143	4 (28)	yes	3612	*cop9-1*	*fus7-1*
emb144	3 (15)	no	4007		*fus9-1*
emb168	2 (55)	yes	5959	*cop1-5*	*fus1-1*
NA	4 (13)	NA	NA	*det1-1*	*fus2*

[a] Assigned by D.W. Meinke as described by Castle et al. (1993); NA, not applicable.

[b] Determined by mapping with visible markers as described in Castle et al. (1993); *det1* map position is as reported by Chory (1992). Chromosome numbers are outside parentheses and approximate map positions are within parentheses.

[c] Assigned by K.A. feldmann as described by Forsthoefel et al. (1992).

[d] References: *emb78-1* and *emb78-2*, Errampalli et al. (1991) and Castle et al. (1993); *cop9-1* Wei and Deng (1992); *cop1-5*, Deng et al. (1992); *det 1-1*, Chory et al. (1989) and Chory and Peto (1990).

[e] Nomenclature of A.J. Müller (Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany), S. Miséra, and G. Jürgens (University of Munich, Germany) for combined collections of *fusca* mutants. Classification of mutants was determined by complementation tests performed by S. Miséra.

The *fusca* Phenotype Is First Evident during Embryogenesis

The most prominent characteristic of *fusca* mutants is the accumulation of anthocyanins in cotyledons of immature embryos. As shown in Figure 1A, *fus6* mutant seeds can be readily distinguished from wild-type seeds in green siliques. Approximately 25% of the seeds produced by heterozygotes after selfing are mutant, as expected for a single recessive mutation. The other *fusca* mutants are similar, although they differ in hue and intensity of anthocyanins accumulated. Mutant embryos from each of the six lines identified following seed transformation are shown in Figure 1B. The pattern of anthocyanin deposition appears to be the same in all *fusca* mutants but is most easily seen with *fus1* (*cop1-5*), as shown in Figure 1C, because it is heavily pigmented. Anthocyanins become visible at the late heart to early torpedo stage at the boundary where the hypocotyl meets the cotyledons. As the embryo matures, pigments accumulate on the outer surfaces of the cotyledons and then on the inner surfaces and margins. Anthocyanins are localized mainly in the epidermis and persist through seed desiccation (Figure 1D).

fusca Mutants Display Different Developmental Potentials

Müller and Heidecker (1968) reported that most *fusca* mutants died before primary leaves formed but that some were capable of reaching maturity. To determine the developmental potential of our *fusca* mutants, we germinated mutant seeds on agar plates maintained under 16-hr light cycles. Wild-type and mutant seedlings at 24 hr and 2 weeks after germination are shown in Figure 2. Mutant seedlings were phenotypically similar immediately following germination (Figures 2A to 2H) but varied widely in the extent of subsequent development (Figures 2I to 2P). Some of these differences appear to be allele specific; others may reflect different functions of *FUSCA* genes in plant development.

Wild-type seeds germinated within 24 to 36 hr of exposure to light after cold treatment. Cotyledons expanded and greened within the next 24 hr (Figure 2A). Wild-type seedlings only transiently accumulated anthocyanin on cotyledon margins and the upper hypocotyl for a few days following germination. Leaves expanded in pairs forming rosettes, which by 2 weeks usually had six to eight leaves and measured ~2 cm across (Figure 2I). Roots were white even when exposed to light in sterile culture. A transition from vegetative to reproductive growth at 3 weeks resulted in bolting and flower formation. Anthocyanins were not visible in mature plants grown under moderate light intensities.

Mutant seeds generally required 3 days to germinate in culture. The most pronounced delay was observed with *fus1* (*cop1–5*) seeds, which often required several weeks. Only *fus9* and *det1* (*fus2*) germinated at the same time as the wild type.

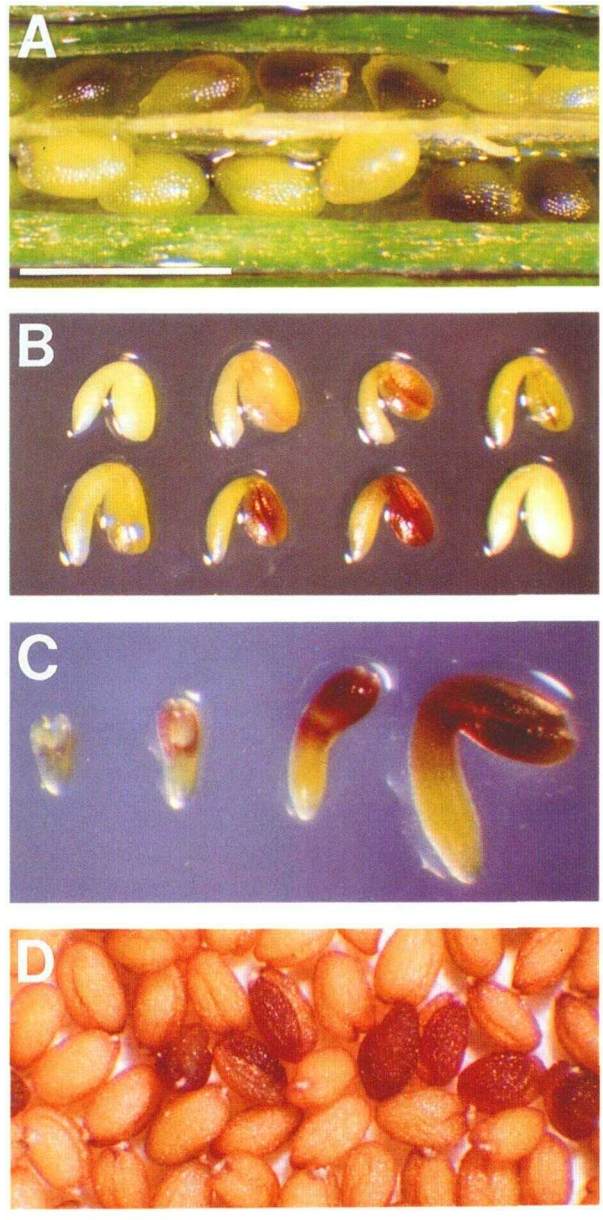


Figure 1. Embryo and Seed Phenotypes of *fusca* Mutants.

(A) Portion of an immature silique from a plant heterozygous for *fus6-1*. Mutant (purple) and wild-type (green) embryos are visible through the translucent seed coat.

(B) Homozygous mutant embryos dissected from immature seeds. Top: wild type, *fus6-1*, *fus8*, and *fus7* (*cop9-1*). Bottom: *fus9*, *fus6-2*, *fus1* (*cop1-5*), and wild type.

(C) Pattern of anthocyanin accumulation in *fus1* (*cop1-5*) embryos at different stages of development.

(D) Dry seed from a plant heterozygous for *fus6-2*. Anthocyanins in mutant seeds persist through desiccation.

Bar in **(A)** = 1 mm for **(A)**, **(B)**, and **(D)** and 0.5 mm for **(C)**.

