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Sucrose availability on the aerial part of the plant promotes morphogenesis and flowering of *Arabidopsis* in the dark

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Summary

Conditions to promote dark morphogenesis and flowering in *Arabidopsis* have previously been limited to liquid cultures and to a few laboratory ecotypes. We have obtained development and flowering of *Arabidopsis* plants under complete darkness by growing them on vertical Petri dishes containing solid agar medium with sucrose. Under these conditions, all the ecotypes tested were able to develop, giving rise to etiolated plants that flowered after producing a certain number of leaves. Dark-grown plants showed similarities with phytochrome-deficient mutants and were different from de-etiolated or constitutive photomorphogenesis mutants such as *det* and *cop*. Late- and early-flowering ecotypes, showing large differences in flowering time and leaf number under long days, flowered with a similar number of leaves when grown in the dark. Rapid dark flowering of late-flowering ecotypes was not an effect of darkness but the result of the interaction between dark and sucrose availability at the aerial part of the plant, since sucrose also had an effect when plants were grown in the light. Gibberellin-deficient and insensitive mutants were delayed in the initiation of flowers in the dark, indicating a role for these hormones in flowering promotion in the dark. The late-flowering phenotype of mutants at different loci of the autonomous and long-day-dependent flowering induction pathways was rescued in dark growth conditions. However, the late-flowering phenotype of *ft* and *fwa* mutants was not rescued by sucrose either in the dark or in the light, suggesting a different role for these genes in flowering induction.

Introduction

Reproductive success of plants largely depends on the correct timing of floral induction. This is the reason why the initiation of flowering is highly regulated by environmental cues exhibiting regular seasonal changes, such as photoperiod and temperature, and by the developmental stage of the plants (Bernier *et al.*, 1993). Environmental cues are perceived by different organs in the plant and promote endogenous stimuli that signal the apical meristem. In photoperiodic plants, leaf exposure to a single inductive photoperiod results in a rapid increase of sucrose levels in leaf exudates (Houssa *et al.*, 1991; Lejeune *et al.*, 1991) and in its rapid accumulation in the apical meristem (Bodson and Outlaw, 1985; Corbesier *et al.*, 1998). Phytohormones such as cytokinins (Lejeune *et al.*, 1988) and gibberellins (Pharis *et al.*, 1987) also seem to be exported by leaves upon flowering induction in different plant species. On the other hand, the developmental stage of the plant seems to determine its competence to respond to those signals through so far unknown mechanisms (Aukerman and Amasino, 1998).

In recent years, a systematic genetic approach has allowed the identification of many of the genes that regulate flowering time in *Arabidopsis* (reviewed in Koornneef *et al.*, 1998; Levy and Dean, 1998). The results of these studies suggest that *Arabidopsis* uses different sets of genes to promote or repress flowering under different environmental conditions. Under inductive long-day photoperiods, flowering is promoted by the function of genes such as *PHYA*, *FHA*, *CO*, *GI*, *LHY* or *FT* (Koornneef *et al.*, 1998; Levy and Dean, 1998), while under non-inductive short-day photoperiods, flowering is completely dependent on gibberellins (Wilson *et al.*, 1992). Genes such as *FCA*, *FPA*, *FVE*, *FY* or *LD*, considered to participate in an autonomous pathway, are important in flowering independently from photoperiod and their function can be substituted by a vernalization treatment (Koornneef *et al.*, 1991; Martínez-Zapater and Somerville, 1990). Flowering repression under short-day conditions largely depends on genes involved in light perception (phytochrome B and related phytochromes) and signal transduction (Hicks *et al.*, 1996; Koornneef *et al.*, 1995). In vernalization-requiring genotypes, flowering repression under regular growing temperatures is provided by dominant alleles at loci

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such as *FRI* and *FLC* (Levy and Dean, 1998; Michaels and Amasino, 1999; Sheldon *et al.*, 1999). Finally, other genes such as *TFL*, function to repress reproductive development irrespective of photoperiod, allowing the plant to reach specific vegetative stages before flowering (Ratcliffe *et al.*, 1998).

The complex interactions among flowering regulatory pathways and the dual roles of light on plant development as an informational cue and as the source of energy, make it difficult to analyse the contribution of every individual pathway independently. One possibility to simplify this analysis is to overcome the need for light by providing plants with a source of energy and growing them in the dark. It is commonly assumed that, when *Arabidopsis* seeds germinate on solid media in Petri dishes in the dark, they cannot develop beyond germination (Chory *et al.*, 1996). However, when grown on stationary liquid cultures containing sucrose or glucose as the carbon source, *Arabidopsis* plants were able to complete their morphogenesis and flower under complete darkness. These results, initially reported by Rédei *et al.* (1974) for the ecotype Columbia, were later extended to a few additional ecotypes (Einkheim, Estland and Landsberg) by Goto (1982), using specific media combinations. More recently, Araki and Komeda (1993) repeated the results reported by Rédei *et al.* (1974), although only for Columbia plants grown in liquid shaken cultures supplemented with sucrose. The fact that the dark growth conditions corrected the late-flowering phenotype of Columbia mutants *co*, *gi* and *ld* was interpreted as due to the lack of light-dependent repression of a default programme for flowering initiation (Araki and Komeda, 1993; Rédei *et al.*, 1974). However, the requirement for a shaken liquid culture and the limitation of its effect to the ecotype Columbia raised the question on whether the dark morphogenesis and flowering observed could be artefacts promoted by the stressful growing conditions.

