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RESEARCH ARTICLE

Phenotype of the Tomato *high pigment-2* Mutant Is Caused by a Mutation in the Tomato Homolog of *DEETIOLATED1*

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Tomato *high pigment* (*hp*) mutants are characterized by their exaggerated photoresponsiveness. Light-grown *hp* mutants display elevated levels of anthocyanins, are shorter and darker than wild-type plants, and have dark green immature fruits due to the overproduction of chlorophyll pigments. It has been proposed that *HP* genes encode negative regulators of phytochrome signal transduction. We have cloned the *HP-2* gene and found that it encodes the tomato homolog of the nuclear protein DEETIOLATED1 (DET1) from Arabidopsis. Mutations in *DET1* are known to result in constitutive deetiolation in darkness. In contrast to *det1* mutants, tomato *hp-2* mutants do not display any visible phenotypes in the dark but only very weak phenotypes, such as partial chloroplast development. Furthermore, whereas *det1* mutations are epistatic to mutations in phytochrome genes, analysis of similar double mutants in tomato showed that manifestation of the phenotype of the *hp-2* mutant is strictly dependent upon the presence of active phytochrome. Because only one *DET1* gene is likely to be present in each of the two species, our data suggest that the phytochrome signaling pathways in which the corresponding proteins function are regulated differently in Arabidopsis and tomato.

INTRODUCTION

Light is a critical environmental signal controlling many aspects of plant development. For example, dark-grown plants display a typical etiolated morphology with elongated hypocotyls, closed apical hooks, and unexpanded cotyledons, whereas plants grown in the light have short hypocotyls, opened apical hooks, and expanded photosynthetically active cotyledons. Light is perceived by a series of photoreceptors that can detect light within a wide spectral range. The phytochromes are the best characterized of these photoreceptors and are able to intercept light primarily within the red and far-red regions of the spectrum (Furuya and Schäfer, 1996). They exist as multigene families, for example, *PHYA* to *PHYE* in Arabidopsis, and each phytochrome is likely to have a specific photoperceptory function during plant development (Quail et al., 1995). In addition, plants contain blue/UV-A–absorbing cryptochromes and UV-B–absorbing photoreceptors.

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Several models for light signal transduction in plants have been proposed. One has been deduced largely by microinjection experiments with tomato and involves G proteins, calcium, and cGMP (Bowler et al., 1994b; Mustilli and Bowler, 1997). Others are based on the genetic analysis of Arabidopsis mutants, such as *deetiolated* (*det*) and *constitutively photomorphogenic* (*cop*), which display characteristics of light-grown plants when grown in complete darkness, for example, reduced hypocotyl length, cotyledon opening and expansion, chloroplast development, and expression of light-induced genes (Chory et al., 1989, 1996; Deng et al., 1991; Chamovitz and Deng, 1996).

Although several *COP* and *DET* genes have been identified, it is not clear how the activities of their gene products are regulated by phytochrome or by the calcium- and cGMP-dependent pathways identified by microinjection (Millar et al., 1994). One problem is that the *cop* and *det* mutants display their strongest phenotypes in the absence of light, and double mutant analyses with phytochrome-deficient mutants indicate that their phenotypes are independent of phytochrome function. Furthermore, many are allelic to seedling-lethal *fusca* mutants (Miséra et al., 1994). Therefore, it is possible that these molecules encode global regulators involved in many plant responses, not only those mediated by light. A more targeted genetic approach to identify components of signal transduction pathways specific for phytochrome could be to isolate mutants with altered

dynamics of light responses rather than mutants with constitutive phenotypes in the absence of light. Only recently have such mutants been described in Arabidopsis (e.g., *spa1* [Hoecker et al., 1998] and *psi2* [Genoud et al., 1998]). In contrast, several light-hypersensitive mutants have been described in tomato (*Solanum lycopersicum*) over the last decades, for example, *high pigment* (*hp-1* and *hp-2*), *atrovi-olacea* (*atv*), and *Intense pigmentation* (*Ip*) (Kendrick et al., 1994).

The monogenic recessive nonallelic *hp-1* and *hp-2* mutants do not display any obvious phenotypes in darkness but are characterized by their exaggerated light responsiveness. For example, they display higher anthocyanin levels, shorter hypocotyls, and more deeply pigmented fruits when compared with wild-type plants. This latter characteristic is due to elevated levels of flavonoids and carotenoids and has resulted in considerable interest from the tomato industry to use these mutations in commercial varieties. *hp-1* was discovered as a spontaneous mutant in 1917 at the Campbell Soup Company farms (Riverton, NJ) (Reynard, 1956) and has been the subject of most studies of *hp* mutants, whereas the *hp-2* mutant was reported in the Italian San Marzano variety in 1975 (Soressi, 1975). Despite some initial confusion, it is now clear that *hp-1* and *hp-2* mutations map to different chromosomes and therefore encode different proteins (Van Tuinen et al., 1997; Yen et al., 1997), even though their phenotypes appear to be identical. Two mutant alleles have been identified at each locus, *hp-1* and *hp-1^{w}*, and *hp-2* and *hp-2^{j}* (Kerckhoffs and Kendrick, 1997; Van Tuinen et al., 1997).

Phenotypes of *hp* mutants appear to be identical to those obtained by ectopic expression of *PHYA* in tomato (Boylan and Quail, 1989). Conversely, double mutant analysis of *hp-1* with PhyA- and PhyB-deficient tomato mutants has demonstrated that the *hp-1* mutation can amplify responses mediated by both phytochromes and that the amplification phenotype is critically dependent upon the presence of an active phytochrome (Peters et al., 1992; Kerckhoffs et al., 1997b). It appears, therefore, that the *hp* mutations affect fairly specifically the responses mediated by phytochrome. The fact that no phenotypic counterparts of *hp* mutants have been isolated thus far in Arabidopsis indicates that the cloning of the *HP* genes is likely to provide important new information about the regulation of photomorphogenesis, because it should identify molecules that act specifically as negative regulators of phytochrome signal transduction.

Furthermore, tomato has become an excellent model system for studying phytochrome function. The phytochrome gene family has been characterized (Pratt et al., 1997), and mutant studies are revealing that the individual tomato phytochromes have functions similar but not identical to their Arabidopsis homologs (Kendrick et al., 1997). Consequently, tomato is now a comparative experimental system to Arabidopsis for studying the roles of individual phytochrome members, and, in addition, tomato promises to reveal the roles of phytochrome in fruit ripening.

In this report, we have analyzed the phenotype of *hp-2* mutants and have cloned the *HP-2* gene. Surprisingly, *HP-2* encodes the tomato homolog of Arabidopsis DET1. Given the very different characteristics of the two mutants, we propose that the signal transduction pathways controlling photomorphogenesis may be wired differently in tomato and Arabidopsis.

RESULTS

Anthocyanin Pigment Production in *hp-2* Mutants

Although the phenotype of the *hp-2* mutant is generally considered to be identical to that of the *hp-1* mutant, it has not been examined in great detail. Therefore, we performed an analysis of *hp-2* mutant characteristics by using *hp-2* and *hp-2^{j}* mutant seedlings grown in the light and in the dark.

Consistent with the light-hypersensitive *hp-2* phenotype, both *hp-2* and *hp-2^{j}* seedlings displayed a light-dependent reduction in hypocotyl length and increase in anthocyanin content (Kerckhoffs et al., 1997a; Figures 1A and 1B). For both responses, the *hp-2^{j}* mutant appears to be the stronger of the two alleles.

To determine the tissue distribution of anthocyanin pigments in *hp-2* and *hp-2^{j}* mutants, we performed light microscopy with hand-cut sections prepared from a range of plant material. In hypocotyls of young *hp-2* seedlings, anthocyanin pigments were present specifically within subepidermal cells, as was previously reported for *hp-1* mutants (Von Wettstein-Knowles, 1968; Figure 2B). This cell specificity for anthocyanin biosynthesis also was found in wild-type seedlings, although in contrast to *hp-2* seedlings, anthocyanin accumulation was only transient and was diluted out after 3 to 4 days (Figure 2A).

In leaves of 3-week-old seedlings, anthocyanin accumulation also was localized to specific cell types in *hp-2* and *hp-2^{j}* mutants. Most notably, pigmentation was observed in the collenchyma and within the basal cells of trichomes (Figures 2D, 2F, and 2H). In wild-type plants grown under the same conditions, anthocyanin pigmentation was not evident (Figures 2C, 2E, and 2G). However, wild-type plants exposed to extreme conditions, such as high light in combination with low temperatures, displayed identical anthocyanin localization patterns (data not shown). This observation indicates that although anthocyanin pigments are overproduced significantly in *hp-2* and *hp-2^{j}* mutants, cell-type specificity nonetheless is not affected.

Chloroplast Biogenesis in *hp-2* Mutants

Immature fruits of *hp-2* and *hp-2^{j}* mutants display very high levels of chlorophyll pigments compared with wild-type

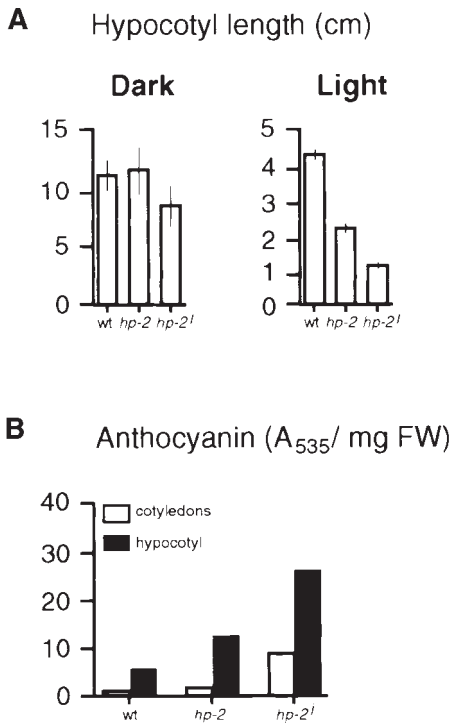

Figure 1. Changes in Hypocotyl Length and Anthocyanin Content in *hp-2* and *hp-2^j* Seedlings Are Light Dependent.

