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Phytochrome D Acts in the Shade-Avoidance Syndrome in Arabidopsis by Controlling Elongation Growth and Flowering Time[1]

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Shade avoidance in higher plants is regulated by the action of multiple phytochrome (phy) species that detect changes in the red/far-red ratio (R/FR) of incident light and initiate a redirection of growth and an acceleration of flowering. The *phyB* mutant of Arabidopsis is constitutively elongated and early flowering and displays attenuated responses to both reduced R/FR and end-of-day far-red light, conditions that induce strong shade-avoidance reactions in wild-type plants. This indicates that phyB plays an important role in the control of shade avoidance. In Arabidopsis phyB and phyD are the products of a recently duplicated gene and share approximately 80% identity. We investigated the role played by phyD in shade avoidance by analyzing the responses of phyD-deficient mutants. Compared with the monogenic *phyB* mutant, the *phyB-phyD* double mutant flowers early and has a smaller leaf area, phenotypes that are characteristic of shade avoidance. Furthermore, compared with the monogenic *phyB* mutant, the *phyB-phyD* double mutant shows a more attenuated response to a reduced R/FR for these responses. Compared with the *phyA-phyB* double mutant, the *phyA-phyB-phyD* triple mutant has elongated petioles and displays an enhanced elongation of internodes in response to end-of-day far-red light. These characteristics indicate that phyD acts in the shade-avoidance syndrome by controlling flowering time and leaf area and that phyC and/or phyE also play a role.

Plant development is strongly influenced by the environment. Of all of the environmental factors, light arguably has the most formative influence on the life history of a plant. Cues from the light environment are involved in the regulation of seed germination, establishment of seedlings, determination of growth habit, and the transition to flowering. Plants have evolved an extensive collection of photoreceptors to perceive information about their light environment.

The phytochromes are a family of plant photoreceptors that absorb mainly in the red and far-red regions of the spectrum (Quail, 1993). The phytochrome molecule consists of a dimer of identical, approximately 124-kD protein moieties, each with a covalently linked tetrapyrrole chromophore (Furuya and Song, 1994). Phytochromes are reversibly photochromic, existing in two photointerconvertible isoforms: the biologically inactive form, Pr, and the active form, Pfr (Furuya and Song, 1994).

All higher plants examined to date contain multiple, distinct phytochrome species that are the products of a divergent gene family (Mathews and Sharrock, 1997). In Arabidopsis, which has been the subject of the most extensive study, there are five phytochromes; they are known as phyA through phyE (Clack et al., 1994). phyA, the product of the *PHYA* gene, is light labile and predominates in etiolated seedlings, where it accumulates to relatively high levels. phyB and phyC are more light stable, with phyB predominating in light-grown tissues (Somers et al., 1991). Phylogenetic analysis of the phytochrome genes of higher plants suggests that duplication of an ancestral gene at about the time of origin of the seed plants led to the divergence of two lineages, one giving rise to the phyA and phyC homologs and the other giving rise to phyB, phyD, and phyE homologs. Subsequent duplications are proposed to have occurred near the time of the origin of flowering plants (Mathews and Sharrock, 1997). In Arabidopsis the phyB and phyD proteins share approximately 80% amino acid sequence identity and are thought to result from a gene duplication in a recent progenitor of the Cruciferae (Mathews and Sharrock, 1997). The phyB and phyD proteins are more closely related to phyE (with approximately 55% identity) than they are to either the phyA or phyC proteins (with approximately 47% identity).

Establishing the roles of the individual phytochrome species has been the subject of extensive research, and much has been revealed from the study of mutants deficient in one or more phytochromes (Whitelam and Devlin, 1997). The most extensive range of phytochrome mutants has been isolated in Arabidopsis. Mutants deficient in phyA and phyB have been described in detail (Nagatani et

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Abbreviations: EOD, end-of-day; phyA to phyE, phytochromes A to E; R/FR, ratio of red light to far-red light.

al., 1993; Parks and Quail, 1993; Reed et al., 1993; Whitelam et al., 1993), and a mutant deficient in phyD has also been identified recently (Aukerman et al., 1997). Etiolated seedlings of the *phyA* mutant fail to demonstrate far-red-induced inhibition of hypocotyl elongation, promotion of cotyledon opening, and/or activation of light-responsive genes (Whitelam et al., 1993; Barnes et al., 1996). Light-grown *phyA* mutant seedlings display a more or less wild-type phenotype, although they are unable to detect a far-red-rich, low-fluence, incandescent day extension that accelerates flowering in wild-type seedlings (Johnson et al., 1994).