Our goal in this work was to set up a culture condition allowing the light-independent development of *Arabidopsis* and identification of the major factors responsible for dark morphogenesis and flowering in this species. Here we show that *Arabidopsis* plants grown on vertical Petri dishes containing solid agar medium with either sucrose or glucose break their skotomorphogenesis and start a morphogenetic process ending with flower development. This dark morphogenetic process is dependent on the availability of a carbon source at the aerial part of the plant and on gibberellin biosynthesis. Many late-flowering ecotypes and mutants tested flower with fewer leaves in the dark growth conditions than in the light, and we show that this flowering promotion is mainly due to the availability of sucrose and not only to the absence of light, as previously proposed (Rédei *et al.*, 1974). The role of the genetic pathways identified by late-flowering mutations is discussed on the basis of the mutant dark-flowering phenotypes.

Results

Dark morphogenesis in Arabidopsis *depends on sugar availability at the aerial part of the plant*

When Landsberg *erecta* (L*er*) seeds were sown in the dark on either vertical or horizontal Petri dishes containing solidified MS medium without sucrose, seedling development followed the classical skotomorphogenic development, giving rise to typical etiolated seedlings (Figure 1a,c). However, when seeds germinated on vertical dishes containing sucrose (1%), the apical meristems of the etiolated seedlings initiated morphogenesis after 2 weeks of culture (Figure 1b). Seeds germinated in the dark in the same medium, but on horizontal plates, could sometimes initiate morphogenesis when the hypocotyl fell down on the agar and had direct contact with the medium (Figure 1d). Taken together, these results indicate that, in the ecotype L*er*, availability of sucrose to the aerial part of the plant can promote morphogenesis in the dark. In agreement with this conclusion, the root system could be excised from the seedlings at different stages of development without affecting the morphogenetic process taking place at the apex (Figure 1e,f).

L*er* seedlings grown on vertical sucrose plates under darkness showed conspicuous flower buds after 6 weeks of culture. When plants of the same ecotype were grown under inductive photoperiods on the same vertical plates, flower buds were already visible after 3 weeks of growth. However, the total number of leaves produced before flowering was not significantly different under both conditions (Figure 2). This delay seems to be the result of a 2-week lag in the initiation of the first pair of leaves and a lower rate of leaf primordia initiation in the dark (Figure 2). After 6 weeks of dark growth, flower buds were apparent in a high proportion of the plants, and by the 7th week all of the dark-grown plants showed flower buds. An increase in the rate of leaf production could be obtained by growing the plants on higher sucrose concentrations (3%), although this higher rate did not result in a significant reduction of the total number of leaves (data not shown). Similar results were also obtained with comparable glucose concentrations (data not shown).

Dark-grown etiolated seedlings initiated their development with the opening of the apical hook and with a limited expansion of cotyledons. After this initial step, the apical meristem started the differentiation of leaf primordia and the elongation of the stem. Roots continued to grow and new roots were initiated from the hypocotyl (Figure 3b). Leaf primordia gave rise to leaves with elongated petioles and unexpanded limbs (Figure 3a,c).