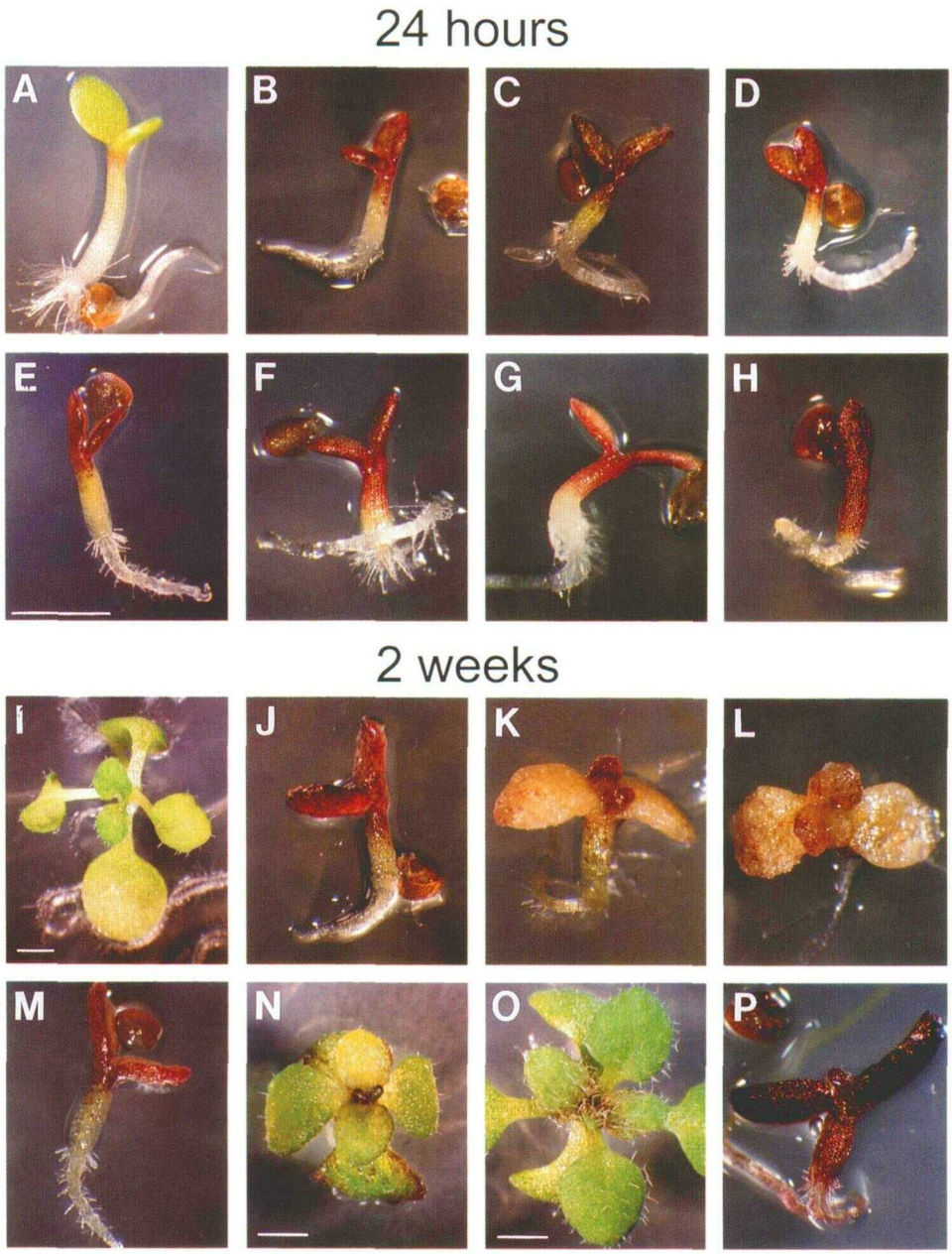


Figure 2. *fusca* Seedling Phenotypes 24 Hr and 2 Weeks after Germination.

(A) through **(H)** Representative seedlings at 24 hr after emergence from the seed coat.
(I) through **(P)** Mutant seedlings with the most advanced phenotypes at 2 weeks after germination.
(A) and **(I)** The wild type is shown.
(B) and **(J)** *fus6-1*.
(C) and **(K)** *fus6-2*.
(D) and **(L)** *fus7* (*cop9-1*).
(E) and **(M)** *fus8*.
(F) and **(N)** *fus9*.
(G) and **(O)** *det1* (*fus2*).
(H) and **(P)** *fus1* (*cop1-5*).
Note the differences in scale at 2 weeks. *fusca* seedlings are much smaller than wild-type seedlings. Bars = 1 mm. The bar in **(E)** applies to all unmarked panels.

Seedling development was severely affected in *fus6-1* (Figures 2B and 2J). At 24 hr after germination, *fus6-1* seedlings had open cotyledons, short roots, and showed some greening (Figure 2B); they rarely developed beyond this stage. The *fus6-2* mutation appeared to be less severe, in that seedlings often developed leaf primordia and had less anthocyanin in the cotyledons (Figures 2C and 2K). The *fus8* mutant (Figures 2E and 2M) was nearly identical to *fus6-1*. Mutant *fus7* (*cop9-1*) seedlings produced short roots and several small leaves before development arrested (Figures 2D and 2L). Seedlings of *fus1* (*cop1-5*) were heavily pigmented, with anthocyanin accumulation even in their roots (Figures 2H and 2P). Development arrested soon after initiation of primary leaves. Mutant *fus9* seedlings developed compact rosettes with no elongation of leaf petioles (Figure 2N). Anthocyanins accumulated on the lower surfaces of leaves and in leaf primordia. Trichomes were predominantly straight, whereas wild-type trichomes are usually branched (Figures 2I and 2N). Occasionally, *fus9* plants produced sterile flowers on stems that did not elongate above the rosette. Roots on *fus9* mutants were green, well developed, and became thick and purple as they aged. The *det1* (*fus2*) plants (Figures 2G and 2O) were dwarfs, showed anthocyanin accumulation on the lower surfaces of leaves, produced green roots, and formed fertile flowers. *fusca* mutants, therefore, exhibit a variety of defects in seedling development.

Accumulation of Anthocyanins Is Not the Cause of Developmental Arrest

To determine whether anthocyanin accumulation caused *fusca* seedlings to be defective, we constructed double mutants defective in anthocyanin biosynthesis. The anthocyanin biosynthetic enzymes in Arabidopsis are encoded by *transparent testa* (*TT*) loci (Sheahan and Rechnitz, 1993). Anthocyanin biosynthesis is not essential in Arabidopsis; *tt* plants develop normally (Koornneef, 1990). Plants with *tt3* mutations are deficient in 3-hydroxyflavanone reductase, which is specific to the anthocyanin branch of the flavonoid biosynthetic pathway (Sheahan and Rechnitz, 1993). Mutations in the *transparent testa, glabra* (*ttg*) locus cause a loss of both anthocyanins and trichomes (Koornneef, 1981). This gene appears to have a regulatory function analogous to the *R* locus involved in anthocyanin production in maize (Lloyd et al., 1992). Double mutant embryos produced by plants heterozygous for *fus6-1* and homozygous for either *tt3* or *ttg* lacked purple pigments, confirming that anthocyanins are indeed the pigments that accumulate in *fusca* mutants. Double mutant seedlings were yellow, failed to green, lacked roots, had small apical domes, and failed to develop after germination in culture. Similar results were obtained with *fus1* (*cop1-5*) *tt3* double mutants. Thus, *fusca* mutants have fundamental defects in development; anthocyanin accumulation is a secondary effect.

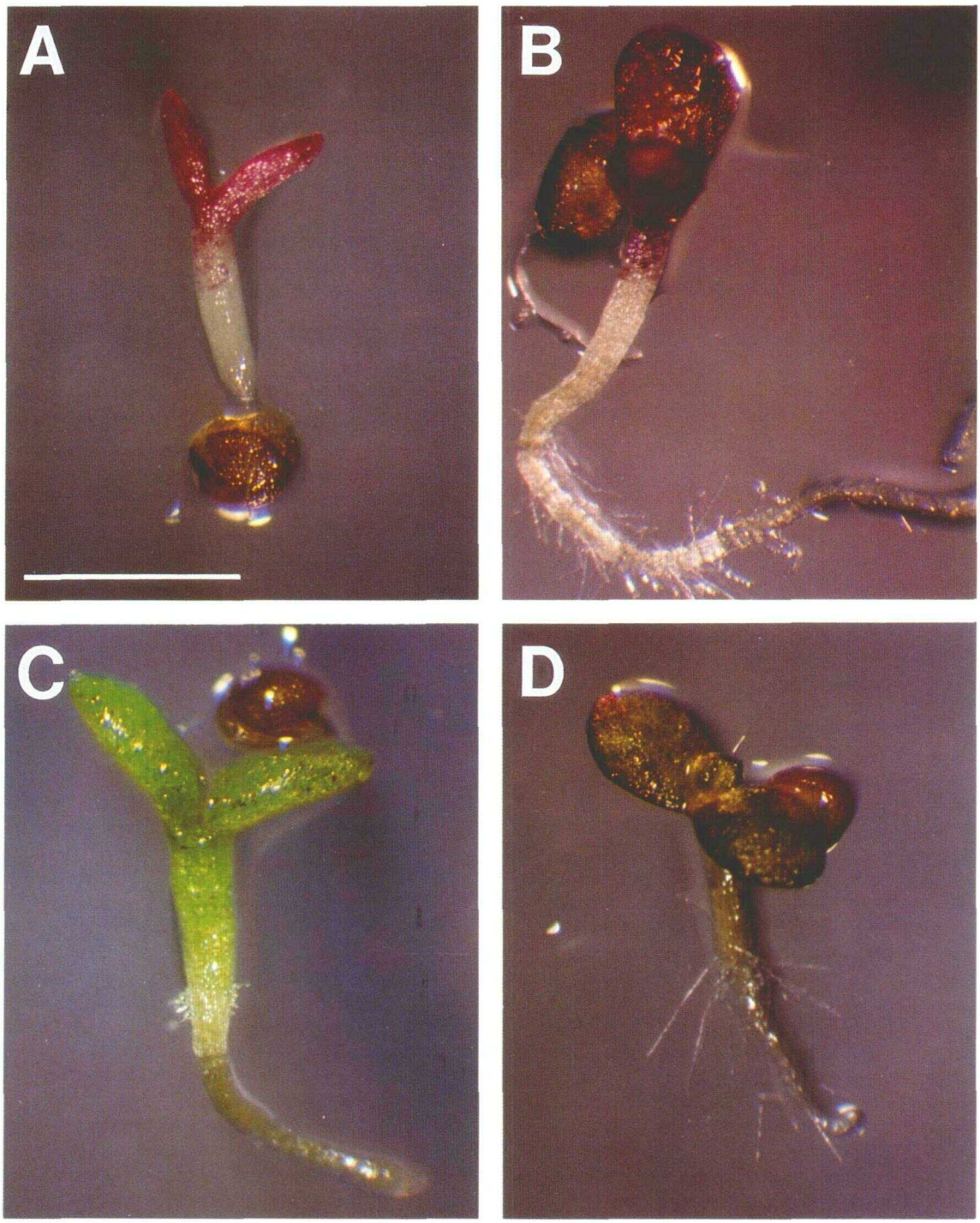
We also looked at *fusca*/albino (*alb*) double mutants to determine whether active photosynthesis contributed to anthocyanin overproduction. Anthocyanin accumulation was not altered by the lack of photosynthetic capability in double mutant embryos produced by plants heterozygous for *alb1* and either *fus6-1* or *fus1* (*cop1-5*). Embryos were white with purple anthocyanins localized in the same pattern as seen in single *fusca* mutants. Development of double mutant seedlings also arrested at the same stage as in single mutants.

