Plant Cell Physiol. 46(1): 213–223 (2005)
doi:10.1093/pcp/pci016, available online at www.pcp.oupjournals.org
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The Different Growth Responses of the *Arabidopsis thaliana* Leaf Blade and the Petiole during Shade Avoidance are Regulated by Photoreceptors and Sugar

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During the shade-avoidance response, leaf blade expansion is inhibited and petiole elongation is enhanced. In this study, we examined the roles of photoreceptors and sugar on the differential growth of the leaf blade and petiole in shade conditions. Under the conditions examined, cell expansion, not cell division, played a major role in the differential leaf growth. The enhanced cell expansion in the leaf blade is associated with an increase in the ploidy level, whereas cell elongation was stimulated in the petiole in dark conditions without an increase in the ploidy level. Analysis of phytochrome, cryptochrome and phototropin mutants revealed that phytochromes and cryptochromes specifically regulate the contrasting growth patterns of the leaf blade and petiole in shade. Examination of the effects of photo-assimilated sucrose on the growth of the leaf blade and petiole revealed growth-promotional effects of sucrose that are highly dependent on the light conditions. The leaf blades of abscisic acid-deficient and sugar-insensitive mutants did not expand in blue light, but expanded normally in red light. These results suggest that both the regulation of light signals and the modulation of responses to sugar are important in the control of the differential photomorphogenesis of the leaf blade and petiole.

Keywords: ABA — *Arabidopsis* — Leaf — Light signals — Shade avoidance — Sucrose

Abbreviations: GUS, β-glucuronidase.

Introduction

In many species of dicotyledonous plants, the leaf consists of a blade and a petiole. Detailed anatomical and mitotic analyses in *Arabidopsis thaliana* (L.) Heynh. have shown that the morphogenesis of each part is mediated by unique developmental processes (Donnelly et al. 1999, Tsukaya et al. 2002). The leaf blade has a wide, flat laminar structure within which several layers of palisade and spongy tissue cells efficiently absorb light. The leaf petiole or stalk supports the leaf blade and delivers it to positions that are more appropriate for photosynthesis. Interestingly, during shade avoidance, leaf blade expansion is inhibited and, at the same time, petiole elongation is enhanced (Nagatani et al. 1991, Reed et al. 1993, Tsukaya et al. 2002). The effects of light conditions on the control of leaf blade expansion and petiole elongation are important mechanisms in the light acclimation of plants.

To plants, light constitutes an indicator of the environment in which the plant grows, an indicator that is gauged by the intensity, quality and direction of incident light, as well as the essential energy source for photosynthesis and the production of sugar. Plants under a canopy perceive decreases in the intensities of blue and red wavelengths and increases in far-red wavelengths (Neff et al. 2000). This change in light quality serves as a warning of competition for sunlight and triggers the shade-avoidance response (Whitelam and Smith 1991). The change in light quality is detected by phytochromes, with the Pfr form of phyB playing a major role in suppressing the shade-avoidance response (Devlin et al. 1999, Franklin et al. 2003a). *Arabidopsis thaliana* contains the phytochrome-encoding genes *PHYA* to *PHYE* (Sharrock and Quail 1989, Nagatani et al. 1993, Reed et al. 1993, Devlin et al. 1998, Devlin et al. 1999, Franklin et al. 2003b). Phytochrome regulates many physiological and developmental responses, such as circadian rhythms, de-etiolation and shade avoidance (Neff et al. 2000). In addition, *A. thaliana* contains genes for blue light receptors, including two cryptochrome (*CRY1* and *CRY2*) and two phototropin (*PHOT1* and *PHOT2*) genes (Ahmad and Cashmore 1993, Huala et al. 1997, Lin et al. 1998, Kagawa et al. 2001, Sakai et al. 2001). Cryptochrome regulates hypocotyl elongation, gene expression and the input for circadian clocks (Cashmore et al. 1999). Phototropin regulates phototropism, chloroplast relocation and stomatal opening (Briggs and Chris-

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Table 1 The areas of the leaf blade and the palisade cells in the leaf blade