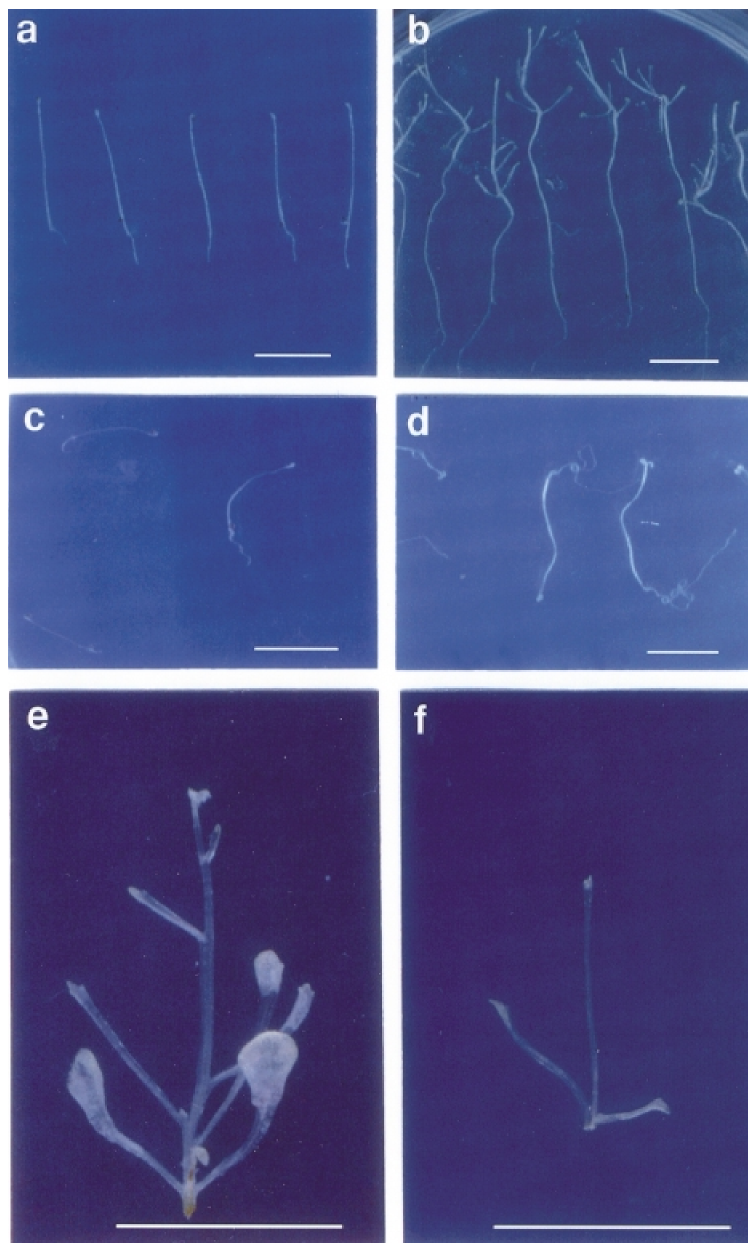

Figure 1. Etiolated seedlings of *Arabidopsis* grown in the dark on different media.
(a) Four-week-old plants grown on vertical Petri dishes containing MS medium. (b) Four-week-old plants grown on vertical Petri dishes containing MS medium supplemented with 1% sucrose. (c) Four-week-old plants grown on horizontal Petri dishes containing MS medium. (d) Four-week-old plants grown on horizontal Petri dishes containing MS medium supplemented with 1% sucrose. (e) Dark flowering of a 5-week-old *Arabidopsis* seedling whose root system was eliminated after the first week of dark development as described in Experimental procedures. (f) Dark flowering of a 5-week-old *Arabidopsis* seedling whose roots and rosette were eliminated after 3 weeks of dark development as described in Experimental procedures. Bar = 1 cm.

Stem elongation was not inhibited as in light-grown plants and leaves were not arranged in a rosette (Figure 3a,c); however, leaf phyllotaxy was not conspicuously altered. After the development of the first 4–5 leaves, internode elongation was further enhanced and leaf petiole elongation was inhibited, in a similar way to what is observed for cauline leaves of light-grown plants (Figure 3d). These leaves were serrated and in some cases bore trichomes with similar distribution as those observed in light-grown plants (Martínez-Zapater *et al.*, 1995). After the development of eight leaf primordia, the shoot apex initiated the production of flowers. Dark-grown flowers had normal number and distribution of whorls and organs. However, petals and stamens failed to elongate to the size of light-grown flowers. The anthers of dark-grown plants did not dehisce, there was no evidence of fertile pollen formation, and seed production was not observed (Figure 3e,f).

Although both carbon availability on the shoot and constitutive photomorphogenesis mutations can overcome skotomorphogenesis arrest, subsequent morphogenesis is very different. Dark-grown plants are strongly etiolated as opposed to the de-etiolated phenotype of *det* and *cop* mutants (Chory *et al.*, 1989; Deng *et al.*, 1991). Furthermore, etiolated dark-grown plants did not show expression of light regulated genes such as *RBCS* and *CAB* (Figure 4). Thus, *Arabidopsis* dark morphogenesis was more similar to the development of mutants with reduced light perception such as double and triple phytochrome

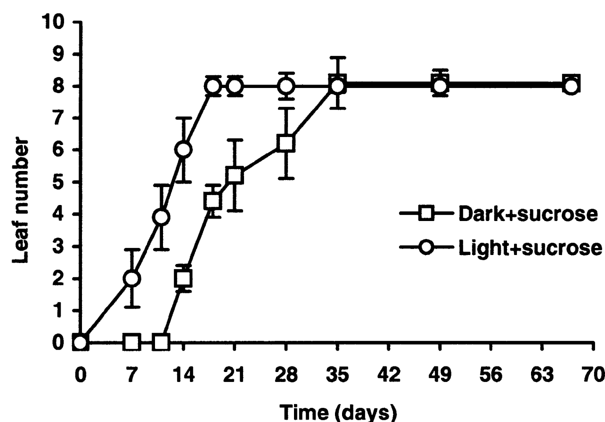

Figure 2. Developmental time course of Landsberg *erecta* plants grown under two different light regimes.
Seedlings were grown on vertical Petri dishes containing MS medium supplemented with 1% sucrose either in the dark or under 16 h photoperiod. Values correspond to the average number of total leaves counted in a sample of 10 plants ± SE.

mutants grown in the light (Aukerman *et al.*, 1997; Devlin *et al.*, 1996). In summary, dark-grown plants followed the developmental phases described for light-grown plants (Schultz and Haughn, 1993) but displayed an etiolated phenotype.