Development of Mutant Seedlings Is Affected by Sugars and Phytohormones

Because anthocyanin biosynthesis is known to be triggered by several environmental and endogenous factors, we looked at the effects of sugars, nutrients, and phytohormones on *fusca* seedlings. To test the effects of sugars, mutant seeds were germinated on nutrient media supplemented with 3% sucrose, glucose, or sorbitol. Sorbitol served as a control for the effects due to osmotic potential, because it is not utilized as a carbon source by plants. A water–agar medium was used as a control for the effects of inorganic salts. The results showed that sugars affected root growth, anthocyanin intensity, greening, and shoot development. These effects were most pronounced in *fus6-1* seedlings, as shown in Figure 3.

Wild-type seedlings grew equally well on glucose and sucrose. Mutant seedlings, however, produced longer roots on sucrose than on glucose. Root growth in *fus6-1*, *fus6-2*, and *fus8* mutants was almost completely inhibited on glucose. Only a small callus at the base of the hypocotyl formed on these seedlings. Anthocyanin retention in all mutants was high on sucrose and glucose, suggesting that *CHS* was still sugar inducible. Mutant seedlings were significantly greener and anthocyanin levels were greatly reduced in the absence of a utilizable sugar. Seeds of *fus1* (*cop1-5*) that were slow to germinate on water and sorbitol plates emerged with almost no anthocyanins present. Apparently, the long imbibition period promoted breakdown of these pigments. One of the most intriguing observations was that several mutants, including *fus6*, showed more leaf development on water–agar plates than on nutrient media, suggesting that *fusca* seedlings may be nutritionally imbalanced.

Responses of mutants to phytohormones were evaluated by germinating mutant and wild-type seeds on standard media containing ABA, GA, cytokinin, or auxin. ABA normally inhibits germination of wild-type seeds in culture. We found that when wild-type seedlings did germinate in the presence of 3 μM ABA, they were developmentally impaired and accumulated anthocyanins. In fact, they were good phenocopies of severe *fusca* mutants. Furthermore, insensitivity to endogenous ABA caused by the ABA-insensitive allele *abi3-3* (Nambara et al., 1992) resulted in increased growth of *fus6-1* mutant seedlings. Double mutants produced by plants heterozygous for *fus6-1* and *abi3-3* produced roots that were several times longer than any produced by single *fus6-1* mutants. Growth of the shoot apex was also enhanced, although leaves



Figures 3. Effects of Sugars and Nutrients on *fus6-1* Seedling Growth.

(A) Dry seeds germinated on nutrient medium with glucose.
(B) Dry seeds germinated on nutrient medium with sucrose.
(C) Dry seeds germinated on nutrient medium with sorbitol.
(D) Dry seeds germinated on water–agar medium.
Seedlings were photographed 2 weeks after germination. Bar = 1 mm.

did not develop. Leaf development of *fus6-1* seedlings was significantly enhanced by the presence of cytokinin in the media. Several sets of small purple leaves with trichomes emerged from the normally inactive apex of mutant seedlings. The effect was most pronounced when curled cotyledon stage embryos dissected from immature green seeds were grown in the presence of cytokinin. Anthocyanin accumulation was also stimulated by cytokinin. Immature *fus6-1* embryos placed on shoot-inducing media with cytokinin and auxin produced an enlarged apical dome which then produced multiple abnormal shoots. The roots of these seedlings were stunted, thick, and green. Mutant *fus9* seedlings responded to GA with elongated hypocotyls and petioles, reduced anthocyanin in leaves and roots, and less chlorophyll in roots. These germination studies demonstrated that *fusca* mutants respond to several factors that induce anthocyanin biosynthesis in wild-type plants.

fusca Mutants Show Altered Responses to Light

In view of the fact that several *fusca* mutants have been described as photomorphogenic mutants (Chory et al., 1989; Deng et al., 1992; Wei and Deng, 1992), we looked at light-regulated responses of our mutants. Wild-type seedlings become etiolated when germinated in the dark: hypocotyls elongate, cotyledons remain small and unopened, and leaves fail to develop. All of the *fusca* mutants in our collection, including the photomorphogenic mutants, failed to show hypocotyl elongation and had open cotyledons when germinated in the dark. Dark-grown seedlings of *fus6-1*, *fus8*, and *fus9* are shown in Figure 4. Anthocyanins were less prominent in dark-grown seedlings but biosynthesis did occur in new leaves.

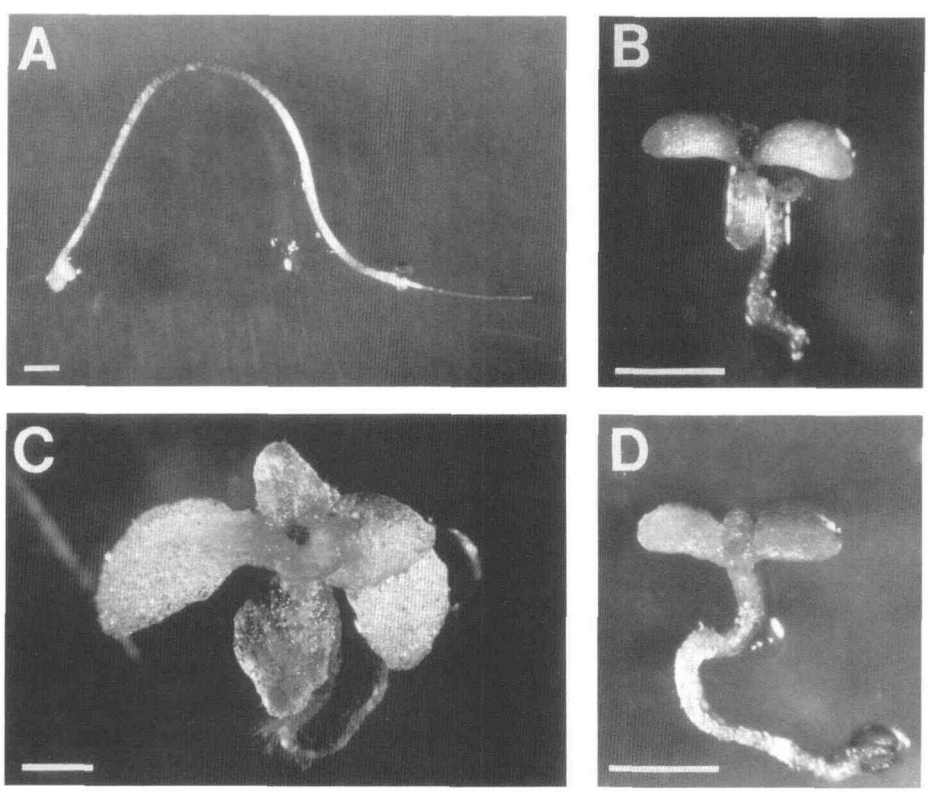


Figure 4. Response of *fusca* Seedlings Germinated in the Dark.