Treatment	Leaf blade area (mm^2, $n \geq 6$)	Cell area (μm^2, $n \geq 250$)		
		Basal [a]	Middle [a]	Apical [a]
Before	16.1 (3.4)	171.3 (50.5)	205.6 (57.0)	242.9 (68.0)
Dark	16.5 (1.2)	182.2 (67.5)	220.2 (62.9)	254.1 (83.7)
White light	31.0 (2.2)	328.5 (96.2)	470.6 (123.2)	343.8 (103.0)

Fourteen-day-old seedlings (Col) that had been incubated in continuous white light (50 μmol m^{-2} s^{-1}) were subjected to the pre-treatment and then placed in darkness for 42 h. The cell areas were determined in the basal, middle and apical portions of the leaf blade. The data are represented as the means (SD).
[a] The partitions were divided equally along with the blade length.

Table 2 The lengths of the leaf petiole and the mesophyll cells in the leaf petiole

Treatment	Leaf petiole length (mm, $n \geq 6$)	Cell length (μm, $n \geq 50$)		
		Basal [a]	Middle [a]	Apical [a]
Before	3.1 (0.2)	28.4 (7.8)	23.2 (5.8)	19.5 (4.2)
Dark	12.2 (0.3)	127.2 (25.9)	88.6 (23.3)	39.4 (10.5)
White light	6.8 (0.8)	78.8 (17.3)	55.2 (16.9)	27.5 (7.1)

Plants were grown as described in Table 1.The lengths of mesophyll cells were determined in the basal, middle and apical portions of the leaf petiole. The data are represented as the means (SD).
[a] The partitions were divided equally along with the petiole length.

tie 2002). However, there is little information of the involvement of these photoreceptor families in leaf blade expansion or petiole elongation, although cryptochrome inhibits hypocotyl elongation and enhances cotyledon expansion in de-etiolated seedlings in the same manner as phytochrome (Neff and Chory 1998). Therefore, to understand light-dependent leaf morphogenesis, it is necessary to investigate the control mechanisms of leaf blade and petiole growth separately, under various light conditions.

In the present study, we also found out the role of photoassimilated sugars in light-dependent leaf development. Many physiological and developmental processes in plants, including leaf growth, are influenced by photoassimilated sugars that act as resources and/or signals (Moore et al. 2003). For example, sugar sensing affects the size of *A. thaliana* rosette leaves (Leon and Sheen 2003, Moore et al. 2003), suggesting that sugars have important roles in leaf growth.

Recent studies have suggested extensive connections between sugar signaling and phytohormone pathways, in which abscisic acid (ABA) acts positively and ethylene acts negatively (Laby et al. 2000, Gibson et al. 2001, Arroyo et al. 2003, Yanagisawa et al. 2003). The relationship between sugar signaling and phytohormone regulation in plant organogenesis differs under varying light conditions. For example, the expression of *ABA2*, which is involved in ABA biosynthesis, is up-regulated by glucose and is stronger in petioles than in leaf blades (Cheng et al. 2002), and the expression of several genes involved in ethylene biosynthesis is induced in response to low light intensity and defects in phyB (Vandenbussche et al. 2003). These observations suggest that the sugar signaling pathway is modulated by the sugar itself and the light signaling system. In contrast, the effects of sugar on the photomorphogenesis of the leaf blade and petiole are unknown.

In this study, to clarify the effects of both light and sugars on the differential growth of leaf blades and petioles, we examined the relative contributions of all available classes of photoreceptor mutations to leaf growth in response to monochromatic blue, red and far-red light. Both phytochrome and cryptochrome were shown to have important roles in the promotion of leaf blade expansion and the inhibition of leaf petiole elongation in light, through cell enlargement. In addition, it was clearly shown that in white light and darkness, sucrose has different effects on the growth promotion of leaf blades and petioles. Furthermore, we suggest that endogenous ABA is required to promote leaf blade expansion in response to blue light but not red light.