Dark morphogenesis is independent from phytochrome and modulated by gibberellins

Etiolated development is the consequence of a low Pr/Pfr ratio or the lack of active phytochrome. This has been suggested to result in higher gibberellin biosynthesis and sensitivity (Reed *et al.*, 1996). Even in the absence of the active Pfr form of phytochrome, there are reports that involve the inactive Pr form in the regulation of some plant developmental responses (Chory *et al.*, 1996). To test whether the red-absorbing form of phytochrome, which accumulates in darkness, or gibberellins were required for dark morphogenesis, we grew different mutants under darkness and compared their development to that of the wild-type plants. The mutants chosen for this study were the long-hypocotyl mutants *hy1* (defective in chromophore biosynthesis: Koornneef *et al.*, 1980; Muramoto *et al.*, 1999) and *hy3* (defective in functional *PHYB* apoprotein: Koornneef *et al.*, 1980; Somers *et al.*, 1991) and gibberellin-deficient (*ga1*, *ga2*, *ga4*, *ga5*: Koornneef and van der Veen, 1980) and insensitive mutants (*gai*: Koornneef *et al.*, 1985).

Dark morphogenesis of phytochrome-deficient mutants was indistinguishable from the dark morphogenesis of wild-type plants, showing similar hypocotyl elongation, seedling etiolation and growth rate (data not shown). Contrary to the early-flowering phenotype described for these mutants in the light (Goto *et al.*, 1991), they flowered with the same number of leaves as the wild-type in the dark (Table 1). Similar results were reported for the *hy2* mutant when grown in shaken liquid culture in the dark (Araki and Komeda, 1993). Altogether, these results suggest that dark flowering is not dependent upon the accumulation of the red-absorbing forms of phytochrome and takes place independently of its signalling pathway.

Gibberellin-deficient and insensitive mutants showed a dwarf phenotype in the dark (Figure 5). Consistently with their light phenotypes, the mutants *ga1-3* and *gai* displayed the strongest dwarfism, mutant *ga2* showed some degree of elongation, and mutants *ga4* and *ga5* did not show conspicuous elongation differences from wild-type (Figure 5). Furthermore, mutants showing stronger dwarfism showed an extended vegetative development before the initiation of flowers and produced more leaves than the wild-type (Table 1). These results were comparable with the flowering behaviour of these mutants in the light where gibberellins accelerate flowering under long-day conditions and are required for flowering under non-inductive short-day conditions. Thus, gibberellin-deficient and insensitive mutants are affected in stem elongation and flowering promotion in the dark as they are in the light.

Sucrose availability accelerates flowering in late-flowering Arabidopsis *ecotypes*

Previous reports on dark morphogenesis in *Arabidopsis* had been limited to the ecotype Columbia and to very specific culture conditions and liquid media (Araki and Komeda, 1993; Goto, 1982; Rédei *et al.*, 1974). Since L*er* plants were able to complete their morphogenesis in our dark growth conditions, we studied the development of other available ecotypes to evaluate the genotype dependence of this dark morphogenetic process. For this purpose we selected some common laboratory ecotypes such as Columbia (Col-0) and Niederzenz (Nd-0), and other ecotypes such as Stockholm (St-0), Leiden (Le-0) and Cape Verde Islands (Cvi-0) with different flowering requirements. All the ecotypes tested initiated dark morphogenesis as for L*er* when germinated and grown in our dark conditions (Table 2). These results indicated that dark morphogenesis is not restricted to specific genotypes, as previously proposed (Araki and Komeda, 1993; Rédei *et al.*, 1974). Interestingly, the large differences in the total number of leaves shown by the different ecotypes when grown in the light were not maintained in darkness (Table 2).

The strong reduction in the number of leaves shown by some ecotypes when grown in darkness could be the result of either the lack of putative light inhibitory effects or the direct availability of sucrose to the aerial part of the plants. In order to elucidate between those possibilities, seedlings were grown on vertical sucrose medium in the