(A) Wild-type seedling showing typical etiolated response.
(B) *fus6-1* seedling germinated in the dark.
(C) *fus9* seedling germinated in the dark.
(D) *fus8* seedling germinated in the dark.
Representative plants were photographed after 2 weeks growth in the dark. All *fusca* mutants failed to etiolate. Bars = 1 mm.

A second aspect of the constitutive photomorphogenic phenotype is chloroplast development in the absence of light. We looked at chloroplast development in light- and dark-grown *fus6-1* mutant and wild-type seedlings, as shown in Figure 5. Wild-type seedlings germinated in the light developed crescent-shaped chloroplasts with stacked thylakoid membranes (Figure 5A). When germinated in the dark, wild-type cotyledons had etioplasts (Figure 5C) with typical prolamellar bodies and prothylakoid membranes (Hoober, 1984). Mutant *fus6-1* seedlings grown in the light had chloroplasts that were round and contained large starch grains (Figure 5B). In contrast to the photomorphogenic mutants, plastids of *fus6-1* seedlings germinated in the dark had prolamellar bodies and circular prothylakoid membranes (Figure 5D). Therefore, chloroplasts of mutant seedlings germinated in the dark clearly differed in morphology from those of wild-type seedlings grown in the light; they more closely resembled etioplasts of wild-type seedlings.

As a further exploration of the light-regulated properties of *fus6-1*, the expression pattern of light-induced nuclear genes was examined. The *det1*, *cop1*, and *cop9* mutants all show expression of light-induced genes in dark-grown plants (Chory et al., 1989; Deng et al., 1991; Wei and Deng, 1992). RNA from wild-type and *fus6-1* seedlings, grown either in continuous light or in the dark, was probed with *CHS*, small subunit of ribulose bisphosphate carboxylase (*RBCS*), and chlorophyll *a/b* binding protein (*CAB*) genes. Wild-type plants expressed all three of these genes in the light; etiolated seedlings did not express any of them (data not shown). *CHS* was expressed in both light- and dark-grown *fus6-1*, as expected because anthocyanin

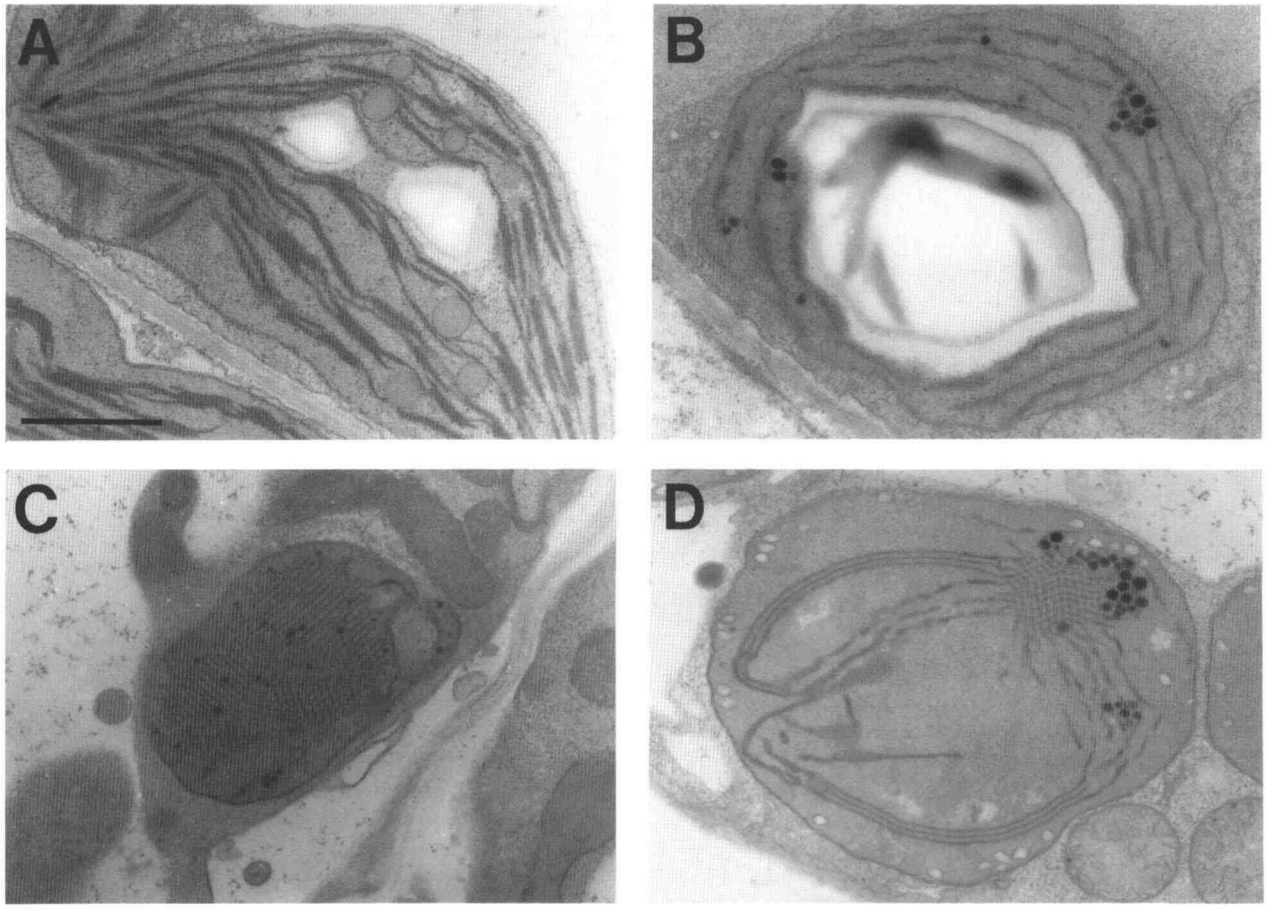


Figure 5. Plastid Development in Wild-Type and *fus6-1* Seedlings.

(A) Chloroplasts in light-grown wild-type cotyledons are crescent shaped and show stacked thylakoid membranes.
(B) Chloroplasts from light-grown *fus6-1* cotyledons are rounder and contain larger starch grains than wild-type chloroplasts.
(C) Wild-type seedlings grown in the dark contain etioplasts with prolamellar bodies and very little prothylakoid membrane.
(D) Etioplasts of *fus6-1* dark-grown seedlings have prolamellar bodies and prothylakoid membranes.
Plastids shown in **(A)**, **(B)**, and **(C)** were from seedlings germinated on sucrose medium. The plastid shown in **(D)** is from a *fus6-1* seedling germinated on water because mutant seeds did not germinate well on 3% sucrose in the dark. Bar = 1 μm.