Etiolated seedlings of the *phyB* mutant are deficient in several responses to red light (Koornneef et al., 1980; Reed et al., 1993). Light-grown seedlings of *phyB* have an elongated, early flowering phenotype characteristic of the shade-avoidance syndrome of wild-type seedlings grown under a low R/FR or in EOD far-red-light treatments. The *phyB* mutant seedlings display attenuated responses to a low R/FR or to EOD far-red light, leading to the proposal that phyB plays a key role in the shade-avoidance response (Nagatani et al., 1991; Whitelam and Smith, 1991). Recently, Hirschfeld et al. (1998) showed that the level of phyC is significantly reduced in *phyB* mutants of Arabidopsis, suggesting that the phenotypes associated with phyB mutant genes may result in part from the attenuation of phyC signaling.

Although some shade-avoidance responses of the *phyB* mutant to a low R/FR or to EOD far-red light (e.g. petiole elongation) are severely attenuated (Nagatani et al., 1991), others, such as reduction in leaf area and the acceleration of flowering, are clearly retained (Robson et al., 1993; Halliday et al., 1994). This implicates the action of phytochromes other than phyB in the control of shade avoidance. Recently, we demonstrated that *phyA-phyB* double mutants respond to EOD far-red light by an acceleration of flowering and by a promotion of elongated internodes between rosette leaves (Devlin et al., 1996). These responses of the *phyA-phyB* double mutant to EOD far-red light are reversible by subsequent treatment with red light, indicating that one or more of phyC, phyD, or phyE controls flowering time and internode elongation (Devlin et al., 1996).

The recent discovery of a naturally occurring mutation within the *PHYD* gene of the Wassilewskija (Ws) ecotype of Arabidopsis has provided an opportunity to study the role of phyD in the response of seedlings to a low R/FR and to EOD far-red-light treatment. The monogenic *phyD* mutant created by introgression of the Ws *PHYD* gene into the Landsberg *erecta* (La-*er*) ecotype showed increased hypocotyl elongation and decreased cotyledon expansion and anthocyanin levels compared with the wild type under a continuously high R/FR, but it showed a wild-type response to EOD far-red light (Aukerman et al., 1997). However, compared with the *phyB* monogenic mutant, the *phyB-phyD* double mutant displayed elongated petioles and early flowering, phenotypes reminiscent of the shade-avoidance syndrome. These results suggest that phyD and phyB have a role in shade avoidance. We have examined the role of phyD in shade avoidance and demonstrate that phyD regulates many but not all of the responses to low R/FR or EOD far-red light previously observed in the *phyB* and *phyA-phyB* mutants.

MATERIALS AND METHODS

Plant Material

All studies were carried out in Arabidopsis Heynh, ecotype La-*er*. The phytochrome mutant alleles used in this study were *phyA-201* (Nagatani et al., 1993), *phyB-1* (Koornneef et al., 1980), *phyB-5* (Reed et al., 1993), and *phyD-1* (Aukerman et al., 1997). All of these mutant alleles, with the exception of *phyD-1*, were originally isolated from mutagenized La-*er* seeds. The *phyD-1* mutation, however, is a naturally occurring allele in the Ws ecotype, and extensive backcrossing of this mutation into the various La-*er* wild-type and mutant lines was performed for introgression into that genetic background. To generate the wild-type and *phyD* mutant near-isogenic lines used here, the Ws (*phyD-1*) line was crossed to the La-*er* wild type. A heterozygous *phyD-1*/+ F_1 progeny plant was backcrossed to La-*er*, and a heterozygous *phyD-1*/+ backcross one (BC1) F_1 plant was identified by PCR (Aukerman et al., 1997). Sequential backcrosses of heterozygous F_1 plants to the La-*er* wild type were performed for six more cycles.