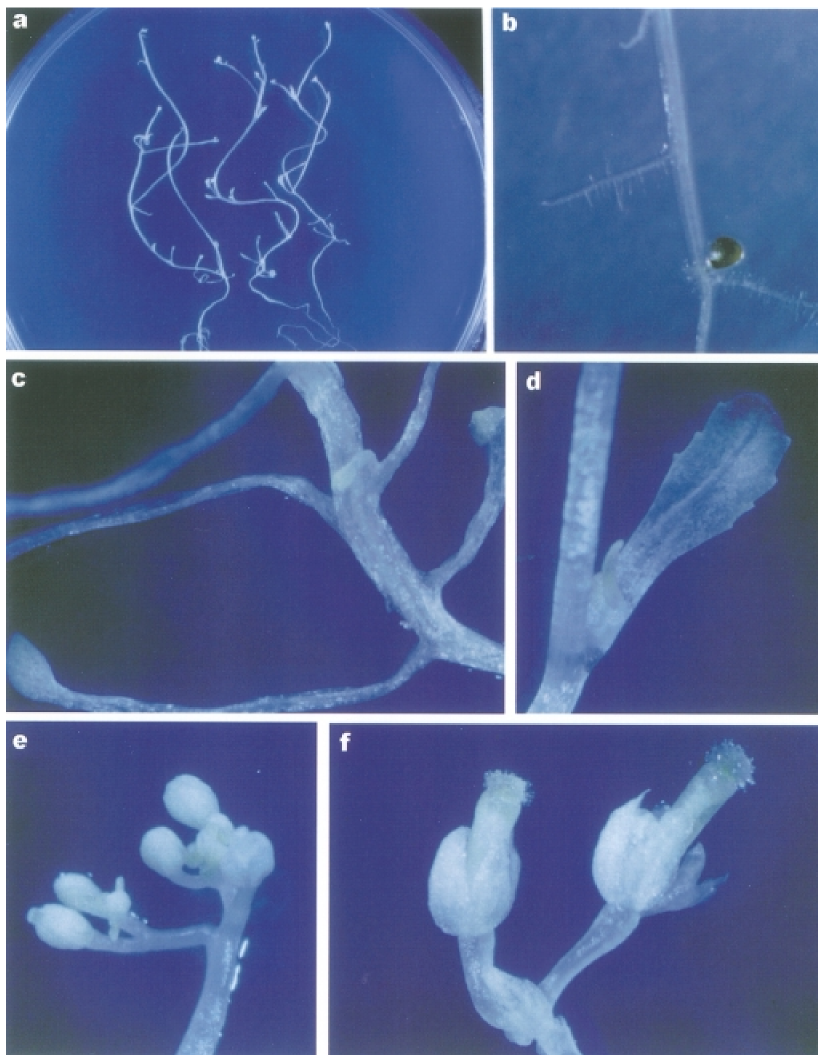

Figure 3. Dark morphogenesis in *Arabidopsis*. All images correspond to 5-week-old plants grown as described in Experimental procedures.
(a) Fully developed etiolated L*er* plants. (b) Close-up of roots developed from the hypocotyl. (c) Detail of the rosette. (d) Detail of a serrated inflorescence leaf. (e) Dark-developed inflorescence. (f) Dark-developed flower showing an elongated pistil and lack of elongation of stamens and carpels.

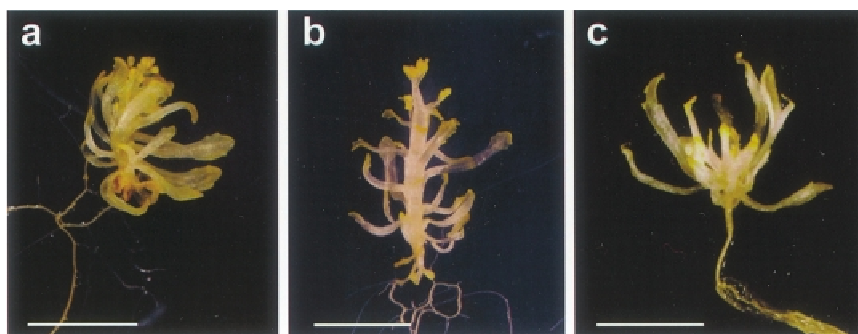

Figure 5. Phenotype of 7-week-old gibberellin-deficient and insensitive mutants grown in the dark.
Bar = 1 cm. (a) *ga1-3*; (b) *ga2*; (c) *gai.*

light. As shown in Table 2, most ecotypes flowered earlier in darkness with sucrose than in light with sucrose, although sucrose produced a significant reduction in the number of leaves in the light. One ecotype (Le-0) showed a similar response to sucrose in the light and in the dark, indicating the existence of genotypic variation in the response to the different environmental conditions. Taken together, these results suggest that dark morphogenesis is not genotype-dependent and that sucrose availability to the aerial part of the plant can have

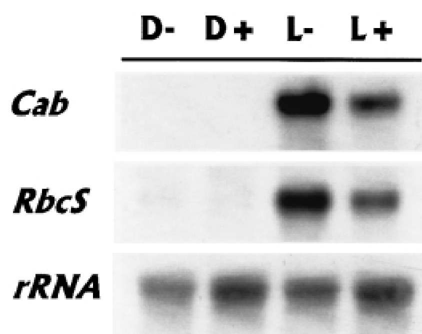

Figure 4. Expression of light-regulated genes in *Arabidopsis* seedlings grown under different conditions.
Total RNA was extracted from *Arabidopsis* L*er* seedlings grown in the dark (D) or in the light (L) with (+) or without (–) sucrose. RNA blot hybridizations were performed with probes for *CAB* and *RBCS* mRNAs.

Table 2. Total number of leaves of different ecotypes grown on vertical Petri dishes under different conditions

	Number of leaves[a]		
Genotype	Light	Light + sucrose	Dark + sucrose
L*er*	9.5 ± 1.1	10.0 ± 1.2	8.4 ± 0.8
Col-0	11.8 ± 1.3[b]	10.0 ± 1.2[c]	9.1 ± 0.6
Nd-0	12.0 ± 1.3[b]	10.0 ± 1.3[c]	8.6 ± 0.8
Cvi	16.7 ± 1.6[b]	12.3 ± 2.8[b,c]	7.6 ± 1.6[d]
Le-0	22.5 ± 2.7[b]	11.6 ± 0.6[c]	11.0 ± 0.8[b]
St-0	25.0 ± 2.0[b]	18.4 ± 3.1[b,c]	9.5 ± 1.0[b,d]

[a]Average leaf number ± standard error ($n = 10$).
[b]Significantly different from wild type L*er* ($P < 0.05$).
[c]Significantly different from light conditions ($P < 0.05$).
[d]Significantly different from light + sucrose conditions ($P < 0.05$).