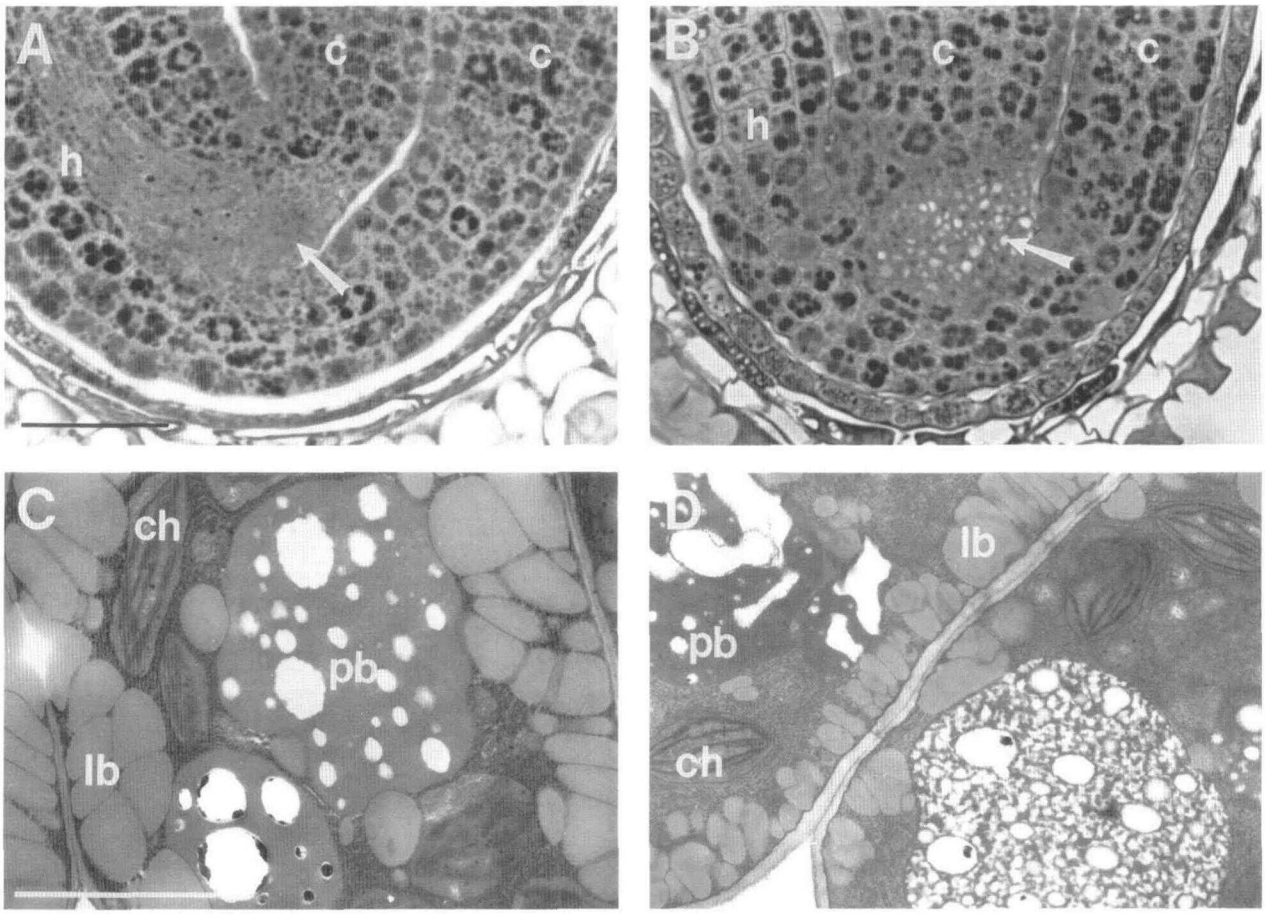


Figure 6. Morphological Characteristics of Wild-Type and *fus6-1* Mutant Embryos.

(A) and **(B)** Light micrographs of late cotyledon stage embryos. In contrast to wild-type embryos **(A)**, mutant *fus6-1* embryos **(B)** contained highly vacuolated cells in the shoot apical meristem (arrows). Abbreviations: c, cotyledon; h, hypocotyl.

(C) and **(D)** Transmission electron micrographs of embryonic cotyledons. Cotyledons of *fus6-1* embryos shown in **(D)** contained normal chloroplasts (ch) and numerous lipid bodies (lb) and protein bodies (pb) characteristic of wild-type embryonic cotyledons as shown in **(C)**.

Bar in **(A)** = 50 μm for **(A)** and **(B)**; bar in **(C)** = 10 μm for **(C)** and **(D)**.

biosynthesis was active. *CAB* message was not detected in either light- or dark-grown *fus6-1* seedlings. *RBCS* was expressed in both light- and dark-grown mutants but at much lower levels than in wild-type light-grown seedlings. Thus, although *fus6-1* shows inhibited hypocotyl growth in the dark, chloroplast development and gene regulation responses differ from those observed in the photomorphogenic mutants.

The Shoot Apical Meristem Is Defective in *fus6-1* Embryos

Mutant embryos were examined by light and transmission electron microscopy to determine whether *fusca* mutations interfered with cellular differentiation during embryogenesis. Emphasis was placed on *fus6-1* because seedling development in this mutant was severely disrupted. We found one intriguing morphological defect. As shown in Figure 6B, cells in the shoot apical meristem of *fus6-1* mutant embryos were highly vacuolated. The entire meristem appeared to be affected. Wild-type embryos (Figure 6A) contained densely cytoplasmic cells in this region. Root apical meristem cells did not show consistent differences from the wild type. The characteristic complement of protein and lipid bodies was present in cotyledons of mutant embryos (Figures 6C and 6D), indicating that other features of cellular differentiation were not disrupted. Chloroplasts also appeared normal in these green (and purple) embryos.

Cloning and Genetic Structure of *FUS6*

To understand the *fusca* phenotype at the molecular and biochemical levels, the *fusca* genes must be cloned and characterized; therefore, we cloned and sequenced *FUS6*. T-DNA insert structures in *fus6-1* and *fus6-2*, as well as plasmid rescue of the *fus6-1* locus, have been described previously (Castle et al., 1993). A fragment containing 1.1 kb of genomic DNA immediately adjacent to the T-DNA insert in *fus6-1* was used to probe an Arabidopsis Wassilewskija (WS) genomic library. A single genomic clone containing 17 kb and the complete *FUS6* locus was repeatedly isolated. Genomic fragments were used to probe a cDNA library made from mRNA isolated from immature siliques. Several cDNA clones were recovered. The longest clone was 1561 bp and appeared to be full length based on RNA gel blot analysis and sequence properties. Both the genomic and cDNA clones were sequenced.

The genetic structure of *FUS6*, with its 7 exons and 6 introns, is shown in Figure 7A. The nucleotide sequence of this region and the predicted amino acid sequence of the cDNA are given in Figure 7B. Exons ranged from 86 to 431 bp and introns ranged from 80 to 627 bp in length. The cDNA contained an open reading frame of 441 amino acid residues beginning with the first methionine residue. Tandem termination codons occurred in the sixth exon. The seventh exon was completely in the 3′ untranslated region. Two polyadenylation sites 41 bp apart were found in sequenced cDNAs. A concatamer of T-DNAs disrupts the first exon in *fus6-1*, strongly suggesting that this mutant represents the null phenotype. No *FUS6* message was detected in RNA prepared from *fus6-1* homozygous mutant seedlings. The insert in *fus6-2* consists of 1.8 kb of T-DNA containing part of the right border region and a portion of pBR322 (Castle et al., 1993).

We used the two T-DNA tagged *fus6* mutants to show that we had cloned the true *FUS6* locus. Extensive gel blot analysis with *FUS6*, T-DNA right border, and pBR322 probes revealed that the insert in *fus6-2* disrupted the 5′ region or first exon of the *FUS6* locus and may have resulted in rearrangements or deletions in this region. The gel blot shown in Figure 8 demonstrates how a *FUS6* probe revealed both wild-type and disrupted fragments in HindIII- and BamHI-digested DNA from both *fus6* mutants. Polymorphisms in the *FUS6* region of *fus6-2* were also seen with EcoRI, SalI, and PstI digests using the same genomic probe. Additional evidence that the T-DNAs tag the *FUS6* locus was provided by restriction fragment length polymorphism mapping. The λ library clone containing the *FUS6* gene mapped to the same genomic location as both *fus6* alleles (P. Dunn, University of Pennsylvania, personal communication).

FUS6 Encodes a Novel Protein Expressed in Light-Grown Plants

The FUS6 protein has a predicted molecular mass of 50.5 kD and a pI of 7.0. Hydropathy plots indicated that FUS6 is generally hydrophilic. Secondary structure analyses indicated that FUS6 is highly α-helical. Furthermore, putative signal and transit sequences were absent, suggesting a cytosolic localization. The predicted amino acid sequence showed no significant homology to sequences in the protein data bases. A match to the translation of a partial random cDNA sequence from rice callus was found in GenBank (accession number C2036A). Translation of the cDNA from rice revealed that it is homologous to the C-terminal 74 residues of FUS6. The rice polypeptide shows 74% identity and 93% similarity to FUS6 from Arabidopsis. At the nucleotide level, the sequences are 73% identical throughout most of the coding region, but diverge significantly in the 3′ untranslated region. Under moderately stringent conditions, a *FUS6* cDNA failed to hybridize to rice genomic DNA but did hybridize to several fragments in oilseed rape, suggesting that *FUS6* may be represented by a small gene family in other crucifers.

FUS6 may represent a new class of proteins essential for plant development. It has no DNA binding motifs, does not show homology to kinases or other regulatory proteins, and is unlike any known metabolic enzyme. Sequence searches for common motifs in FUS6 revealed several consensus protein kinase C (PKC) phosphorylation sites. One of these sites near the C terminus is surrounded by basic residues (RSARK), making it an especially strong candidate for PKC phosphorylation (Woodgett et al., 1986). In the N-terminal region, FUS6 contains a potential ATP/GTP binding site (AALYKGRT) with a conserved substitution of arginine for lysine. A single potential glycosylation site (NATF) and possible metal binding sites are present in FUS6 as well. The significance of these motifs in FUS6 remains to be determined.