A heterozygous *phyD-1*/+ BC7 F_1 plant was selfed and F_2 plants were screened by PCR to identify *PHYD+*/*PHYD+* and *phyD-1*/*phyD-1* homozygous lines. Experiments were performed using two independent BC7 F_3 or F_4 seed lots of each genotype. To generate the near-isogenic *phyB* and *phyB-phyD* mutant lines, the original La-*er phyB-1* mutant was first crossed and then backcrossed to the La-*er* wild type twice to decrease the effects of unlinked mutations resulting from the mutagenesis, and a homozygous *phyB-1* BC2 F_2 plant was identified phenotypically. This plant was crossed to Ws (*phyD-1*), the doubly heterozygous F_1 was backcrossed to the *phyB-1* line, and six more sequential backcrosses to the *phyB-1* line were performed. Two independent BC7 F_2 plants with each of the homozygous genotypes (*phyB-PHYD+* or *phyB-phyD*) were identified and used in these experiments. To generate the *phyA-phyB* and *phyA-phyB-phyD* near-isogenic lines, the *phyA-201-phyB-5* line of Reed et al. (1994) was crossed to Ws (*phyD-1*), and a similar backcrossing schedule to those described above was followed, using the *phyA-201-phyB-5* line as the recurrent parent. Two independent BC7 F_2 plants of the *phyA-phyB* and *phyA-phyB-phyD* genotypes were identified.

Growth Conditions

Seeds were sprinkled onto a moistened mixture of three parts peat compost to one part horticultural silver sand and chilled for 4 d at 4°C. Seedlings were germinated under 16-h light/8-h dark cycles for 7 d. Uniformly sized seedlings were then transplanted with even spacing into pots containing the same compost/sand mixture and placed in the experimental conditions.

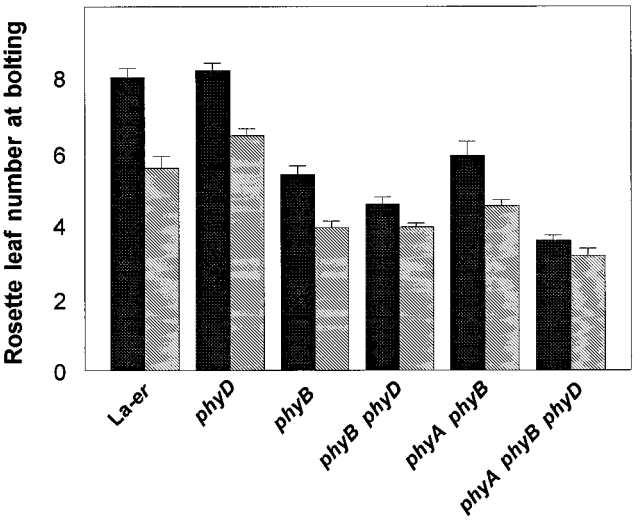

Figure 1. Effect of R/FR on flowering time in La-*er*, *phyD*, *phyB*, *phyB-phyD*, *phyA-phyB*, and *phyA-phyB-phyD*. Seedlings were grown in continuous light of either high R/FR (solid bars) or low R/FR (hatched bars). Flowering time was measured as the number of rosette leaves produced at bolting. Error bars represent SE.

Light Sources

Control conditions used in the EOD far-red experiments were 8 h of warm-white fluorescent light (photon irradiance, 400–700 nm, 102 μmol m^{-2} s^{-1}). Plants given the EOD far-red treatment received far-red light (photon irradiance, 700–800 nm, 57 μmol m^{-2} s^{-1}) obtained by filtering the output of 500-W tungsten halogen lamps (Haloline, Osram-Sylvania, Towanda, PA) through 10 mm of flowing water and one layer (3 mm) of black Plexiglas (FRF 700, West Lakes Plastics, Lenni, PA).