Table 1. Total number of leaves of different mutants grown on vertical Petri dishes in darkness

Genotype	Number of leaves[a]
L*er*	7.6 ± 1.2
hy1	7.8 ± 0.4
hy3	8.1 ± 0.4
ga1-3	23.0 ± 1.8[b]
ga2	18.6 ± 2.5[b]
ga4	7.8 ± 1.5
ga5	7.4 ± 0.8
gai	12.0 ± 2.0[b]

[a]Average leaf number ± standard error ($n = 10$).
[b]Significantly different from wild type L*er* ($P < 0.05$).

Table 3. Total number of leaves of different late-flowering mutants grown on vertical Petri dishes under different conditions

	Number of leaves[a]		
Genotype	Light	Light + sucrose	Dark + sucrose
Ler	9.5 ± 0.4	10.0 ± 0.9	8.2 ± 0.8
fca	20.4 ± 0.4[b]	12.3 ± 0.8[b,c]	11.6 ± 0.5[b]
fpa	18.4 ± 1.6[b]	15.7 ± 0.4[b,c]	10.4 ± 0.7[b,d]
fve	21.4 ± 0.6[b]	12.5 ± 0.8[b,c]	8.3 ± 0.7[d]
co-3	15.4 ± 0.6[b]	12.1 ± 0.6[c]	8.4 ± 0.6[d]
gi-3	14.2 ± 2.3[b]	10.4 ± 0.4[c]	9.1 ± 0.2
ft	16.3 ± 2.0[b]	20.0 ± 0.7[b,c]	16.7 ± 0.5[b,d]
fwa	17.4 ± 0.7[b]	21.1 ± 1.2[b,c]	15.4 ± 1.4[b,d]

[a]Average leaf number ± standard error ($n = 10$).
[b]Significantly different from wild type L*er* ($P < 0.05$).
[c]Significantly different from light conditions ($P < 0.05$).
[d]Significantly different from light + sucrose conditions ($P < 0.05$).

important effects on the flowering time of different *Arabidopsis* ecotypes both in the light and in the dark. Furthermore, the drastic reduction in the number of leaves observed in the dark growth conditions should be attributed to the interaction of both the lack of light and the direct availability of sucrose.

Arabidopsis *late-flowering mutants flower with a similar number of leaves as the wild-type in dark growth conditions*

The flowering promotion observed for late-flowering *Arabidopsis* ecotypes in the dark led us to investigate the effect of these growing conditions on late-flowering mutants, most of them isolated in the ecotype L*er* (Koornneef *et al.*, 1991). We selected a set of late-flowering mutants representing the genetically identified regulatory pathways (Koornneef *et al.*, 1998; Levy and Dean, 1998). The results of this analysis are shown in Table 3. Mutants affected in genes involved in the autonomous pathway of flowering transition (*fca*, *fpa*, *fve*) flowered in darkness with a phenotype much closer to that of the wild-type L*er*. Mutants affected in genes involved in the long-day inductive pathway (*co*, *gi*, *ft*, *fwa*) showed a differential behaviour. On one hand, mutants *co* and *gi* flowered in the dark with a similar number of leaves as the wild-type L*er*. On the other hand, mutants *ft* and *fwa* maintained their late-flowering phenotype under dark conditions (Table 3). These experiments were repeated with additional *FT* (*ft-2* and *ft-3*) and *FWA* (*fwa-2*) mutant alleles with similar results (data not shown), indicating that the observed effects are not the consequence of specific mutations.

Late-flowering mutants were also grown on vertical sucrose dishes in the light to determine whether the availability of sucrose at the aerial part of the plant could correct their flowering delay in the light. As shown in Table 3, late-flowering mutants, showing a closer to wild-type flowering phenotype in darkness, also displayed a significant acceleration of flowering when sucrose was provided on vertical dishes in the light. As previously

shown for the ecotypes, the flowering promoting effect of sucrose was slightly higher in the dark than in the light. Consistently with their dark phenotype, mutants *ft* and *fwa* also showed a late-flowering phenotype in the light, characterized by a significant increase in the number of leaves (Table 3). Similar results were observed when using the additional mutant alleles mentioned above.