(A) Hypocotyl length (in centimeters) of wild-type (wt), *hp-2*, and *hp-2^j* tomato seedlings grown at 25°C for 5 days in absolute darkness (Dark) or in a 16-hr-light and 8-hr-dark photoperiod (Light). Error bars indicate ±SE.

(B) Anthocyanin content (per milligram fresh weight [FW]) from cotyledons and hypocotyl of wild-type (wt), *hp-2*, and *hp-2^j* light-grown tomato seedlings.

Values are the means of 10 seedlings, and the experiments were repeated three times.

fruits (Figures 2I and 2J). In *hp-1* mutants, this darker pigmentation appears to be due to a higher number of chloroplasts per cell (Yen et al., 1997). In the leaves of *hp-2* and *hp-2^j* mutants, chloroplast number did not appear to be affected; indeed, chlorophyll content was not higher than in wild-type leaves (data not shown). However, whereas roots of wild-type seedlings were white, the roots of *hp-2* and *hp-2^j* seedlings were green, indicating the presence of chloroplasts. Indeed, confocal imaging of root sections revealed chlorophyll fluorescence within the cortex and pericycle cells of *hp-2* and *hp-2^j* mutant roots but not in wild-type roots (Figures 3A and 3B).

Electron microscopic observations of wild-type roots revealed the presence of only a few small amyloplasts in most cell types (Figure 3C). By contrast, in *hp-2* and *hp-2^j* roots, cortical cells contained large numbers of plastids with very well-developed thylakoid membrane systems similar to those observed in leaves (Figure 3E), whereas plastids within pericycle cells were less developed and fewer in number (Figure 3D).

hp-2 Mutants Display Only Minor Dark Phenotypes

Neither *hp-1* nor *hp-2* mutants have been reported to display dark phenotypes, such as reduced hypocotyl length, opened apical hooks, or enlarged cotyledons, except for very small reductions in hypocotyl length in *hp-1^w* and *hp-2^j* seedlings that are not statistically significant (Kerckhoffs et al., 1997a; Figure 1A). Nonetheless, electron microscopic observations revealed the presence of partially developed chloroplasts in both *hp-2* and *hp-2^j* mutants (Figure 4), a well-known characteristic of Arabidopsis *cop* and *det* mutants (Chory et al., 1989; Deng et al., 1991). Prolamellar bodies were generally absent (*hp-2^j*) or reduced in size (*hp-2*), and prothylakoid membranes were apparent (particularly in *hp-2^j*). The presence within the same plastid of both prothylakoid membranes and prolamellar bodies has not been reported previously for Arabidopsis *cop* and *det* mutants and may be a result of hypersensitivity in the *hp* mutant background to the active phytochrome normally present in mature seeds.

Gene Expression Is Deregulated in the Light

To examine the effects of *hp-2* and *hp-2^j* mutations on gene expression, we performed RNA gel blot analysis of light-regulated gene expression by using *CHS* (encoding chalcone synthase) and *CAB* (encoding the chlorophyll *a*/*b* binding protein) gene fragments as probes. In agreement with the weak phenotypes of *hp-2* and *hp-2^j* seedlings grown in darkness, we did not observe any dramatic alteration of *CHS* and *CAB* gene expression. Nonetheless, *CHS* and *CAB* mRNA levels were slightly higher in plants carrying the *hp-2^j* allele when compared with *hp-2* and wild-type seedlings (Figure 5). Interestingly, *CHS* mRNA appeared to be of a slightly smaller size than that found in light-grown material, perhaps indicating differential light-dependent splicing.

Consistent with the exaggerated photoresponsiveness of *hp-2* and *hp-2^j* mutants, *CHS* mRNA levels were significantly enhanced compared with wild-type seedlings after light irradiation for 48 hr (Figure 5). *hp-2^j* seedlings contained higher levels of *CHS* transcripts than did *hp-2*, and *CHS* mRNA was particularly abundant in hypocotyls. Detailed time-course experiments indicated that *CHS* gene expression was both anticipated and stronger in *hp-2* and *hp-2^j* mutants compared with wild-type seedlings (A.C. Mustilli and C. Bowler, manuscript in preparation). In contrast to those of *CHS*, *CAB* mRNA levels were higher in cotyledons than in hypocotyls and were slightly lower in light-exposed

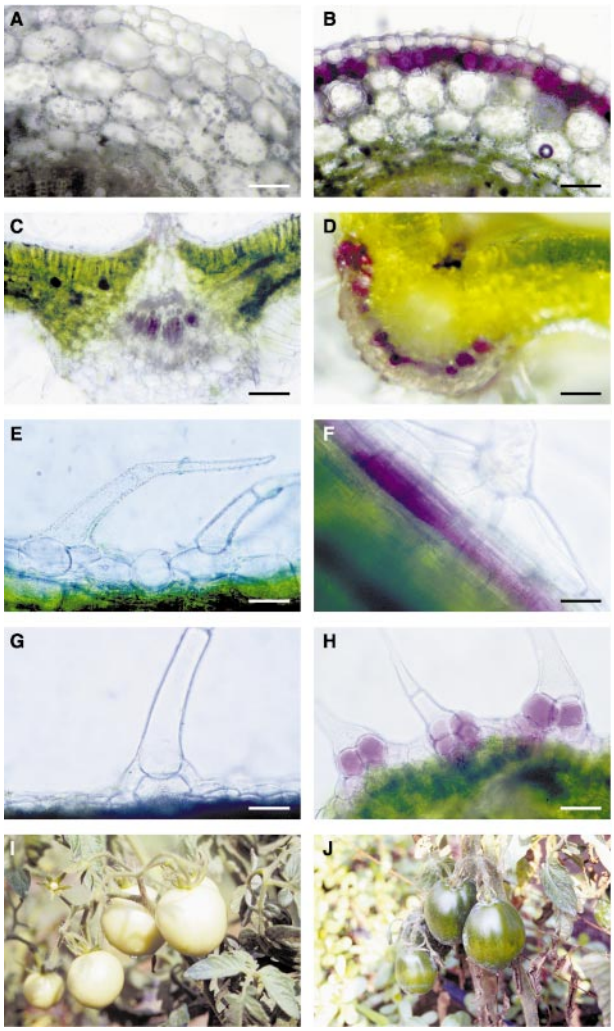

Figure 2. Phenotypes of *hp-2* Seedlings.

(A) and **(B)** Transverse sections of hypocotyls of wild-type **(A)** and *hp-2* **(B)** seedlings.
(C) and **(D)** Transverse sections of young leaves of wild-type **(C)** and *hp-2* **(D)** seedlings.
(E) and **(F)** Longitudinal sections through vascular tissue in leaves of wild-type **(E)** and *hp-2* **(F)** seedlings.
(G) and **(H)** Trichomes on the adaxial side of leaves of wild-type **(G)** and *hp-2* **(H)** seedlings.
(I) and **(J)** Immature fruits of wild-type **(I)** and *hp-2* **(J)** plants.
In *hp-2* seedlings, anthocyanin pigments are located within the subepidermal cells of hypocotyls **(B)** and within the collenchyma (**[D]** and **[F]**) and basal cells of trichomes **(H)** on the upper surface of leaves. Note that trichomes directly above the vasculature do not contain anthocyanin **(F)**. Hand-cut sections were derived from 3-week-old greenhouse-grown seedlings. *hp-2^j* mutants displayed the same phenotypes as did *hp-2* plants, but the *hp-2^j* mutant phenotypes were slightly more severe (data not shown). Bars in **(A)** to **(D)** = 100 μm; bars in **(E)** and **(F)** = 20 μm; bars in **(G)** and **(H)** = 50 μm.

hp-2 and *hp-2^j* mutant seedlings when compared with the wild type (Figure 5).

Phenotype of the Tomato *hp-2* Mutant Is Caused by Mutation in *DET1*

The *hp-2* mutation has been mapped previously using restriction fragment length polymorphism (RFLP) analyses of a segregating population derived from a cross between the *hp-2* mutant (*S. lycopersicum*) and *S. pennellii.* Mapping data with a second backcross (BC-2) population indicated a position close to the centromere of chromosome 1, within a cluster of several RFLP markers (Balint-Kurti et al., 1995; Broun and Tanksley, 1996; Van Tuinen et al., 1997). We discovered that the CT151 RFLP marker included in this cluster had a high degree of similarity to the Arabidopsis *DET1* gene, mutations in which were previously identified as causing the deetiolated phenotype of *det1* mutants (Pepper et al., 1994). Therefore, we examined whether the *hp-2* mutant phenotype was a result of mutation in the tomato *DET1* (*TDET1*) gene.