The R/FR cabinets were the same as those described in detail by Keiller and Smith (1989), and continuous irradiation was used. The high R/FR cabinet provided a photon irradiance, 400–700 nm, of 85 μmol m^{-2} s^{-1} and a R/FR of 5.64. The low R/FR cabinet provided the same photon irradiance but a R/FR of 0.15.

All light measurements were made using a PSII spectroradiometer (Li-Cor, Lincoln, NE).

Measurements of Growth and Flowering

All measurements were made after plants had completed bolting, and the data represent the means ± SE from 15 plants. Internode and petiole lengths were measured with a ruler. Petiole lengths were determined for the largest fully grown leaf, and internode lengths were measured for the internode between rosette leaves 5 and 6. Flowering time was recorded as the number of rosette leaves at inflorescence production. As previously described (Devlin et al., 1996), rosette leaves were readily distinguished from cauline leaves on the basis of their morphology. Leaf area was measured for the largest fully grown leaf using a leaf area meter (LI-3000, Li-Cor).

RESULTS

Effect of Reduced R/FR and EOD Far-Red Light on Flowering Time

Previously it was observed that *phyB* and *phyA-phyB* mutant seedlings display an acceleration of flowering in response to low R/FR or to EOD far-red-light treatment, indicating the action of a phytochrome other than phyA or phyB (Robson et al., 1993; Halliday et al., 1994; Devlin et al., 1996). We examined the effect of phyD deficiency on these responses to determine whether phyD plays a role in the control of flowering time in response to alterations in light quality.

Wild-type and mutant seedlings were grown under a continuously high R/FR for 10 d and were then either maintained under these conditions or transferred to conditions of low R/FR. Wild-type seedlings showed a pronounced acceleration in flowering in response to a low R/FR, and the *phyD* mutant behaved in exactly the same way (Figs. 1 and 2). Under a high R/FR, the *phyB* mutant flowered considerably earlier than the wild type and displayed a reduced response to a low R/FR (Figs. 1 and 2). The *phyB-phyD* double mutant flowered earlier than *phyB* under a high R/FR and displayed a more reduced response to a low R/FR, although an acceleration of flowering was still apparent. Similarly, compared with the *phyA-phyB* double mutant, the *phyA-phyB-phyD* triple mutant flowered earlier under high R/FR conditions and showed a reduced response to a low R/FR (Figs. 1 and 2). This response, although very small, was still statistically significant.

We also examined the role of phyD in the flowering response of the *phyA-phyB* double seedlings to EOD far-

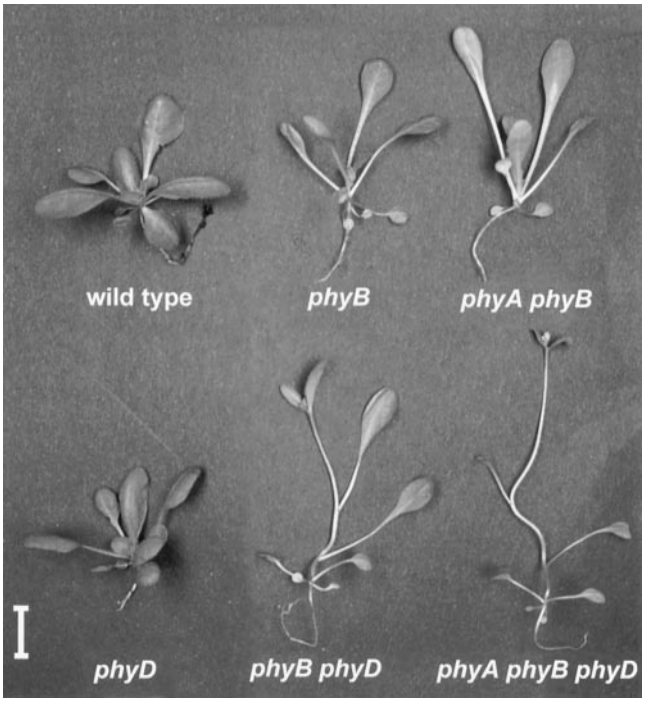

Figure 2. Phenotypes of La-*er*, *phyD*, *phyB*, *phyB-phyD*, *phyA-phyB*, and *phyA-phyB-phyD* grown in continuous light of high R/FR for 30 d. Scale bar = 1 cm.