Clues about protein function can often be ascertained by looking at gene expression patterns. To determine the expression pattern of *FUS6* in Arabidopsis, we hybridized a *FUS6* cDNA probe to total RNA from mature plants and young seedlings. The blot shown in Figure 9 revealed that leaves, flowers, immature siliques, and light-grown roots all expressed a *FUS6* transcript of ~1.6 kb. *FUS6* message was not detected in etiolated wild-type seedlings. Because mutant seeds produced defective seedlings when germinated in the dark, *FUS6* must carry out an essential function prior to germination.

DISCUSSION

Eukaryotic organisms must respond to a complex array of environmental and developmental signals with specific biochemical responses. Many components of signal transduction pathways in animal systems have been examined in detail. These include receptors, G-proteins, kinases, second messenger molecules, and transcription factors. One of the most well characterized pathways involves transduction of multiple growth signals from the environment, relayed through the G-protein RAS, to a downstream phosphorylation cascade and nuclear gene expression (reviewed by Marx, 1993). Many of the same signal transduction components described in animal systems are also found in plants (Boss and Morré, 1989;

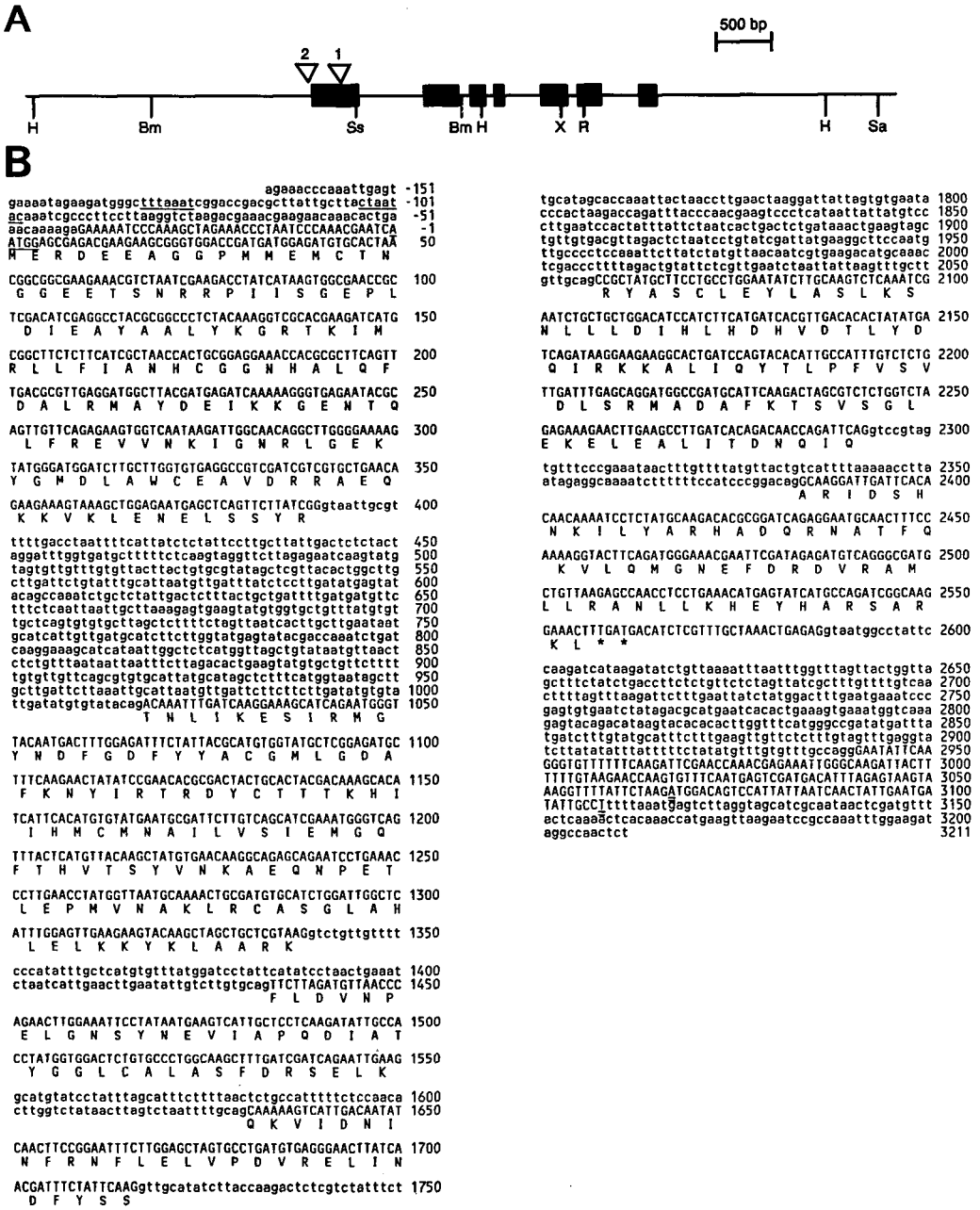


Figure 7. Structure and Sequence of the *FUS6* Locus.

(A) Restriction map and genetic structure of the *FUS6* genomic region. Solid boxes represent exons. The seventh exon is in the 3′ untranslated region. Transcription is from left to right. Triangles indicate the known T-DNA insertion site in *fus6-1* (1) and the estimated site for *fus6-2* (2). Restriction sites are H, HindIII; Bm, BamHI; Ss, SstI; X, XbaI; R, EcoRI; and Sa, SalI.

(B) Nucleotide sequence and predicted amino acid sequence of *FUS6*. Nucleotides corresponding to the cDNA are shown in capital letters. Numbering begins at A in the ATG of the proposed translation start site. Stars represent stop codons. Consensus TATA, transcriptional, and translational start sites are underlined (Joshi, 1987). Polyadenylation sites are double underlined. The entire sequence shown has been submitted to GenBank as accession number L26498.

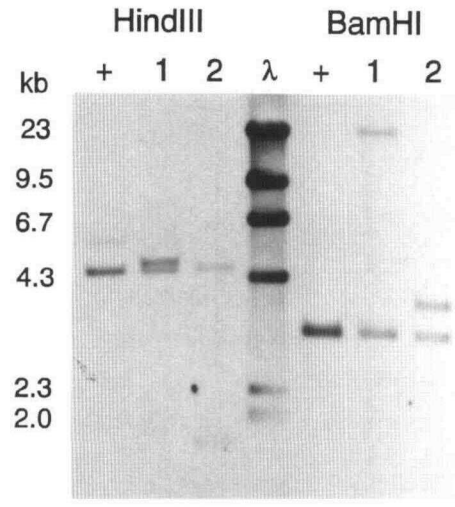


Figure 8. DNA Gel Blot Showing Disruption of the *FUS6* Locus.

DNA (2 μg) was digested with HindIII or BamHI, electrophoresed, blotted, and probed with a 0.9-kb SstI-BamHI fragment containing the first intron and second exon of *FUS6* (see Figure 7A). The lanes designated (+) contain DNA from wild-type plants; lanes 1, DNA from *fus6-1* heterozygotes; lanes 2, DNA from *fus6-2* heterozygotes. Lanes containing the wild-type DNA show a single band containing two copies of the genomic fragments at *FUS6*. Heterozygotes show a new band indicating T-DNA disruption of one of the wild-type fragments. Additional faint bands in HindIII lanes are due to incomplete digestion and were not seen on repeated gel blots. Both disrupted bands of *fus6-1* hybridized to a left border T-DNA probe. The small T-DNA fragment in *fus6-2* appears to have caused deletions or rearrangements resulting in HindIII and BamHI restriction sites near the T-DNA–plant junction (Castle et al., 1993). There are also internal HindIII and BamHI sites in the truncated insert. The *FUS6* probe detected fragments outside of the insert for the digests shown. Molecular size standards are HindIII-digested λ DNA.

Trewavas and Gilroy, 1991). The use of mutants with altered growth and development has allowed partial elucidation of transduction pathways involved in phytohormone action (Finkelstein and Somerville, 1990; Klee and Estelle, 1991; Kieber et al., 1993), environmental responses (Okada and Shimura, 1992), and light-induced development (Chory, 1992, 1993) in higher plants. Our observations that *fusca* mutants of Arabidopsis had altered responses to both endogenous factors (sugars and phytohormones) and environmental signals (nutrients and light) is consistent with a model that *FUSCA* gene products function as critical links in multiple signal transduction pathways with profound effects on plant growth and development.