Comparison of CT151 with Arabidopsis *DET1* revealed that CT151 has homology to the 3′ end of *DET1.* We isolated a full-length cDNA encoding *TDET1* by using 5′ rapid amplification of cDNA ends (Loh et al., 1989). Subsequently, the *TDET1* gene was isolated from genomic libraries in λ DASH and λ FIXII. Alignment of the *TDET1* cDNA and genomic sequences revealed the presence of 10 introns, which is similar to the number found in Arabidopsis (Figure 6A). Nine introns are located in the same positions as those of the Arabidopsis *DET1* gene, whereas intron 2 of *TDET1* is not present in the Arabidopsis homolog (Figures 6A and 6B; Pepper et al., 1994). Comparative protein sequence analysis between Arabidopsis DET1 and TDET1 shows 81.3% similarity and 74.8% identity (Figure 6B). There are no major differences between the sequences, except for a small deletion of 16 amino acids at the center of *TDET1*, suggesting that the tomato and Arabidopsis genes are true homologs.

The function of DET1 in Arabidopsis is not known, although the protein is largely hydrophylic and contains a bipartite nuclear localization signal (NLS) (Figure 6B). As expected, DET1 is found predominantly in the nucleus in Arabidopsis cells (Pepper et al., 1994), and the high conservation of TDET1 within the NLS (Figure 6B) indicates that TDET1 is likely to be nuclear localized in tomato as well. Indeed, fusions of TDET1 with the green fluorescent protein (GFP) confirmed that the tomato protein also can mediate nuclear translocation (Figure 6C).

A *DET1* homolog is not present in the yeast genome or in any prokaryotic genome sequenced to date (data not shown). However, mouse and human expressed sequence tags with homology to *DET1* have been identified (Figure 6B), as well as a Drosophila homolog (S. Pimpinelli, personal communication).

TDET1-encoding cDNAs from the wild type and *hp-2* and *hp-2^j* mutants were amplified with specific primers from leaf mRNA (see Methods). In the *hp-2^j* TDET1 cDNA, we found a C-to-T mutation in exon 11 (nucleotide 1640), which gave rise to the substitution of a conserved proline for a serine residue in the C-terminal region of the protein (amino acid 498) (Figures 6A and 6B). In the *hp-2* mutant, we found an alternative splicing of intron 10, which led to a deletion of the first three amino acids (Gly, Pro, and Glu) of exon 11 within the second putative NLS (amino acids 478 to 480), presumably resulting in mislocalization of the protein (Figures 6A and 6B). To determine the site of the mutation that caused alternative splicing, we directly sequenced the 3′ end of the *TDET1* gene isolated from the *hp-2* mutant. We found an AG-to-TG substitution in the consensus 3′ splice junction of intron 10 (Figure 6D). These results demonstrate that the phenotype of the *hp-2* and *hp-2^j* mutants is caused by mutation in the *TDET1* gene.

Expression analysis of *TDET1* mRNA in wild-type seedlings revealed no major differences between dark- and light-grown material (Figure 6E and data not shown). Conversely, no major differences were observed in *TDET1* steady state mRNA levels between the wild type and the two mutants (data not shown). However, the presence of transcripts deriving from the mutated gene in the *hp-2* mutant could be readily detected in reverse transcriptase–polymerase chain reaction (RT-PCR) products because the mutation results in the generation of a PvuII restriction site (CAGCTG) at the junction between exons 10 and 11 (Figures 6D and 6E). RT-PCR and subsequent restriction enzyme digestion revealed that *TDET1* mRNA in the *hp-2* mutant was a mixture of mutated and wild-type transcripts (Figure 6E), indicating that at a reduced efficiency the mutated gene can be spliced normally and that this mutant is therefore not a null. RT-PCR under quantitative conditions followed by DNA sequencing revealed that the *hp-2* mutant contains ~10% correctly spliced *TDET1* mRNA (data not shown).

Comparison of Tomato *hp-2* and Arabidopsis *det1* Mutants

As is clear from the results presented in Figures 1 to 5, *hp-2* mutants are phenotypically different from *det1* mutants. Most conspicuously, they do not display dark phenotypes, such as reduced hypocotyl length, opened apical hooks, or enlarged cotyledons, whereas these were selection criteria for the isolation of *det* and *cop* mutants in Arabidopsis (Chory et al., 1989; Deng et al., 1991). Conversely, *det1* mutants can even develop true leaves and floral buds in prolonged darkness. No such phenotypes are observed in *hp* tomato seedlings grown in the dark.

Arabidopsis *det1* mutants also are characterized by high-level expression of light-regulated genes such as *CHS* and *CAB* in the dark, whereas in the light, *CHS* and *CAB* gene expression is similar to that observed in wild-type plants (Chory et al., 1989). In tomato *hp-2* mutants, dark expression of *CHS* and *CAB* genes is only very slight, and deregulation of gene expression is essentially light dependent (Figure 5).

Also in the light, *det1* mutant phenotypes contrast with those of *hp* mutants. Although both are small and have reduced apical dominance, *det1* mutants are pale green (Chory and Peto, 1990), whereas *hp* mutants are deeply pigmented (Figure 2).

Despite the above-mentioned differences, plastid development in darkness in the cotyledons of *hp-2* and *hp-2^j* seedlings (Figure 4) is similar to that observed in Arabidopsis

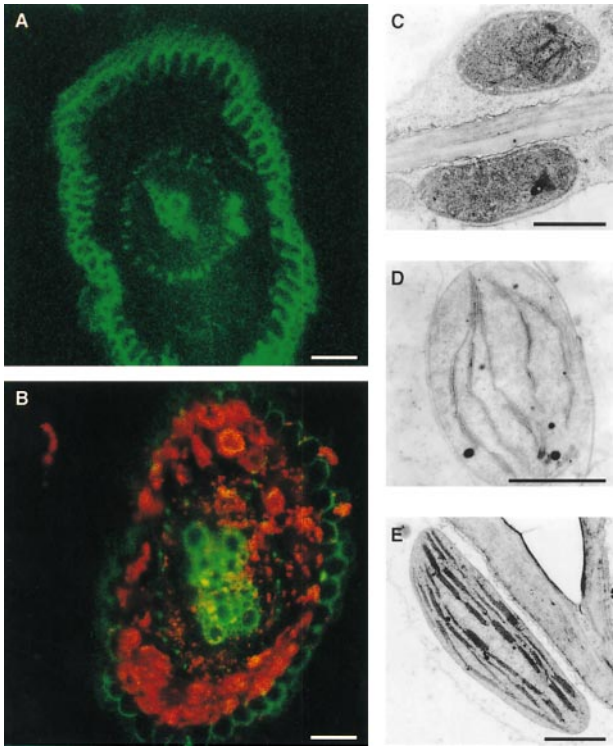

Figure 3. Roots of Light-Grown *hp-2* Seedlings Contain Fully Developed Chloroplasts.

(A) and **(B)** Confocal images from representative roots of wild-type **(A)** and *hp-2* **(B)** seedlings. Images are three-dimensional reconstructions of Z optical sections 1.5 to 2 μm thick. Cell wall autofluorescence is shown in green pseudocolor, and chlorophyll autofluorescence is indicated in red.

(C) to **(E)** Electron microscopic images from roots of wild-type **(C)** and *hp-2* (**[D]** and **[E]**) seedlings. Electron microscopic images of plastids were taken from cortical cells in **(C)** and **(E)** and pericycle cells in **(D)**.

hp-2^j roots displayed the same phenotypes as shown here for *hp-2* mutants, but the *hp-2^j* phenotypes were slightly more severe (data not shown). Bars in **(A)** and **(B)** = 100 μm; bars in **(C)** to **(E)** = 1 μm.

det1 mutants (Chory et al., 1989). The development of chloroplasts in the roots of these mutant seedlings grown in the light (Figure 3) also is consistent with the phenotype of *det1* mutants (Chory and Peto, 1990) and is even more pronounced. However, whereas in Arabidopsis *det1* mutants this observation was interpreted as indicating an alteration in cell-type specificity, in tomato this is not the case because, unlike in Arabidopsis, old roots of wild-type tomato plants commonly display some chloroplast development (data not shown). Similarly, whereas in Arabidopsis *det1* mutants anthocyanins are ectopically produced in all cell types (Chory and Peto, 1990; Miséra et al., 1994), the cell-type specificity of anthocyanin production is maintained very clearly in tomato *hp-2* mutants (Figure 2). Consequently, the phenotypes of *hp-2* and *hp-2^j* mutants can be interpreted as being due to exaggerated responses to light rather than to alterations of cell-type specificity resulting from ectopic expression.

det1 seedlings have been reported to display a strongly enhanced expression of stress-related genes such as those encoding pathogenesis-related (PR) proteins and glutathione reductase (Mayer et al., 1996). To examine whether this was also the case in *hp-2* and *hp-2^j* mutants, we hybridized RNA gel blots with a probe encoding tomato *PR-1b1* (Tornero et al., 1997). Although hypocotyls of *hp-2* seedlings reproducibly displayed *PR-1b1* gene expression at slightly higher levels compared with wild-type seedlings, this effect was very weak compared with that observed in Arabidopsis *det1* mutants and has never been observed in *hp-2^j* mutants (Figure 5). Nonetheless, this result indicates that, as with *det1*, the *hp-2* mutation does not affect only light-regulated genes. An alternative explanation is that there is considerably more cross-talk between different regulatory pathways than is currently appreciated. In Arabidopsis, *CAB* gene expression recently has been shown to be regulated by low temperature in a light-independent manner (Capel et al., 1998).