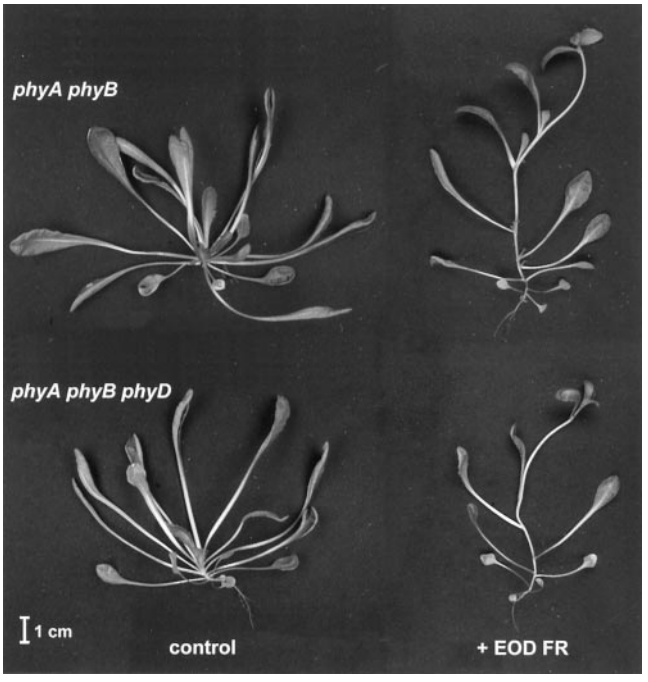

Figure 3. Appearance of internodes in *phyA-phyB* and *phyA-phyB-phyD* in response to EOD far-red-light treatment. Seedlings were grown for 60 d in either 8 h of light/16 h of dark (control) or with the same photoperiods plus 15 min of EOD far-red light.

red-light treatment. The seedlings were grown in control (8 h) photoperiods with or without an EOD far-red-light treatment, and flowering time was measured as leaf number at bolting. As observed previously (Devlin et al., 1996), the *phyA-phyB* double mutant displayed a pronounced acceleration of flowering in response to EOD far-red light. The *phyA-phyB-phyD* triple mutant flowered earlier than the *phyA-phyB* double mutant under control conditions (Figs. 3 and 4a). Furthermore, the response of the *phyA-phyB-phyD* triple mutant to EOD far-red-light treatment was less than that seen for the *phyA-phyB* double mutant (Figs. 3 and 4a). Nevertheless, the triple-mutant seedlings still showed a clear acceleration of flowering in response to EOD far-red-light treatment.

Effect of EOD Far-Red Light on Internode Length

In response to EOD far-red-light treatments the *phyA-phyB* double mutant displayed a promotion of internode elongation such that the seedlings no longer had a rosette appearance (Devlin et al., 1996). In the present study, the internode elongation response was compared in the *phyA-phyB* double mutant and the *phyA-phyB-phyD* triple mutant. The *phyA-phyB* double mutant retained the appearance of a rosette plant under control conditions but showed a pronounced promotion of internode elongation in response to EOD far-red-light treatment (Figs. 3 and 4b). The *phyA-phyB-phyD* triple mutant also retained the appearance of a rosette plant under control conditions and showed a pronounced promotion of internode elongation in response to EOD far-red-light treatment (Figs. 3 and 4b). The extent of the internode elongation response to EOD far-red light was greater in *phyA-phyB-phyD* than in *phyA-phyB* mutant seedlings (Figs. 3 and 4b).

Effect of EOD Far-Red Light on Petiole Length

Consistent with previous findings (Devlin et al., 1996), the *phyA-phyB* double mutant showed a reduction in petiole length in response to EOD far-red-light treatments (Fig. 4c). Following growth under control conditions the *phyA-phyB-phyD* triple mutant displayed longer petioles than the *phyA-phyB* double mutant (Fig. 4c). In response to EOD far-red-light treatments, the *phyA-phyB-phyD* triple mutant showed a more marked reduction in petiole length than the *phyA-phyB* double mutant (Fig. 4c).