Discussion

Dark morphogenetic arrest is overcome by the availability of sucrose at the aerial part of the plant

We have shown that *Arabidopsis* plants, irrespective of their genetic background, can initiate morphogenesis in the dark if sucrose or glucose are provided to their aerial part. Although dark morphogenesis had previously been reported in *Arabidopsis* (Araki and Komeda, 1993; Goto, 1982; Rédei *et al.*, 1974), this process was found to be restricted to very specific liquid culture conditions and to a few ecotypes, what made it not generally applicable. Dark morphogenesis of wild-type plants on vertical Petri dishes gives rise to etiolated plants that do not express light-regulated genes, differing from the constitutive photomorphogenesis shown by *det* and *cop* mutants (Chory *et al.*, 1989; Deng *et al.*, 1991) and from the phenotype described for dark-grown plants on liquid medium (Araki and Komeda, 1993). It is therefore possible that, as proposed by Araki and Komeda (1993), the dark expression of light-inducible genes observed in liquid shaken dark cultures of Columbia plants could be due to a side-effect of shaking or submergence stress. Thus, carbon availability at the apex could trigger morphogenesis at the shoot apical meristem in the absence of light, while light would be required to modulate the extent of organ elongation and expansion and the greening and development of chloroplasts (Fankhauser and Chory, 1997). The observation that dark morphogenesis does not take place on horizontal sucrose plates indicates that carbon is not efficiently mobilized from the roots to the apex in the dark. This could be due to a reduced sink strength of the shoot apical meristem of etiolated seedlings formed by a very small number of cells (Ho, 1988) or to the requirement for a light-dependent signal.

Sucrose and darkness accelerate flowering

Growth on vertical dishes in darkness promoted a reduction in the number of leaves in most of the late-flowering ecotypes and mutants tested. A similar reduction had been reported for late-flowering mutants *ld*, *co* and *gi* in dark liquid cultures (Araki and Komeda, 1993; Rédei *et al.*, 1974). This reduction has previously been explained as the result of the lack of a putative light inhibitory effect (Rédei *et al.*, 1974) or as the result of the stressful conditions created by submergence and shaking (Araki and Komeda, 1993). By growing late-flowering ecotypes and mutants on vertical sucrose plates in the light, we have shown that an important part of the promoting effect of darkness is due to the sucrose present in the medium. Moreover, the slight increase in the number of leaves observed in the light even on vertical sucrose plates could indirectly result from the more extensive vegetative development shown by the plants. Light promotes leaf expansion (Robson *et al.*, 1993) and expanding leaves would act as strong photo-assimilate sinks (Herbers and Sonnewald, 1998) competing with the apical meristem and delaying the flowering transition. Additionally, the existence of flowering inhibitory signals produced by the developing leaves in the light cannot be ruled out.

Several observations suggest a role for photo-assimilate mobilization in the regulation of flowering time (see Bernier *et al.*, 1993 for a review). On one hand, exogenous sucrose treatments promote flowering of some species under non-inductive conditions (Friend *et al.*, 1984). On the other hand, plants induced to flower show a very early accumulation of endogenous sucrose at the shoot apical meristem (Bodson and Outlaw, 1985), and, in *Arabidopsis*, photoperiod inductive treatments cause a large, early and transient increase in carbohydrate export from leaves (Corbesier *et al.*, 1998). These increases in sucrose export levels are generally the result of carbohydrate mobilization rather than increased photosynthesis (Lejeune *et al.*, 1991; Lejeune *et al.*, 1993). Our observations on the effects of sucrose applied to the aerial part of the plant are consistent with a role for sucrose in the regulation of plant morphogenesis and flowering. Although some reports suggest a role for sucrose as a signalling molecule in assimilate partitioning (Chiou and Bush, 1998), further studies will be required to elucidate whether sucrose has a specific role as a signalling molecule in regulating these developmental programmes.

The delay in flowering initiation shown by gibberellin-deficient and insensitive mutants in the dark indicates that these hormones are required to promote flowering under these conditions. Because *ga1-3* mutants can still synthesize some traces of gibberellins (Zeevaart and Talón, 1992) and cannot germinate without a gibberellin treatment, we cannot establish whether the observed gibberellin requirement is absolute. Gibberellins have been shown to be completely required for *Arabidopsis* flowering under non-inductive photoperiods since *ga1-3* plants are unable to flower under short-day conditions (Wilson *et al.*, 1992). Furthermore, gibberellin applications promoted flowering of *Arabidopsis* plants grown under non-inductive photoperiodic conditions (Langridge, 1957). Thus, gibberellins could be considered as fundamental to flowering when there are no environmental inductive stimuli. The promo-

tional effects of sucrose and gibberellins on flowering time are consistent with their synergistic effect on the regulation of *LFY* expression (Blázquez *et al.*, 1998) and could suggest the occurrence of interactions between the gibberellin and sugar signal transduction pathways as has been pointed out in other systems (Perata *et al.*, 1997).

Autonomous and photoperiodic floral induction pathways are partially over-ridden by sucrose

The flowering delay of ecotypes St-0 or Le-0 is the result of dominant alleles at loci *FRI* and *FLC* and can be corrected by vernalization (Koornneef *et al.*, 1994; Lee *et al.*, 1994). A vernalization treatment also rescues the late-flowering phenotype caused by mutations at loci *FVE, FCA* or *FPA* (Martínez-Zapater and Somerville, 1990) and all these loci have been grouped in the autonomous flowering induction pathway (Koornneef *et al.*, 1998). Interestingly, in all these genotypes, the availability of sucrose at the apex has major effects in promoting flowering, especially in the dark. It is tempting to propose that sucrose, in a similar way as vernalization (Michaels and Amasino, 1999; Sheldon *et al.*, 1999), could promote flowering by regulating expression of the flowering repressor *FLC*.