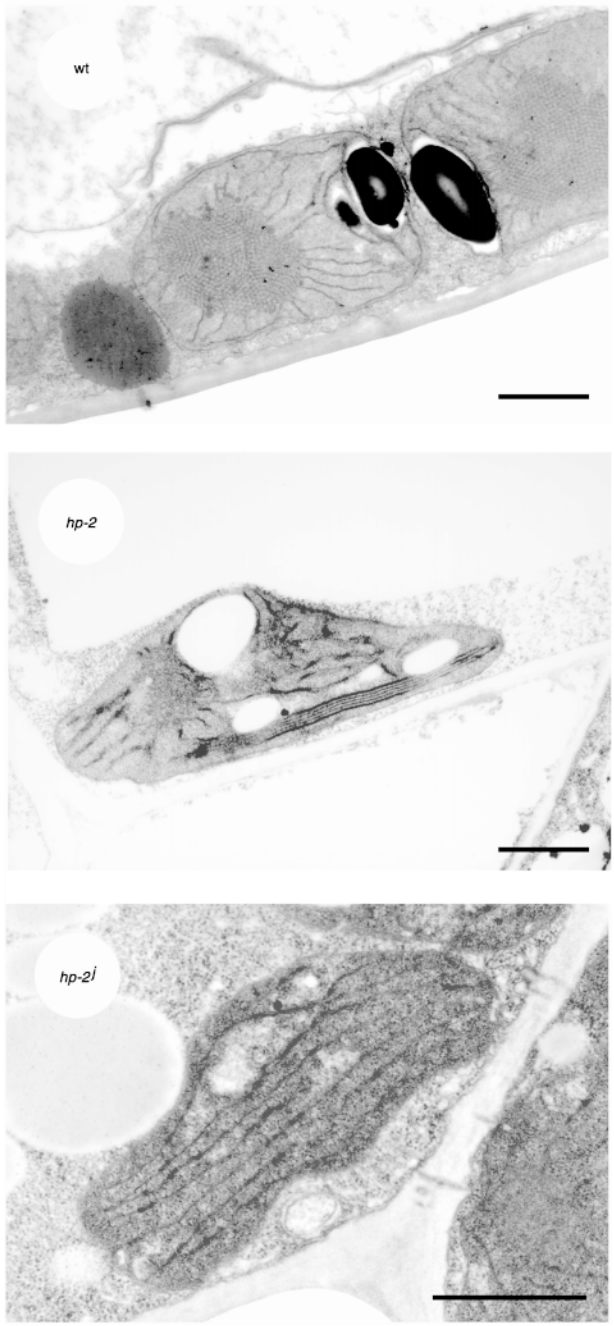

Figure 4. Partial Plastid Development in Dark-Grown *hp-2* and *hp-2^j* Mutant Seedlings.

Electron microscopy of representative plastids from cotyledons of 5-day-old dark-grown wild-type (wt), *hp-2*, and *hp-2^j* tomato seedlings is shown. Bars = 1 μm.

Cytokinin Can Phenocopy the *hp* Mutant

Previous observations in Arabidopsis have shown that a *det1* mutant phenotype can be phenocopied by the exogenous application of cytokinin (Chory et al., 1994). To determine the effects of cytokinin treatment in tomato, we treated wild-type seedlings with different concentrations of cytokinin in the dark and in the light (Figure 7). Interestingly, cytokinin does not phenocopy the *hp-2* mutation in tomato. In dark-grown seedlings, although hypocotyls are shorter in the presence of cytokinin, apical hook opening, cotyledon expansion, and anthocyanin biosynthesis were not observed (Figures 7A and 7B), even after prolonged periods (up to 3 weeks; data not shown). However, in the light, cytokinin can phenocopy the *hp-2* mutation: seedlings displayed shorter and thicker hypocotyls and accumulated high levels of anthocyanin (Figures 7A to 7C). These results therefore indicate that at least as far as the effects of cytokinin are

concerned, the *hp* mutation in tomato is equivalent to the *det1* mutation in Arabidopsis.

Double Mutant Analyses

Double mutant analysis with *det1* and phytochrome-deficient mutants in Arabidopsis indicates that the *det1* mutation is completely epistatic to photoreceptor mutations (Chory, 1992). This has been interpreted as meaning that DET1 acts downstream of phytochrome. To determine the relationship between TDET1 and phytochrome function, we examined the effects of the *hp-2* mutation in an *aurea* (*au*) mutant background, a phytochrome chromophore-deficient mutant (Terry and Kendrick, 1996). In contrast to its counterpart double mutant in Arabidopsis, *au hp-2* is similar to the single mutant *au.* For example, hypocotyls are elongated and anthocyanin accumulation is limited (Figures 8A to 8C). Although in genetic terms this result therefore infers that *au* is epistatic to *hp-2*, we nonetheless consider it more likely that TDET1 acts downstream of phytochrome, as proposed for Arabidopsis, but that its activity as a negative regulator is strictly dependent upon the presence of active phytochrome. This requirement is clearly not observed in Arabidopsis (Chory, 1992). The small but significant reduction in hypocotyl length and the small increase in anthocyanin found in the *au hp-2* double mutant compared with *au* (Figure 8) are likely, therefore, to be a result of hypersensitivity caused by the *hp-2* mutation toward the low amounts of functional phytochrome present in the *au* mutant, which is estimated to be ~3% of wild-type levels (Parks et al., 1987; Adamse et al., 1989). Similar results have been found in the *au hp-1* double mutant (Peters et al., 1992; Kerckhoffs et al., 1997b).

Only One *DET1* Homolog in Tomato

The fact that the *hp-2* mutant phenotype is strictly light and phytochrome dependent could suggest that in tomato there is either a redundancy in *DET1* gene function or that the regulatory networks controlling photomorphogenesis are wired differently compared with those in Arabidopsis. To identify whether there may be other *DET1* homologs in tomato, DNA gel blot analysis was performed with tomato genomic DNA at medium stringency. The results indicate that, as in Arabidopsis (Pepper et al., 1994), there is only one *DET1* gene in tomato (Figure 6F).

DISCUSSION

Ever since the photobiology of *hp* mutants was first described (Adamse et al., 1989), it has been thought that their exaggerated responsiveness to light must be a result of a mutation in a key negative regulator of the amplification steps in phytochrome signal transduction. Cloning of *HP* genes therefore promised to identify new components functioning specifically in phytochrome signal transduction pathways. In this report, we have demonstrated that the *HP-2* gene encodes the tomato homolog of DET1. This is surprising, given the dramatic differences between Arabidopsis *det1* and tomato *hp-2* phenotypes. Most conspicuously, *hp-2* mutants do not display any strong visible phenotypes in darkness, whereas deetiolation in the dark was the selection scheme used to isolate *det1* mutants. Furthermore, whereas *det1* mutations are epistatic to phytochrome mutations, analysis of the *au hp-2* double mutant reveals that the appearance of the *hp-2* mutant phenotype is strictly dependent upon the presence of active phytochrome.

The fact that mutation of *TDET1* in tomato is not manifested strongly in the dark or in the absence of functional photoreceptors may be related to the observation that in tomato, anthocyanin biosynthesis is strictly light dependent, whereas in Arabidopsis, it can be induced in the absence of light (Chory et al., 1989; Deng et al., 1991; Peters et al., 1992; Miséra et al., 1994; Pepper et al., 1994; Kwok et al., 1996; Kerckhoffs et al., 1997b). This may also explain why no constitutive deetiolated mutants, such as *cop* and *det*,

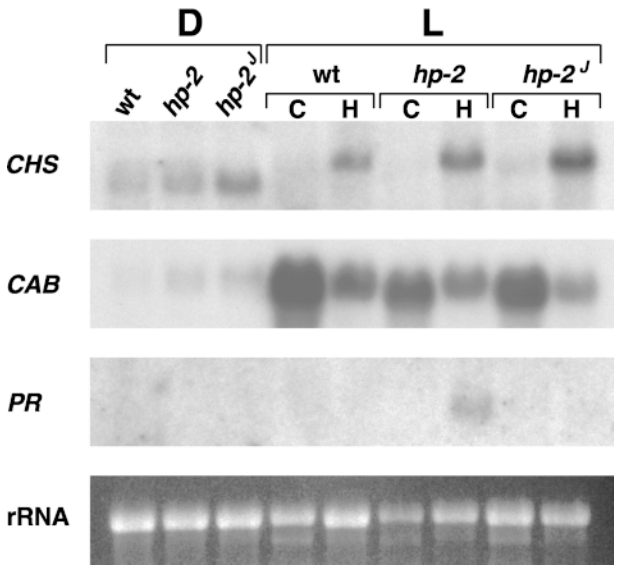

Figure 5. Gene Expression in Wild-Type, *hp-2*, and *hp-2^J* Seedlings.

Seedlings were grown at 25°C for 5 days in absolute darkness (D), followed by 2 days in continuous white light (L). RNA was extracted from whole seedlings (dark) or from cotyledons (C) and hypocotyls (H) (light). Modifications in gene expression in *hp-2* and *hp-2^J* seedlings compared with wild-type seedlings (wt) are shown to be principally light dependent. Ten-microgram samples of total RNA were loaded on gels and analyzed for expression of *CHS1* (*CHS*), *CAB6* (*CAB*), and *PR1-1b1* (*PR,* for pathogenesis related) genes after RNA gel blotting. The 30S rRNA is shown as a control for loading.