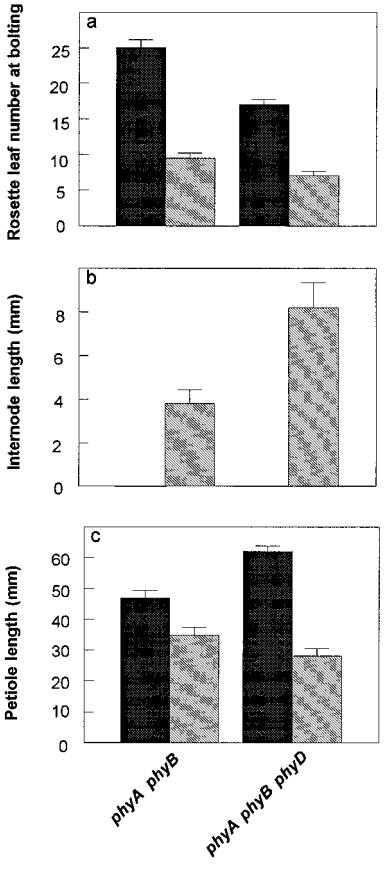

Figure 4. Responses to EOD far-red light in *phyA-phyB* and *phyA-phyB-phyD* mutants. Seedlings were grown with 8-h light/16-h dark photoperiods (control, black bars) or in the same photoperiods plus 15 min of EOD far-red light (hatched bars). a, Effect of EOD far-red light on flowering time in *phyA-phyB* and *phyA-phyB-phyD* measured as the number of rosette leaves produced at bolting. Error bars represent SE. b, Effect of EOD far-red light on internode elongation in *phyA-phyB* and *phyA-phyB-phyD*. The length of the internode between "rosette" leaves 5 and 6 was measured after bolting had occurred. Error bars represent SE. c, Effect of EOD far-red light on petiole length in La-*er*, *phyD*, *phyB*, *phyB-phyD*, *phyA-phyB*, and *phyA-phyB-phyD*. Petiole length from the largest fully grown leaf was measured after bolting had occurred. Error bars represent the SE.

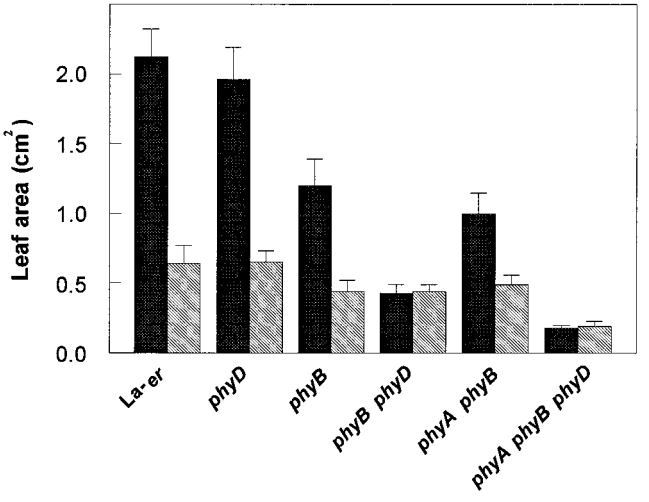

Figure 5. Effect of R/FR on leaf area. La-*er*, *phyD*, *phyB*, *phyB-phyD*, *phyA-phyB*, and *phyA-phyB-phyD* were grown in continuous light of either high R/FR (black bars) or low R/FR (hatched bars). Leaf area of the largest fully grown leaf was measured after bolting had occurred. Error bars represent the SE.

Effect of Reduced R/FR on Leaf Area

In response to a low R/FR, wild-type seedlings displayed a decrease in leaf area (Fig. 5). The *phyB* mutant, although having a reduced leaf area compared with the wild type, also showed a pronounced reduction in leaf area in response to low R/FR (Fig. 5). When grown under high R/FR conditions, the *phyB-phyD* double mutant displayed small leaves and showed no further decrease in leaf area in response to a reduction in the R/FR (Fig. 5). The *phyA-phyB* double mutant behaved identically to *phyB*, displaying a marked decrease in leaf area under low R/FR. The phenotype of the *phyA-phyB-phyD* triple mutant was similar to that of the *phyB-phyD* double mutant, displaying a constitutively reduced leaf area and showing no further reduction in area in response to a low R/FR (Fig. 5).