The results of the analysis of late-flowering mutants in the photoperiod-dependent pathway are consistent with the existence of two partially different functions within the pathway. On one hand, the late-flowering phenotype of *co* and *gi* mutants is completely corrected under darkness and partially corrected by exogenous sucrose in the light. Since the whole pathway is dependent on photoperiod length, it could be expected that genes in the pathway were dispensable in darkness. Furthermore, the partial correction of the late phenotype in the light suggests an additive role for sucrose with respect to the function of these two genes. This possibility is compatible with the putative participation of sucrose in the autonomous pathway proposed above. On the other hand, the late-flowering phenotype of *ft* and *fwa* mutants is not corrected by sucrose, either in the dark or in the light, which even causes a further delay. This lack of effect of exogenous sucrose suggests that FT and FWA functions are required in flowering transition in steps that are downstream of sucrose. This is in agreement with the results of the characterization of double mutants *ft lfy* and *fwa lfy* which indicate a role for these genes in the control of flower meristem identity (Ruiz-García *et al.*, 1997).

Experimental procedures

Plant material

The *Arabidopsis* ecotype Landsberg *erecta* (L*er*) was the parent strain of most of the mutants used in this work. This wild type and mutant lines *hy1-1*, *hy3-1*, *ga1-3*, *ga2-1*, *ga4-1*, *ga5-1*, *gai-1, fca-1, fpa-1, fve-1, co-3*, *gi-3*, *ft-1, ft-2, ft-3*, *fwa-1* and *fwa-2* were kindly provided by Maarten Koornneef (University of Wageningen, The Netherlands) or by the Nottingham Stock Center (Nottingham, UK). Seeds belonging to ecotypes Nd-0, Col-0, Cvi-0, Le-0 were purchased from Albert Kranz (Kranz and Kirchheim, 1987).

Growth conditions

Plants were aseptically grown from seeds that had been surface-sterilized for 10 min with 30% commercial bleach containing 0.1% triton X-100 and washed with sterile water three times before sowing on 9 cm diameter Petri dishes containing the different media solidified with agar 0.8%. The basal medium used was MS mineral and vitamin solution (Murashige and Skoog, 1962), 0.5 g l^{-1} MES buffer, pH 5.7 with KOH. This medium was supplemented with sucrose (1–3%) or glucose (1–3%) when indicated. To grow seedlings in the dark, after sterilization and sowing, seeds were exposed to white-cool light (50 μE m^{-2} sec^{-1}) for 12 h to trigger germination and then wrapped with aluminium foil. Wrapped Petri dishes were stored vertically in a dry and dark room at 24°C. The lower half of the plates was aseptically sealed with parafilm before wrapping to avoid contamination due to water condensation. The upper half was sealed with transpirable tape (3M Micropore) to facilitate gas exchange. For light conditions, the same procedure was followed but vertical unwrapped plates were placed in a growth chamber with a long-day photoperiod of 16 h of white-cool light (50 μE m^{-2} sec^{-1}) at 24°C. Germination of plants homozygous for the *ga1-3* or the *ga2-1* alleles, which require exogenous gibberellins, was induced after sterilization by incubation with 100 μM GA3, for 7 days at 4°C, in darkness. After this treatment, seeds were rinsed with four changes of sterile water and sown and treated as described above.

Morphological analysis

Flowering behaviour was recorded as the number of total leaves produced. A total of 10 plants grown in two independent Petri dishes were analysed for each genotype. Mean values were compared using a *t* test. To study the growth rate under dark conditions, sets of aluminium-wrapped plates were prepared and opened for analysis at different times. Every experiment was repeated at least three times with similar results. The role of the root system in dark morphogenesis was analysed by a surgical experiment. *Arabidopsis* plants growing in darkness were cut below the shoot apex either 1 week after sowing (only cotyledons are conspicuous) or at a later stage of development (3 weeks after sowing). The excised basal parts were eliminated and the plates were sealed and wrapped again to study the development of the excised apices. All these manipulations were performed under safe green light and uncut controls were grown and manipulated in parallel to identify any effect due to the experimental procedure.

Northern blot analysis

Total RNA was isolated as described by Capel *et al.* (1998). Approximately 10 μg of each sample was loaded and separated on a formaldehyde–agarose gel and transferred to a nylon membrane (Hybond N, Amersham International, UK) according to Sambrook *et al.* (1989). The probes used were the *Apa–Sac*I fragment from the cDNA corresponding to the *Arabidopsis*

*Lhcb*1*3 (CAB) gene (Karlin-Neumann *et al.*, 1988), the *Eco*RI–*Bam*HI fragment from *Arabidopsis Rbs* S1A gene (Krebbers *et al.*, 1988) and the *Eco*RI fragment from *Arabidopsis* 18S rRNA (Pruitt and Meyerowitz, 1986). Probes were labelled by random primer extension with (α-^{32}P)dCTP (Feinberg and Vogelstein, 1983). Membranes were hybridized at standard conditions (Sambrook *et al.*, 1989) and exposed to X-ray film (Hyperfilm-MP, Amersham International, UK).

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