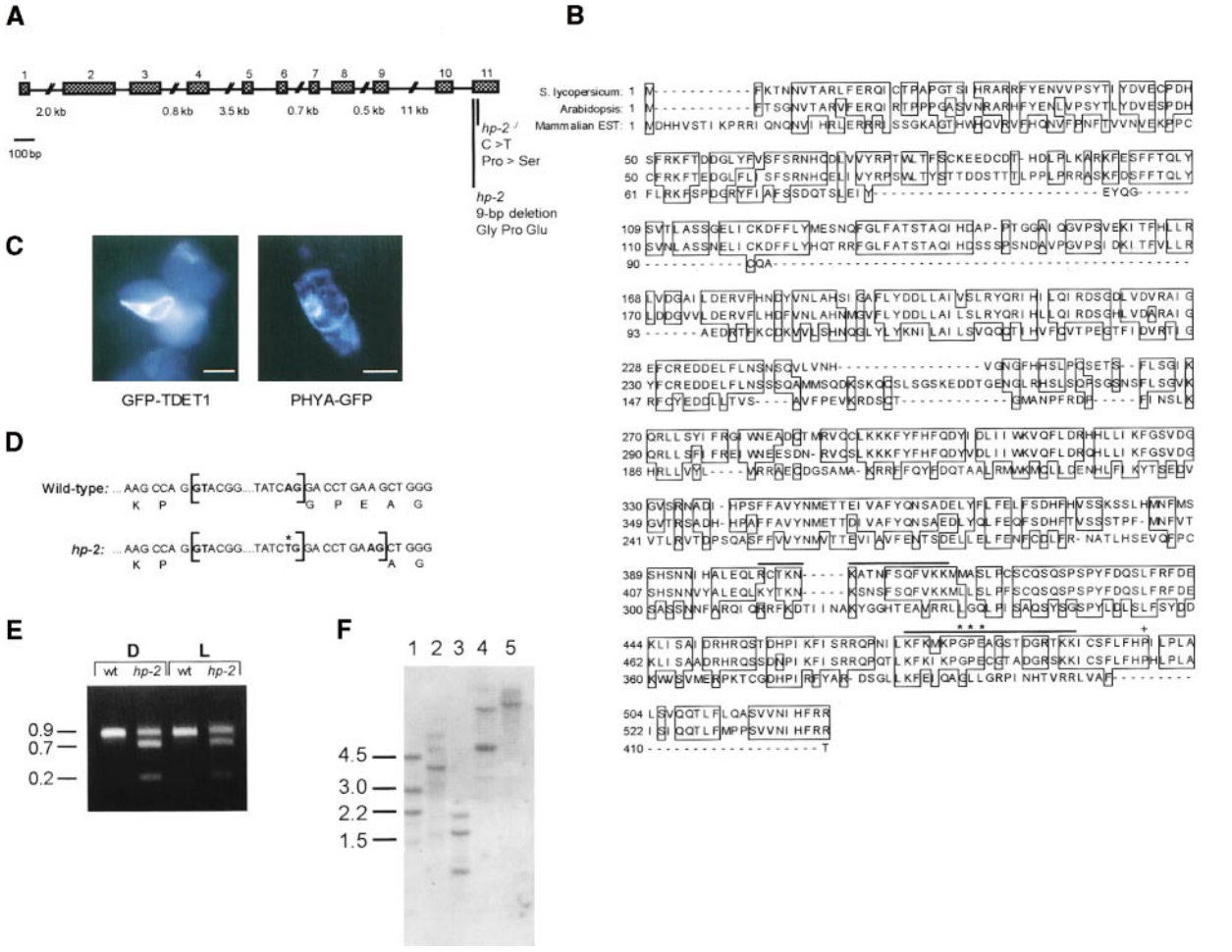

Figure 6. Structure of *TDET1* and Sites of *hp-2* and *hp-2^j* Mutations.

(A) Exon–intron structure of tomato *TDET1* gene and sites of mutations in the *hp-2* and *hp-2^j* alleles. Stippled boxes indicate exons, and slashes indicate introns. The scale bar is indicated.

(B) Alignment of the deduced amino acid sequences of *S. lycopersicum* TDET1, Arabidopsis DET1, and the mammalian expressed sequence tags (ESTs) with homology to DET1 (determined using the Clustal method [Higgins and Sharp, 1988]). The putative bipartite NLS is overlined. The amino acids missing in the *hp-2* mutant are indicated with asterisks, and the amino acid substitution in *hp-2^j* is denoted by a plus sign. The mammalian sequence is a compilation of derived amino acid sequences from mouse and human ESTs (GenBank accession numbers AA756238, AA236057, AA050184, and W64359). Boxed residues indicate conserved amino acids; dashes indicate arbitrary insertions.

(C) Targeting of the GFP–TDET1 fusion protein to nuclei of tobacco BY-2 suspension-cultured cells. A non-nuclear-targeted fusion protein, PHYA–GFP, is shown as a control. See Methods for more details. Scale bars = 20 μm.

(D) Donor and acceptor splicing sites of intron 10 from the wild type and *hp-2* mutant. Brackets indicate splice sites, dots indicate internal intron sequence (data not shown), and amino acids are shown in the one-letter code. The asterisk indicates the mutated nucleotide in the *hp-2*–derived sequence.

(E) Detection of mutated *TDET1* mRNA sequences in dark (D)- and light (L)-grown *hp-2* seedlings. A 0.9-kb *TDET1* fragment was amplified by reverse transcriptase–polymerase chain reaction (RT-PCR) from the wild type (wt) and the *hp-2* mutant and subsequently digested with PvuII, as described in Methods. The *hp-2* mutant sequence can be detected by PvuII digestion, which yields 0.7- and 0.2-kb fragments. Wild-type and mutant sequences were verified by DNA sequencing. RT-PCR was performed under nonquantitative conditions. Molecular lengths are shown at left in kilobases.

(F) DNA gel blot analysis of wild-type tomato genomic DNA. DNA was digested with EcoRI (lane 1), EcoRV (lane 2), DraI (lane 3), HindIII (lane 4), and BamHI (lane 5). A 1.8-kb *TDET1* cDNA fragment was used as probe, and hybridization patterns were fully consistent with the presence of a single *TDET1* gene in tomato, based on the genomic sequence (GenBank accession numbers AJ224356 and AJ224357). The only additional hybridizing bands at lower stringencies were nonspecific organellar DNA fragments (data not shown). Molecular lengths are shown at left in kilobases.

have ever been reported in tomato. Conversely, seedling-lethal mutants that display high levels of anthocyanin in the embryos of developing seeds, such as Arabidopsis *fusca* mutants, also have never been observed in tomato, even though they are commonly found in mutant screens in Arabidopsis and often represent strong alleles of *cop* and *det* mutants (Miséra et al., 1994; Kwok et al., 1996). The *brown seed* (*bs*) mutants are perhaps the most analogous in tomato (Rick, 1990). Although one *bs* mutation has been found to map to the vicinity of the *HP-2* gene on chromosome 1 (Balint-Kurti et al., 1995; Broun and Tanksley, 1996; Van Tuinen et al., 1997), the brown coloration of *bs* seeds is due to enhanced pigmentation of the endosperm and not of the embryo, and *bs* is not mutated in the *TDET1* gene (data not shown).

Detailed analysis of *hp-2* mutant phenotypes in darkness has revealed that, as in *det1* mutants, plastids show partially developed morphologies. Furthermore, several light-dependent responses also have escaped attention previously. For example, *hp-2* roots display some chloroplast development, and stress-related genes such as *PR-1b1* are partially deregulated. However, the observation that the tissue specificity of anthocyanin biosynthesis is not affected in *hp-2* mutants is in notable contrast to *det1* mutants (Chory and Peto, 1990; Miséra et al., 1994).

It could be argued that the discrepancies observed between *hp-2* and *det1* mutants could result if the *hp-2* and *hp-2^j* mutant alleles were weaker than were the reported *det1* alleles. Whereas the *hp-2* mutation is certainly not null (Figure 6E), whether the *hp-2^j* mutation is null awaits further analysis, although it should be noted that the mutated proline residue in the *hp-2^j* mutant is conserved in both plant sequences. We believe, however, that the absence of several well-known *det1* phenotypes in *hp-2* and *hp-2^j* mutants (as discussed above) is unlikely to be due to the severity of the *det1* alleles compared with the *hp* alleles because (1) these are qualitative not quantitative differences (e.g., altered tissue specificities in *det1* mutants); (2) weak alleles of *det1* in Arabidopsis (e.g., *det1-1*) display visible dark phenotypes (Pepper et al., 1994); (3) even heterozygous *det1* plants show clearly deregulated gene expression in the dark (Pepper et al., 1994); and (4) in tomato, cytokinin can phenocopy an *hp* mutant phenotype rather than a *det1* mutant phenotype (Figure 7). Furthermore, some of the phenotypes of *hp-2* and *hp-2^j* mutants are actually stronger than those observed in *det1* mutants, for example, chloroplast development in the dark and in the roots of light-grown seedlings. Consequently, whereas the role of DET1 as a specific negative regulator for photomorphogenesis can be questioned in Arabidopsis, the dark- and light-dependent phenotypes of *hp* mutants in tomato are consistent with a specific role for DET1 in regulating light responses in this species.

It is also possible that different threshold amounts of DET1 protein are needed for different light-regulated responses in tomato compared with Arabidopsis, because the mutant phenotypes clearly demonstrate that phytochrome action in etiolated seedlings is under constraint of the DET1 protein.