DISCUSSION

The shade-avoidance syndrome constitutes one of the most prominent roles for the phytochromes in higher plants. In Arabidopsis the most noticeable shade-avoidance responses were the promotion of elongation growth and flowering and the reduction in leaf area. The elongated, early flowering phenotype of the *phyB* mutant, coupled with its attenuated response to low R/FR or EOD far-red-light treatments, had indicated a major role for phyB in the control of shade-avoidance responses (Smith and Whitelam, 1997). However, phyB-null mutants still retained responses to a reduced R/FR, demonstrating the involvement of phytochromes other than phyB (Robson et al., 1993). Because the *phyA-phyB* double mutant also retained marked shade-avoidance responses, a significant role for phyA can be excluded.

We have exploited the discovery of a null mutation within the *PHYD* gene in Arabidopsis ecotype Ws to investigate the role of phyD in the shade-avoidance syndrome. The observation that the *phyB-phyD* double mutant flowered earlier and had more elongated petioles than the monogenic *phyB* mutant (Aukerman et al., 1997) suggests that phyD makes a significant contribution to the shade-avoidance response. The high degree of sequence conservation between phyB and phyD (Mathews and Sharrock, 1997) and the similar patterns of expression of the *PHYB* and *PHYD* promoters (Goosey et al., 1997) are consistent with a similarity in function of these phytochromes.

Although in our experiments and those of Aukerman et al. (1997) the monogenic *phyD* mutant displayed a wild-type flowering time phenotype, the *phyB-phyD* double mutant flowered considerably earlier than the *phyB* mutant. Furthermore, compared with the monogenic *phyB* mutant, the *phyB-phyD* double mutant displayed a reduced response to a low R/FR. Likewise, we observed that for plants grown under 8-h photoperiods, the *phyA-phyB-phyD* triple mutant flowered much earlier than the *phyA-phyB* double mutant. Furthermore, the *phyA-phyB-phyD* triple mutant displayed a reduced flowering response to EOD far-red-light treatment compared with the *phyA-phyB* double mutant. These observations indicate a role for phyD similar to that of phyB in the control of flowering time in response to light quality. However, the absence of a detectable mutant phenotype in monogenic *phyD* seedlings suggests that there is a redundancy of function. Furthermore, the retention of an early flowering response to EOD far-red-light treatments in the *phyA-phyB-phyD* triple mutant indicates the participation of other phytochromes in this response.

For plants growing under continuous high R/FR, the *phyA-phyB-phyD* triple mutant displayed an extremely early flowering phenotype. The channeling of available resources into flowering at such an early stage most likely accounts for the dramatic reduction in the size of *phyA-phyB-phyD* plants. A consequence of this extremely early flowering behavior under high R/FR is that a response of the *phyA-phyB-phyD* triple mutant to low R/FR was barely detectable. As a result the analysis of the effects of EOD far-red-light treatments provided a more sensitive assay for the retention of shade-avoidance responses in the *phyA-phyB-phyD* triple mutant. Flowering of the *phyA-phyB-phyD* triple mutant was much slower under the 8-h photoperiods used for analysis of responses to EOD far-red light, and under these conditions the triple mutant produced a substantial rosette.

Redundancy of phyD function was also observed with respect to the control of leaf area by R/FR. The actions of phyB and phyD alone can fully account for these observed responses, with the role of phyD being revealed only in the absence of phyB. Thus, whereas the monogenic *phyD* mutant displays a wild-type leaf-area phenotype under all conditions tested, the *phyB* mutant displays a reduced leaf area following growth under high R/FR conditions. The *phyB* mutant shows a further reduction in leaf area in response to low R/FR, in agreement with the results of Robson et al. (1993). *PhyB-phyD* double-mutant plants grown under high R/FR conditions displayed a reduced leaf area equivalent to that of the *phyB* mutant grown under low R/FR conditions. Furthermore, leaf area in the

phyB-phyD double mutant showed no further reduction in response to low R/FR.