The structures of *FUSCA* gene products suggest that these essential proteins have novel functions. The FUS6 protein described here appears to be cytosolic, may be phosphorylated, and shows no significant homology to other proteins in the data bases. The COP1 (FUS1) protein has an unusual combination of a zinc binding domain and a region similar to a domain found in both G-proteins (Deng et al., 1992) and a Drosophila transcription factor–associated protein (Dynlacht et al., 1993). Thus, COP1 (FUS1) may act in the nucleus at the level of gene regulation. A third *FUSCA* gene, *COP9* (*FUS7*), has been cloned, and it encodes a small protein distinct from both COP1 and FUS6 (X.-W. Deng, personal communication). Differences in protein structures and mutant phenotypes may reflect the varied roles that *FUSCA* genes play in signal transduction networks. FUSCA proteins may act sequentially, as in phosphorylation cascades, interactively as protein–protein complexes, or in different branches of overlapping pathways.

Our approach of looking at *fusca* mutants during embryogenesis and seed development revealed that *FUSCA* genes perform critical functions before germination. Many of the defects seen in mutant seedlings may result from aberrations initiated during embryo development. The first indication of abnormalities is the appearance of anthocyanins in developing mutant embryos. We have shown here through analysis of double mutants that anthocyanin production is a secondary effect of *fusca* mutations. Accumulation may be induced by cellular stress or by inappropriate interpretation of environmental and cellular signals. The only morphological abnormality found in *fus6-1* mutant embryos was vacuolization of cells in the shoot apical meristem, which correlated with a subsequent lack of shoot development after germination. Several shoot apical meristem mutants have been described in Arabidopsis, but none appears to show abnormal vacuolization or anthocyanin accumulation (Leyser and Furner, 1992;

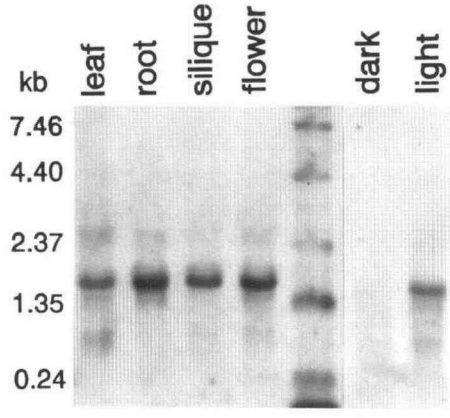


Figure 9. Expression Pattern of *FUS6* in Wild-Type Plants.

Total RNA was prepared from leaves, flowers, and siliques of wild-type plants grown in soil, roots grown in liquid culture, and light- and dark-grown sterile seedlings as described in Methods. RNA (20 μg) was electrophoresed through a 1.2% agarose gel, blotted onto nylon membrane, and hybridized with a full-length *FUS6* cDNA cloned fragment. The detected *FUS6* transcript is 1.6 kb. Molecular size standards are an RNA ladder (fifth lane; Gibco BRL) detected with labeled λ DNA.

Medford et al., 1992; Sung et al., 1992; Barton and Poethig, 1993). The primary function of FUSCA proteins may be to prepare embryos for growth after germination. Aside from desiccation tolerance, little is known about the mechanisms embryos employ to prepare for new conditions encountered outside of the seed. Preparation could involve setting up systems for responding to new environmental and developmental signals. This transduction network may not be necessary during early stages of embryogenesis, and this may explain why morphogenesis is not severely disrupted in *fusca* embryos.

fusca mutants show pleiotropic effects, suggesting that the *FUSCA* genes are needed for normal responses to phytohormones, carbohydrates, light, and developmental signals. Exposure to high levels of ABA in the light causes wild-type seedlings to phenocopy severe *fusca* mutants. Insensitivity to endogenous ABA in *fus6-1 abi3-3* double mutants resulted in significantly increased root development. These results suggest that *fus6* mutants may either produce too much ABA or may be overly sensitive to growth inhibition caused by this hormone. Partial release of meristematic growth in *fus6* mutants induced by applied cytokinin suggests that mutant seedlings either lack sufficient cytokinins for shoot development or are somewhat insensitive to endogenous cytokinins. In contrast, the deetiolated phenotype of dark-grown *det1* (*fus2*) mutants can be phenocopied by high cytokinins (Chory, 1993), suggesting that this *fusca* mutant may be more sensitive to endogenous phytohormone. The *fusca* mutants are unlike known phytohormone biosynthetic mutants (Klee and Estelle, 1991). Thus, it appears to be perception and response pathways that are affected in *fusca* mutants.

fusca mutations can also lead to interference of light-regulated responses. This has been established in previous reports where *cop1*, *cop9*, and *det1* were characterized as photomorphogenic mutants (Chory et al., 1989; Deng and Quail, 1992; Wei and Deng, 1992). All of the *fusca* mutants that we examined failed to etiolate when germinated in the dark. However, dark-grown *fus6-1* seedlings did not show light-independent chloroplast development or the same pattern of gene expression seen in *det1* and *cop* mutants. Because *fus6* mutants do not develop normally in the light, inhibition of hypocotyl elongation in the dark may reflect a more fundamental defect in growth that is established during embryo development. It should be noted that germination studies are generally conducted with seeds that have developed on plants grown in the light. Light signals and plant hormones control many of the same responses in seed and vegetative plant development (Funckes-Shippy and Levine, 1985; Chory, 1993). FUS6 appears to have a smaller role than DET1 in light transduction pathways and a different role in mediating responses to hormones. Furthermore, the *fus6-1* mutant described here appears to have a null phenotype based on the site of T-DNA insertion and the failure of mutant seedlings to develop leaves. In contrast, the *cop1* and *det1* mutants examined for light responses were able to grow into mature plants (Chory et al., 1989; Deng and Quail, 1992).

Partial gene or protein activity due to weak alleles may affect the extent to which responses are affected. Chory (1993) has proposed that DET1 protein acts as an intermediate, linking light and cytokinin transduction pathways. In addition, it appears to be involved in repression of cell-type–specific expression of genes and chloroplast development in the light (Chory and Peto, 1990). Deng et al. (1992) have proposed that COP1 functions as a light-inactivatable master switch that represses photomorphogenic processes in the dark. It is not clear from this model why *cop1* mutations are deleterious or lethal in the light. Our results suggested that, as FUSCA proteins, COP1 and DET1 may carry out their primary functions prior to seedling germination and light-responsive growth and that partial gene activity allows for growth of mutant plants as dwarfs.

Phenotypic descriptions of *fus6* mutants suggest that the *FUS6* gene product is an essential regulatory protein. *FUS6* is expressed in mature wild-type plants, suggesting that it has an essential role throughout plant development. A *FUS6* homolog expressed in rice callus indicates that the gene is present in monocots as well as dicots and may be widespread in the plant kingdom. The function of FUS6, however, is not easily inferred by homology to other proteins or structural motifs. The hydrophilic and α-helical properties of FUS6 suggest a cytosolic localization. One possible model is that FUS6 is regulated by PKC, perhaps as part of a cascade with other FUSCA proteins. PKC has been linked to cytokinin responses in barley (Selivankina et al., 1990; Pereverzeva and Selivankina, 1991) and tobacco (Dominov et al., 1992). FUS6 could be important in relaying plant hormone and other signals from membrane-associated PKC to cytoplasmic factors. This could lead directly to biochemical responses or to downstream gene regulation.

The initial model that *fusca* mutants have a primary defect in metabolic processes that results in excess precursors entering the anthocyanin biosynthetic pathway (Müller and Heidecker, 1968) is not supported by our results. The *FUS6* and *COP1* genes do not appear to encode metabolic enzymes. Nevertheless, the growth of *fusca* seedlings in culture is influenced by sugars and inorganic salts, suggesting that metabolic imbalances are associated with the *fusca* phenotype. Growth and development of these mutants are so severely disrupted that many cellular processes are likely to be disturbed. Our data support a model in which a small number of FUSCA proteins function as critical components in the transduction of several environmental and cellular signals during plant development.

METHODS

Arabidopsis Mutants

Six *Arabidopsis thaliana fusca* mutants were recovered following T-DNA insertional mutagenesis of Wassilewskija (WS) seeds (Feldmann and

Marks, 1987; Feldmann, 1991; Castle et al., 1993). Lines 578, 2817, and 4759 were identified during screening for embryo-defective mutants as described by Castle et al. (1993). Lines 3612, 4007, and 5959 were identified from germination tests by K. Feldmann and associates (University of Arizona, Tucson). Because they are lethal, the recessive *fusca* mutants were maintained as heterozygotes that produced 25% mutant seeds following self-pollination. T-DNA tagging status was determined by kanamycin ratio tests, nopaline analysis, and DNA gel blot analysis as described previously (Errampalli et al., 1991; Castle et al., 1993). Pairwise crosses were made between these *fusca* heterozygotes to determine complementation groups. S. Miséra (University of Munich, Germany) conducted complementation tests for the assignment of each of the mutants used in this study to *fus* groups. Homozygous *det1-1* seed was provided by J. Chory (The Salk Institute, San Diego, CA). The *det1-1* mutation was induced by ethyl methanesulfonate in the Columbia ecotype. Homozygous marker lines used for double mutant analysis (*alb1*, *lu tt3*, and *ttg yi*, in the Landsberg *erecta* background) were obtained from M. Koornneef (Agricultural University, Wageningen, The Netherlands). The abscisic acid (ABA)-insensitive mutant, *abi3-3* (Columbia ecotype), was obtained from P. McCourt (University of Toronto, Canada). All soil-grown plants were maintained in a growth room under 16-hr light cycles and watered daily with nutrients as described by Heath et al. (1986).