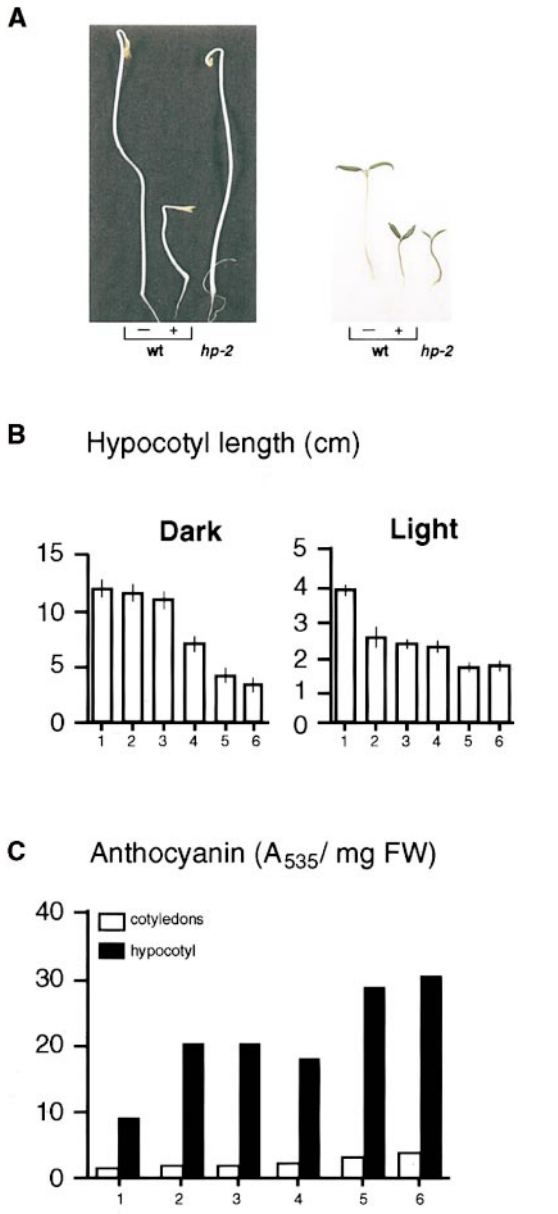

Figure 7. Cytokinin Can Phenocopy the *hp-2* Mutant Phenotype in Light-Grown Tomato Seedlings.

(A) Phenotype of wild-type (wt) seedlings grown in the absence (−) or the presence (+) of 500 μg/L benzoaminopurine compared with *hp-2* seedlings grown in the dark (left) and in the light (right).

(B) and **(C)** Hypocotyl length (in centimeters) and anthocyanin content (per mg fresh weight [FW]), respectively, of wild-type tomato seedlings grown in the presence of 0, 1, 5, 20, 100, and 500 μg/L benzoaminopurine (bars 1 to 6). Seedlings were grown at 25°C for 5 days in absolute darkness (Dark) or in a 16-hr-light and 8-hr-dark photoperiod (Light; **[C]**). In **(B)**, values are the mean of 10 seedlings. Highly similar results were obtained with the cytokinin zeatin (data not shown). Error bars indicate ±SE.

The availability of clearly null mutant alleles in tomato should resolve these issues. We are currently in the process of generating such mutants by transposon mutagenesis.

The most dramatic difference between *hp-2* and *det1* mutants is therefore the fact that *hp-2* mutations are able to sensitize light responses, whereas *det1* mutations cannot. For example, whereas light inducibility of *CHS* gene expression in *hp-2* mutants is more than double that observed in wild-type seedlings (Figure 5), in *det1* mutants, *CHS* gene photoregulation is essentially lost, which is the result of both higher expression levels and ectopic expression in all cell types in the dark (Chory and Peto, 1990; Miséra et al., 1994). Therefore, we propose that even though the function of the DET1 protein is likely to be identical in both tomato and Arabidopsis, the regulatory circuitry in which it operates is not the same in the two plants—in tomato, activation of photomorphogenesis is strictly dependent upon a light stimulus, whereas in Arabidopsis, photomorphogenic programs can be uncoupled from the light. Most simply, the signal transduction pathways regulating photomorphogenesis in Arabidopsis may have a low basal activity in darkness (i.e., a flux or dark current), whereas in tomato, they may be completely inactive under the same conditions. This hypothesis is reinforced further by the very different epistatic relationships of *hp-2* and *det1* mutations with mutations affecting phytochrome function.

It will be extremely interesting to determine how TDET1 activity is desensitized by light and to examine how its activity is influenced by calcium and cGMP, which are the second messengers identified in tomato as being involved in phytochrome control of anthocyanin biosynthesis and chloroplast development, respectively (Bowler et al., 1994a). Based on our knowledge of the workings of these pathways, we could hypothesize that the main desensitizer of TDET1 activity in anthocyanin-producing cells of leaves and hypocotyls is cGMP, thus resulting in the enhanced anthocyanin biosynthesis and concomitant reduction in chloroplast development (via reciprocal control) in *hp-2* mutants (Bowler et al., 1994b). In other tissues, such as in roots and immature fruits, TDET1 activity may be principally modulated by calcium, therefore resulting in enhanced chloroplast development in *hp-2* mutants. Microinjection experiments should determine the validity of such hypotheses.

The function of DET1 remains a mystery. Although it is a nuclear protein, it has not been reported to bind DNA directly (Pepper et al., 1994), and its ability to influence the expression of a large number of genes indicates that it may regulate transcription at a higher level, perhaps by organizing repressive regions of chromatin in the dark (Chory et al., 1996; Mustilli and Bowler, 1997). The fact that *DET1* homologs have now been found in mouse, human, and Drosophila but not in yeast or prokaryotes would indicate a role specific for multicellular organisms, perhaps as a general regulator of chromatin structure. Biochemical and cell biological experiments will be necessary to assess this possibility.

Based on the findings presented in this study, it is likely

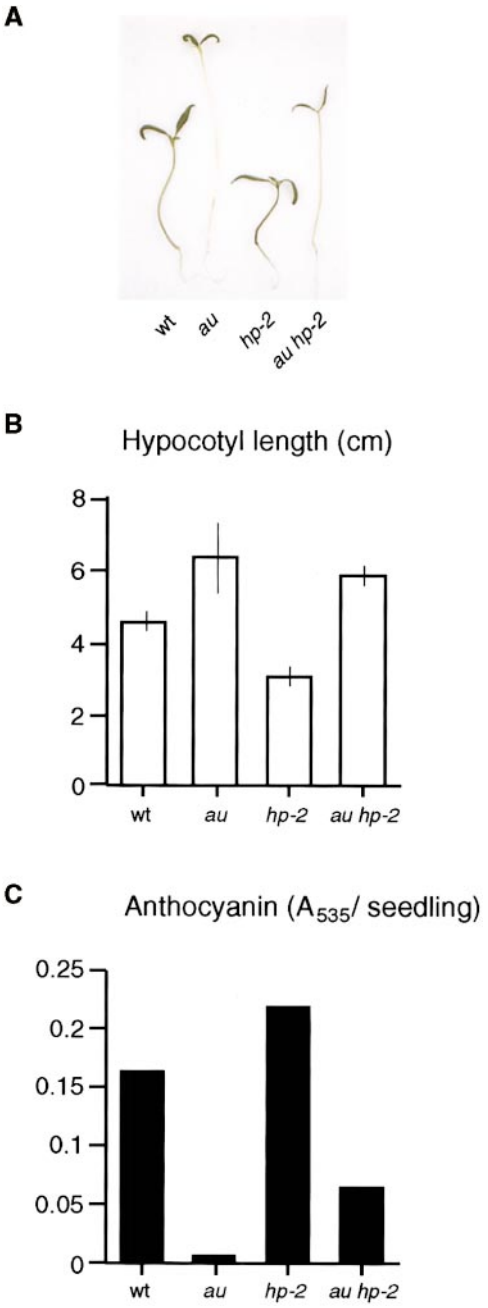

Figure 8. The *au* Mutation Is Epistatic to *hp-2.*

(A) Light-grown phenotypes of wild-type (wt), *au*, *hp-2*, and *au hp-2* tomato seedlings.

(B) and **(C)** Hypocotyl length (in centimeters) and anthocyanin accumulation (per seedling), respectively. Seedlings were grown at 25°C for 6 days under a 16-hr-light and 8-hr-dark photoperiod. Values are the mean of 15 seedlings, and the experiments were repeated three times. In **(B)**, error bars indicate ±SE.

that only a few key regulators are responsible for controlling light responses in all higher plants. The isolation and characterization of other *COP*/*DET*/*FUSCA* homologs in tomato therefore will be of great interest in investigating whether alteration of their functions also results in light-hypersensitive phenotypes, such as those seen in *hp-1* and *atv* mutants. The availability of such genes in tomato, as well as information from mutational analysis, would allow an evaluation of their role within the calcium- and cGMP-dependent phytochrome signaling pathways previously elucidated by microinjection in this species and may allow the construction of a single unified model for phytochrome signal transduction.