The phenotype of the *phyA-phyB-phyD* triple mutant was very similar to that of the *phyB-phyD* double mutant, indicating that phyA played little or no role in controlling this response. Thus, phyD action can fully account for the responsiveness of the monogenic *phyB* mutant and the *phyA-phyB* double mutant to R/FR. In these experiments we observed that the *phyB* mutation led to a reduction in leaf area in plants grown under high R/FR conditions. This is contrary to the observations of Robson et al. (1993), who observed an increase in leaf area as a result of the *phyB* mutation. We assume that the different behavior of the *phyB* mutant is a reflection of the different growth conditions used in the two studies.

Previously, Aukerman et al. (1997) had shown that petiole length under continuous light was unaffected by the presence of the *phyD* mutation, but that the elongated petiole phenotype seen in *phyB* mutants was exaggerated in a *phyB-phyD* double mutant. This indicated that phyD also played a role in the control of petiole elongation but that phyD was redundant in the presence of phyB. In the present study we observed that for plants grown under 8-h photoperiods the *phyA-phyB-phyD* triple mutant displayed longer petioles than did the *phyA-phyB* double mutant. However, unlike flowering time, for this response the deficiency of phyD did not phenocopy the response of the *phyA-phyB* double mutant to EOD far-red-light treatment. The petioles of the *phyA-phyB* double mutant showed reduced elongation in response to EOD far-red light, an apparently correlative growth effect that accompanied the elongation of internodes between "rosette" leaves (Devlin et al., 1996).

In the present study the *phyA-phyB-phyD* triple mutant maintained a normal rosette habit, albeit with longer petioles, when grown under control conditions. The triple mutant also showed a greater reduction in petiole length in response to EOD far-red light than was observed for the *phyA-phyB* double mutant. This was correlated with an increased internode elongation response in the *phyA-phyB-phyD* triple mutant compared with the *phyA-phyB* double mutant. These observations suggest that phyD played a role not just in the inhibition of petiole elongation, but also in the inhibition of internode elongation. However, the action of phyD in controlling internode elongation was revealed only when both phyA and phyB were absent and plants received EOD far-red-light treatments (data not shown). This suggests that phytochromes other than phyA, phyB, and phyD were active in the maintenance of the rosette habit for plants grown under control conditions.

The shade-avoidance syndrome in Arabidopsis is clearly regulated by a complex interaction of signals from multiple phytochrome species. The finding that phyD plays a significant role in the control of shade-avoidance responses is interesting in view of the high degree of amino acid sequence identity that it shares with phyB, the major phytochrome controlling shade avoidance in Arabidopsis. However, for all responses examined, the *phyD* mutant phenotype was apparent only in the absence of phyB (Aukerman et al., 1997). Thus, although phyD played a role in the shade-avoidance syndrome in Arabidopsis, this role appeared to be redundant in the presence of phyB. This investigation has also demonstrated the action of phytochromes other than phyA, phyB, and phyD in the control of flowering time and internode elongation in response to light quality.

We recently isolated a *phyE* mutant of Arabidopsis after the mutagenesis of *phyA-phyB* seed and screening of the M_2 population for individuals that constitutively displayed elongated internodes (Devlin et al., 1998). In addition to having elongated internodes, the *phyA-phyB-phyE* triple mutant was also early flowering compared with the *phyA-phyB* double mutant, indicating that, like phyB and phyD, phyE was a pleiotropic regulator of shade-avoidance responses (Devlin et al., 1998). The similarity of function of phyB, phyD, and phyE correlates with a shared phylogeny, in that phyB, phyD, and phyE form a related subgroup within the phytochromes of dicots (Mathews and Sharrock, 1997). However, the functions of these three phytochromes did not overlap completely, and the *phyA-phyB-phyD* triple mutant was readily distinguishable from the *phyA-phyB-phyE* triple mutant. The availability of these various phytochrome mutants will allow the creation of the *phyA-phyB-phyD-phyE* quadruple mutant, which will provide the opportunity to assess the functions of phyC.

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