In Vitro Growth Conditions

Dry seed and green siliques were surface sterilized for 1 min in 95% EtOH, 6 min in 20% commercial bleach with 0.1% Tween-20, and washed several times with sterile water. Dry seeds and embryos dissected from immature green seeds were plated on a standard germination medium of Murashige and Skoog basal salts with Gamborg's B5 vitamins (Sigma), 1% sucrose, 0.5 g/L Mes, and 0.8% phytagar (Gibco BRL) at pH 5.7. Plates with dry seed were kept at 4°C in the dark for 3 to 7 days before being exposed to either 16-hr light cycles or continuous light for 24 to 36 hr followed by total darkness. Cold treatment was omitted when dissected embryos were plated. Sugar effects were tested with 3% sucrose, glucose, or sorbitol added to standard medium and on a simple water and phytagar medium. Phytohormones were added to standard medium after autoclaving at the following concentrations: ABA, 1 and 3 μM; indole-3-acetic acid, 2 μM; N^6-[2-*iso*-pentenyl]adenine, 4 μM; gibberellic acid (GA), 1 μM. Shoot-inducing media contained 0.1 mg/mL naphthaleneacetic acid and 1 mg/mL benzyladenine. Wild-type seedlings for RNA isolation were grown for 14 days on 3% glucose. Root tissue for RNA analysis was harvested from wild-type seedlings grown in 500-mL flasks for 3 weeks in 100 mL Gamborg's B5 medium with sucrose (Gibco BRL) at 80 rpm under continuous light.

Immature Silique cDNA Library Synthesis

Total RNA was prepared from immature siliques of mixed stages from WS Arabidopsis soil-grown plants by a modified guanidinium isothiocyanate method (Chirgwin et al., 1979; Kingston, 1990). Frozen silique tissue was ground with liquid nitrogen, suspended in 5 volumes of buffer (4 M guanidinium isothiocyanate, 25 mM sodium citrate, pH 7.0, 1.5% sarkosyl, 100 mM 2-mercaptoethanol, 0.8% Sigma antifoam A), homogenized for 1 min using a polytron, and centrifuged for 20 min at 15,000 rpm at 4°C. The supernatant was filtered through Miracloth (Calbiochem, San Diego, CA), layered over 2 volumes CsCl buffer (5.7 M CsCl, 100 mM EDTA, pH 7.0), and centrifuged for 24 hr at 25,000 rpm at 20°C in a SW40 rotor (Beckman, Fullerton, CA) or 14 hr at 40,000 rpm in a SW60 rotor (Beckman). The RNA pellet was washed with 70% EtOH, resuspended in water, and precipitated with sodium acetate and ethanol. Poly(A)$^+$ RNA was purified from total RNA using MagneSphere particles (Promega). First-strand cDNA was synthesized with oligo(dT) primers and SuperScript reverse transcriptase (Gibco BRL). Second-strand cDNA was synthesized by the method of Gübler and Hoffman (1983). The ends of the cDNA were made blunt with T4 DNA polymerase (Gibco BRL). After phenol/chloroform extraction and ethanol precipitation, the reaction mixture was treated with RNAse A and RNAse T1 (Sigma), phenol/chloroform extracted, and size selected (0.5 to 6 kb) by isolation from an agarose gel. EcoRI-NotI adapters (Promega) were added to the cDNA and phosphorylated with polynucleotide kinase (Gibco BRL). Excess adapters were removed with a spin column (Sephacryl S-400; Promega). The cDNA was ligated into EcoRI digested, dephosphorylated, λgt10 (Promega), packaged with Gigapack II Gold (Stratagene), and plated with C600hfl (Promega). The primary library contains 7.5 × 10^6 plaque-forming units with an average insert size of 0.96 kb.

Cloning and Sequencing of *FUS6*

The flanking region from *fus6-1* was recovered by plasmid rescue as described by Castle et al. (1993). A 1.6-kb probe from inside the T-DNA to the 3′ BamHI site in *FUS6* was used to probe a λGem11 genomic library (Du Pont Experimental Station, Wilmington, DE) made from Arabidopsis WS DNA. A restriction map of the region was prepared and subclones in M13 were sequenced with Sequenase 2.0 (U.S. Biochemicals). Over 4 kb encompassing the entire *FUS6* gene was sequenced in both directions. The immature silique cDNA library was initially screened with the same 1.6-kb plasmid rescue clone and subsequently with a 2.2-kb BamHI-SstI fragment containing the first exon. The longest cDNA was sequenced in both directions by M13 subcloning and using synthetic oligonucleotides prepared by the DNA Core Facility (Oklahoma State University). Homology searches were done by electronic mail to the NCBI Blast server (Altschul et al., 1990). Protein sequence analysis was conducted with MacVector 4.0 and GCG programs (Devereux et al., 1984) at the Oklahoma State University DNA Core Facility.

RNA and DNA Gel Blot Analyses

Total RNA was prepared from wild-type leaves, flowers (buds and open), and siliques from soil-grown plants, liquid culture grown roots, and light- and dark-grown sterile seedlings as described for cDNA library construction. Total RNA (20 μg) was electrophoresed through 1.2% agarose with formaldehyde, as described by Selden (1987), with 100 ng/μL ethidium bromide in the samples. Gels were vacuum blotted onto Magna NT membrane (Micron Separations Inc., Westborough, MA) and UV cross-linked. Hybridization was conducted using a *FUS6* cDNA probe in 50% formamide, 2% blocking reagent (Genius; Boehringer Mannheim), 5 × SSC (1 × SSC is 0.15 M NaCl, 0.15 M sodium citrate, pH 7.0), 0.2% SDS, 0.1% sarkosyl, 0.1 mg/mL yeast tRNA at 42°C. The probe was random prime labeled with nonradioactive digoxigenin dUTP and detected with chemiluminescent substrate (LP530; Boehringer Mannheim). The same method was used for detection of chalcone synthase (*CHS*), small subunit of ribulose

bisphosphate carboxylase (*RBCS*), and chlorophyll *a/b* binding protein (*CAB*) expression in 5 to 10 μg total RNA prepared from wild-type and *fus6-1* seedlings germinated on standard medium in continuous light or dark. An RNA ladder (Gibco BRL) was used for size measurements.

Arabidopsis genomic DNA was prepared, digested, blotted, and detected as described previously (Errampalli et al., 1991; Castle et al., 1993). An SstI-BamHI 0.9-kb genomic *FUS6* fragment containing the first intron and the second exon was used to probe the DNA gel blot in Figure 8. Genomic DNA from other species was digested with HindIII and hybridized to a full-length *FUS6* cDNA probe.

Light and Electron Microscopy

Wild-type and light-grown *fus6-1* seedlings used for electron microscopy were germinated on a 3% sucrose medium. Dark-grown *fus6-1* seedlings were germinated on water–agar medium. Plates were maintained under 16-hr light cycles or continuous darkness. Cotyledons from 10-day-old seedlings were fixed for 2 hr in 2% glutaraldehyde, 0.1 M phosphate buffer at room temperature, and washed three times in phosphate buffer. Wild-type and homozygous mutant seeds were removed from immature siliques of soil-grown plants, punctured, and fixed for 4 hr. All tissues were postfixed in buffered 1% osmium tetroxide, dehydrated in a graded ethanol series, embedded in plastic resin, sectioned, and stained with uranyl acetate and lead citrate for transmission electron microscopy by the Electron Microscopy Laboratory at Oklahoma State University.

Mature green wild-type and mutant seeds from soil-grown plants heterozygous for *fus6-1* were prepared for light microscopy as described by Yeung (1984) and Yeung and Law (1987). Seeds were embedded in plastic resin, sectioned, stained with amido black and periodic acid/Schiff's reagent to show protein bodies and cell walls by E. Yeung (University of Calgary, Canada).

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