METHODS

Plant Material and Growth Conditions

The *hp-2* and *hp-2^j* exaggerated photoresponse mutants, the *au hp-2* double mutant, and the corresponding wild-type tomato seeds (*Solanum lycopersicum* cv Money Maker) were kindly provided by R.E. Kendrick and M. Koornneef (Wageningen Agricultural University, The Netherlands). Seeds were surface sterilized and directly sown in magenta boxes (Sigma) containing 4.3 g/L Murashige-Skoog salts (Sigma) and 0.8% agar. After 2 days pregermination in darkness, seedlings were grown at 25°C either in a 16-hr-light, 8-hr-dark photoperiod or in continuous dark, as appropriate. For cytokinin treatment, seeds were sown in the presence of different concentrations of benzylaminopurine (Sigma).

Anthocyanin Assays

Anthocyanins were extracted from cotyledons, hypocotyls, and whole seedlings with 0.5 mL of acidified (1% HCl) methanol for 48 hr in darkness with shaking. The extracts were separated by the addition of 0.4 mL of H_2O and 1 mL of chloroform, followed by centrifugation for 5 min at 3000 rpm. The absorbance of the upper phase was determined spectrophotometrically at 535 nm (A_{535}), and the anthocyanin content was calculated as (A_{535})/mg fresh weight or (A_{535})/seedling.

Isolation and Hybridization of Nucleic Acids

Total RNA was prepared from tomato leaves according to Verwoerd et al. (1989). RNA gels (10 μg per lane) were blotted onto Hybond N^+ membranes (Amersham) and hybridized with random primed probes (see below). Hybridization was performed for 24 hr at 50°C in phosphate buffer (7% SDS, 0.5 M $NaPO_4$, pH 7.2, and 1 mM EDTA), followed by 20-min washes in 40 mM $NaPO_4$, pH 7.2, 1% SDS, and 1 mM EDTA. As probes, we used the tomato chalcone synthase gene *CHS1* (O'Neill et al., 1990), the chlorophyll *a*/*b* binding protein gene *CAB6* (Piechulla et al., 1991), and the pathogenesis-related protein gene *PR-1b1* (Tornero et al., 1997). All RNA gel blots were repeated at least twice, using different samples.

DNA extraction from tomato leaves was performed as described by Dellaporta et al. (1983). Five-microgram samples were digested with a range of different restriction enzymes overnight and run on 0.8% agarose gels. Alkaline DNA gel blotting onto nylon Hybond N^+ membranes was as described by the supplier (Amersham). The 1.8-kb fragment of tomato *DEETIOLATED1* (*TDET1*) cDNA, labeled by random priming, was used as probe. Hybridization conditions were as described above.

Isolation and Sequencing of Genomic Clones, cDNAs, and Polymerase Chain Reaction Products

Reverse transcription of the 5′ sequence of the *TDET1* mRNA was performed using the 5′ rapid amplification of cDNA ends system (Gibco BRL) with total tomato leaf RNA with the oligonucleotide 5′-CATCAACACTGCCAAAC-3′, derived from the sequence of the restriction fragment length polymorphism (RFLP) marker CT151. An 0.8-kb polymerase chain reaction (PCR) product containing the 5′ end of the *TDET1* cDNA was obtained after two different amplification reactions with TaqI polymerase (Perkin Elmer) by using two CT151-derived nested primers (5′-GAAAGCAGCCGTTGCT-3′ and 5′-AGTTCATCATCTTCACGGC-3′, respectively) with the provided anchor primer. The 0.8-kb fragment was directly sequenced on both strands with Thermosequenase (Amersham).

Total cDNA from the wild type (cv Money Maker) and the corresponding *hp-2* and *hp-2^j* mutants were obtained by reverse transcription (RT) using avian myeloblastosis virus reverse transcriptase (Promega) of poly(A) mRNA isolated from leaves by using oligo(dT) Dynabeads (Dynal, Skøyen, Norway). From these total cDNAs, *TDET1* cDNA sequences were PCR amplified with specific primers (5′-GTATGATTCACTAGTTTAATGCTGCTGAAAG-3′ and 5′-CCCATACTAGTCGTCTTGGCACTCTATCAAG-3′) by using the Expand High Fidelity system (Boehringer Mannheim) and subcloned in pBluescript SK+ (Stratagene, La Jolla, CA) as SpeI fragments; four independent clones were sequenced on both strands. The wild-type *TDET1* cDNA and genomic sequences have GenBank accession numbers AJ224356, AJ224357, and AJ222798.

For the RT-PCR analysis presented in Figure 6E, *TDET1* sequences were amplified from total cDNAs derived from dark- and light-grown wild-type and *hp-2* seedlings by using the primers 5′-GTCTGCCTCAATCAGAAACTTCTTTCC-3′ and 5′-GTGATTTCTAGGTTGATTTCAATCTAGAG-3′. PCR products were subsequently purified, digested with PvuII, and run on 1% agarose gels.

Tomato genomic libraries in λ DASH and λ FIXII (kindly provided by, respectively, J. Giovannoni, Texas A&M University, College Station, TX, and the Tomato Genome Center, Rehovot, Israel) were screened using standard methods (Sambrook et al., 1989) with a ^{32}P-labeled CT151 fragment as a probe. Overlapping fragments from different recombinant phages were subcloned in pBluescript SK+ for sequencing of both strands.

Oligonucleotides 5′-GAAGGTAATTTTATATTAAACATAGAATAGA-3′ and 5′-GTGATTTCTAGGTTGATTTCAATCTAGA-3′ were used for amplification of the 3′ end of the *TDET1* gene from *hp-2* genomic DNA. The 1.3-kb PCR product was directly sequenced on both strands beginning with the primer 5′-CAAATCGGTAACATAT-3′ by using a Thermosequenase kit (Amersham).

Microscopy

To observe anthocyanin pigment localization in leaves and hypocotyls of the wild type and *hp* mutants, we prepared hand-cut sections from 3-week-old greenhouse-grown plants and observed them by light microscopy using a Zeiss (Jena, Germany) Axiolab microscope.

For confocal microscopy, roots from 10-day-old seedlings were sectioned by hand, placed on a cover slip, and observed using a Zeiss LSM410 confocal microscope. For excitation, we used the 488-nm argon laser with a DBSP 488/543 dichroic mirror (Zeiss). Wall autofluorescence was detected with a BP 515- to 565-nm emission filter (Zeiss), and chlorophyll autofluorescence was visualized simultaneously with an LP 570-nm emission filter (Zeiss).

For electron microscopic analysis of cotyledons, seedlings were grown in darkness for 7 days, and samples were taken in absolute darkness. Cotyledons were fixed for 1 hr with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, rinsed in buffer, and postfixed overnight in 2% osmium tetroxide at 4°C. Subsequently, samples were dehydrated in a graded ethanol series, rinsed in propylene oxide, and embedded in Epon resin (Electron Microscopy Sciences, Fort Washington, PA). Samples were then polymerized at 65°C overnight. Thin sections were stained with uranyl acetate and lead citrate and examined on a transmission electron microscope (model 400; Phillips, The Netherlands). For analysis of root samples, roots from 10-day-old seedlings were prepared for electron microscopy as described previously (Chory et al., 1989).

Green Fluorescent Protein Fusion Constructs and Transient Transfection

The full-length *TDET1* coding region was cloned as a translational fusion downstream of a green fluorescent protein (*GFP*) gene variant, *mGFP4*, which contains modified codons engineered to mutate the cryptic intron splicing sites present within the wild-type *GFP* sequence (Haseloff et al., 1997). The fusion gene was under control of the 35S cauliflower mosaic virus promoter and nopaline synthase terminator in the pBI121-derived vector pBIN35S-mGFP4 (Haseloff et al., 1997). The construct *PHYA–GFP* was generated in the same binary vector and contains the full-length rice *PHYA* sequence (Kay et al., 1989) in a translational fusion upstream of *mGFP4.* This fusion protein is not targeted to the nucleus (F. Fenzi and C. Bowler, unpublished results).

Transient transfection of heterotrophic *Nicotiana tabacum* BY-2 suspension-cultured cells (Nagata et al., 1992) was performed using the Biolistic PDS-1000/He gene gun (Du Pont) and standard protocols recommended by the manufacturer. Twenty-four hours after particle bombardment, cells were observed in an epifluorescence microscope (Zeiss Axiolab) by using a ×20 objective and filter sets HQ:GFP (for TDET1–GFP) and GFP (for PHYA–GFP) from Chroma Technology Corp. (Brattleboro, VT).

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REFERENCES

Adamse, P., Peters, J.L., Jaspers, P.A.P.M., Van Tuinen, A., Koornneef, M., and Kendrick, R.E. (1989). Photocontrol of anthocyanin synthesis in tomato seedlings: A genetic approach. Photochem. Photobiol. **50,** 107–111.

Balint-Kurti, P.J., Jones, D.A., and Jones, J.D.G. (1995). Integration of the classical and RFLP linkage maps of the short arm of tomato chromosome 1. Theor. Appl. Genet. **90,** 17–26.

Bowler, C., Neuhaus, G., Yamagata, H., and Chua, N.-H. (1994a). Cyclic GMP and calcium mediate phytochrome phototransduction. Cell **77,** 73–81.

Bowler, C., Yamagata, H., Neuhaus, G., and Chua, N.-H. (1994b). Phytochrome signal transduction pathways are regulated by reciprocal control mechanisms. Genes Dev. **8,** 2188–2202.

Boylan, M.T., and Quail, P.H. (1989). Oat phytochrome is biologically active in transgenic tomatoes. Plant Cell **1,** 765–773.

Broun, P., and Tanksley, S.D. (1996). Characterization and genetic mapping of simple repeat sequences in the tomato genome. Mol. Gen. Genet. **250,** 39–49.

Capel, J., Jarillo, J.A., Madueño, F., Jorquera, M.J., Martínez-Zapater, J.M., and Salinas, J. (1998). Low temperature regulates Arabidopsis *Lhcb* gene expression in a light-independent manner. Plant J. **13,** 411–418.

Chamovitz, D.A., and Deng, X.-W. (1996). Light signaling in plants. Crit. Rev. Plant Sci. **15,** 455–478.

Chory, J. (1992). A genetic model for light-regulated seedling development in *Arabidopsis.* Development **115,** 337–354.

Chory, J., and Peto, C.A. (1990). Mutations in the *DET1* gene affect cell-type–specific expression of light-regulated genes and chloroplast development in *Arabidopsis.* Proc. Natl. Acad. Sci. USA **87,** 8776–8780.

Chory, J., Peto, C., Feinbaum, R., Pratt, L., and Ausubel, F. (1989). Arabidopsis thaliana mutant that develops as a light-grown plant in the absence of light. Cell **58,** 991–999.

Chory, J., Reinecke, D., Sim, S., Washburn, T., and Brenner, M. (1994). A role for cytokinins in de-etiolation in *Arabidopsis.* Plant Physiol. **104,** 339–347.

Chory, J., Chatterjee, M., Cook, R.K., Elich, T., Fankhauser, C., Li, J., Nagpal, P., Neff, M., Pepper, A., Poole, D., Reed, J., and Vitart, V. (1996). From seed germination to flowering, light controls plant development via the pigment phytochrome. Proc. Natl. Acad. Sci. USA **93,** 12066–12071.

Dellaporta, S.L., Wood, J., and Hicks, J.B. (1983). A plant mini-preparation: Version II. Plant Mol. Biol. Rep. **1,** 19–21.

Deng, X.-W., Caspar, T., and Quail, P.H. (1991). *cop1*: A regulatory locus involved in light-controlled development and gene expression in *Arabidopsis.* Genes Dev. **5,** 1172–1182.

Furuya, M., and Schäfer, E. (1996). Photoperception and signaling of induction reactions by different phytochromes. Trends Plant Sci. **1,** 301–307.

Genoud, T., Millar, A.J., Nishizawa, N., Kay, S.A., Schäfer, E., Nagatani, A., and Chua, N.-H. (1998). An Arabidopsis mutant hypersensitive to red and far-red light signals. Plant Cell **10,** 889–904.

Haseloff, J., Siemering, K.R., Prasher, D.C., and Hodge, S. (1997). Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. Proc. Natl. Acad. Sci. USA **94,** 2122–2127.

Higgins, D.G., and Sharp, P.M. (1988). CLUSTAL: A package for performing multiple sequencing alignment on a microcomputer. Gene **73,** 237–244.

Hoecker, U., Xu, Y., and Quail, P.H. (1998). *SPA1*: A new genetic locus involved in phytochrome A–specific signal transduction. Plant Cell **10,** 19–33.

Kay, S.A., Keith, B., Shinozaki, K., and Chua, N.-H. (1989). The sequence of the rice phytochrome gene. Nucleic Acids Res. **17,** 2865–2866.

Kendrick, R.E., Peters, J.L., Kerckhoffs, L.H.J., Van Tuinen, A., and Koornneef, M. (1994). Photomorphogenic mutants of tomato. Biochem. Soc. Symp. **60,** 249–256.

Kendrick, R.E., Kerckhoffs, L.H.J., Van Tuinen, A., and Koornneef, M. (1997). Photomorphogenic mutants of tomato. Plant Cell Environ. **20,** 746–751.

Kerckhoffs, L.H.J., and Kendrick, R.E. (1997). Photocontrol of anthocyanin biosynthesis in tomato. J. Plant Res. **110,** 141–149.

Kerckhoffs, L.H.J., De Groot, N.A.M.A., Van Tuinen, A., Schreuder, M.E.L., Nagatani, A., Koornneef, M., and Kendrick, R.E. (1997a). Physiological characterization of exaggerated-photoresponse mutants of tomato. J. Plant Physiol. **50,** 578–587.

Kerckhoffs, L.H.J., Schreuder, M.E.L., Van Tuinen, A., Koornneef, M., and Kendrick, R.E. (1997b). Phytochrome control of anthocyanin biosynthesis in tomato seedlings: Analysis using photomorphogenic mutants. Photochem. Photobiol. **65,** 374–381.

Kwok, S.F., Piekos, B., Miséra, S., and Deng, X.-W. (1996). A complement of ten essential and pleiotropic Arabidopsis *COP*/*DET*/*FUS* genes is necessary for repression of photomorphogenesis in darkness. Plant Physiol. **110,** 731–742.

Loh, E.Y., Elliott, J.F., Cwirla, S., Lanier, L.L., and Davis, M.M. (1989). Polymerase chain reaction with single-sided specificity: Analysis of T cell receptor δ chain. Science **243,** 217–220.

Mayer, R., Raventos, D., and Chua, N.-H. (1996). *det1*, *cop1*, and *cop9* mutations cause inappropriate expression of several gene sets. Plant Cell **8,** 1951–1959.

Millar, A.J., McGrath, R.B., and Chua, N.-H. (1994). Phytochrome phototransduction pathways. Annu. Rev. Genet. **28,** 325–349.

Miséra, S., Müller, A.J., Weiland-Heidecker, U., and Jürgens, G. (1994). The *FUSCA* genes of *Arabidopsis*: Negative regulators of light responses. Mol. Gen. Genet. **244,** 242–252.

Mustilli, A.C., and Bowler, C. (1997). Tuning in to the signals controlling photoregulated gene expression in plants. EMBO J. **16,** 5801–5806.

Nagata, T., Nemoto, Y., and Hasezawa, S. (1992). Tobacco BY-2 cell line as the "HeLa" cell in the cell biology of higher plants. Int. Rev. Cytol. **132,** 1–30.

O'Neill, S.D., Tong, Y., Spörlein, B., Forkmann, G., and Yoder, J.I. (1990). Molecular genetic analysis of chalcone synthase in *Lycopersicon esculentum* and an anthocyanin-deficient mutant. Mol. Gen. Genet. **224,** 279–288.

Parks, B.M., Jones, A.M., Adamse, P., Koornneef, M., Kendrick, R.E., and Quail, P.H. (1987). The *aurea* mutant of tomato is deficient in spectrophotometrically and immunochemically detectable phytochrome. Plant Mol. Biol. **9,** 97–107.

Pepper, A., Delaney, T., Washburn, T., Poole, D., and Chory, J. (1994). *DET1*, a negative regulator of light-mediated development and gene expression in Arabidopsis, encodes a novel nuclear-localized protein. Cell **78,** 109–116.

Peters, J.L., Schreuder, M.E.L., Verduin, S.J.W., and Kendrick, R.E. (1992). Physiological characterization of a high-pigment mutant of tomato. Photochem. Photobiol. **56,** 75–82.

Piechulla, B., Kellmann, J.-W., Pichersky, E., Schwartz, E., and Förster, H.-H. (1991). Determination of steady-state mRNA levels of individual chlorophyll a/b binding protein genes of the tomato *cab* gene family. Mol. Gen. Genet. **230,** 413–422.

Pratt, L.H., Cordonnier-Pratt, M.-M., Kelmenson, P.M., Lazarova, G.I., Kubota, T., and Alba, R.M. (1997). The phytochrome gene family in tomato (*Solanum lycopersicum* L.). Plant Cell Environ. **20,** 672–677.

Quail, P.H., Boylan, M.T., Parks, B.M., Short, T.W., Xu, Y., and Wagner, D. (1995). Phytochromes: Photosensory perception and signal transduction. Science **268,** 675–680.

Reynard, G.B. (1956). Origin of Webb Special (Black Queen) in tomato. Rep. Tomato Genet. Coop. **6,** 22.

Rick, C.M. (1990). TGSC stocklists. Rep. Tomato Genet. Coop. **40,** 44–64.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Soressi, G.P. (1975). New spontaneous or chemically-induced fruit ripening tomato mutants. Rep. Tomato Genet. Coop. **25,** 21–22.

Terry, M.J., and Kendrick, R.E. (1996). The *aurea* and *yellow-green-2* mutants of tomato are deficient in phytochrome chromophore synthesis. J. Biol. Chem. **271,** 21681–21686.

Tornero, P., Gadea, J., Conejero, V., and Vera, P. (1997). Two PR-1 genes from tomato are differentially regulated and reveal a novel mode of expression for a pathogenesis-related gene during the hypersensitive response and development. Mol. Plant-Microbe Interact. **10,** 624–634.

Van Tuinen, A., Cordonnier-Pratt, M.-M., Pratt, L.H., Verkerk, R., Zabel, P., and Koornneef, M. (1997). The mapping of phytochrome genes and photomorphogenic mutants of tomato. Theor. Appl. Genet. **94,** 115–122.

Verwoerd, T.C., Decker, B.M., and Hoekema, A. (1989). A small-scale procedure for the rapid isolation of plant RNAs. Nucleic Acids Res. **17,** 2362.

Von Wettstein-Knowles, P. (1968). Mutations affecting anthocyanin synthesis in the tomato. Hereditas **60,** 318–346.

Yen, H., Shelton, A., Howard, L., Vrebalov, J., and Giovannoni, J. (1997). The tomato *high-pigment* (*hp*) locus maps to chromosome 2 and influences plastome copy number and fruit quality. Theor. Appl. Genet. **95,** 1069–1079.