Results

Organogenetic and cellular analyses of leaf blade and leaf petiole growth

To examine the effects of light irradiation on the growth of leaf blades and petioles, 14-day-old wild-type seedlings grown under continuous white light were exposed to far-red light for 15 min and then placed in darkness for 6 h. These processes are referred to as 'pre-treatment'. Following the pre-treatment, the seedlings were cultivated for 42 h under continuous irradiation with light of various intensities and qualities. Tables 1 and 2 show the areas and lengths of leaf blades and petioles before and after cultivation in white light or darkness for 42 h, respec-

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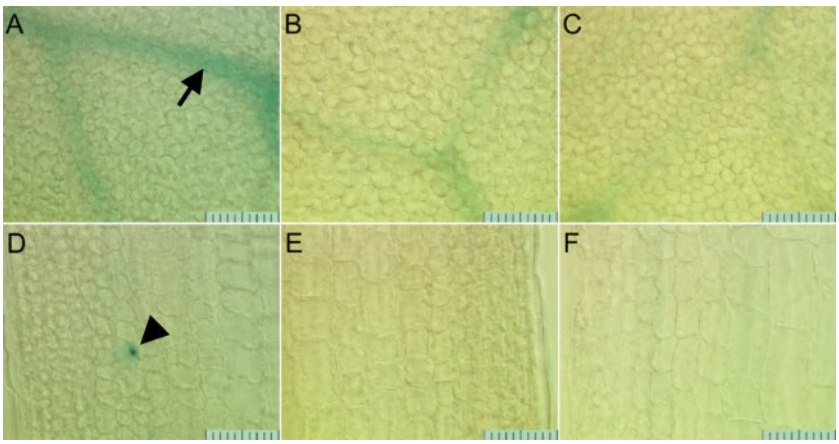

Fig. 1 Expression and localization of *cyc1At::GUS* activity in leaf blades (A, B and C) and petioles (D, E and F). Fourteen-day-old seedlings of transgenic plants were subjected to the pre-treatment, after which the fifth rosette leaves were stained for GUS activity before (A and D) and after 42 h of incubation under either continuous white light (50 μmol m^{-2} s^{-1}; B and E) or in darkness (C and F). The *GUS* activity was examined for the fifth rosette leaves from 10 plants. The arrow indicates the GUS-expressing vein and the arrowhead indicates a GUS-positive cell. Bars indicate 10 μm.

tively. During the dark treatment, the expansion of leaf blades almost completely ceased, whereas the leaf blades nearly doubled in area during white light treatment (Table 1). The leaf petioles elongated by 9.1 mm in darkness and 3.7 mm in white light (Table 2). These results confirmed our earlier observations (Tsukaya et al. 2002).

To investigate the roles of cell division and cell expansion in leaf blade expansion and petiole elongation during the treatment period (Tables 1 and 2), the sizes of cells in the basal, middle and apical portions of leaf blades and petioles were measured. Measurements were made on paradermal images. As shown in Table 1, the increase in the area of the leaf blade promoted by white light correlated with an increase in the areas of the palisade cells. The areas of the cells in the middle portion of the leaf blades increased most dramatically. The increase in the leaf petiole length in darkness also correlated with an increase in the length of the mesophyll cells (Table 2), with the mesophyll cells in the basal portion of the leaf petiole elongating more than those in the apical portion. Because this trend was evident in both white light and darkness, these results suggest that light modulates the extent of the cell expansion in petioles without affecting the spatial growth pattern.

Next, we monitored the cell proliferation activity using a *cyc1At::GUS* reporter construct, which is a specific marker for the G_2/M phase of the cell cycle (Donnelly et al. 1999), using the light treatments described in Fig. 1. When the fifth rosette leaves were analyzed for β-glucuronidase (GUS) activity just before and after these treatments, GUS-positive cells were occasionally detected among the mesophyll cells (Fig. 1A, D) but not at all among the pavement cells. Some stomata-forming meristemoids and vein procambial cells had GUS activity before the white light or dark treatment (data not shown). In contrast, after 48 h of either white light or dark treatment, almost no GUS activity was detectable (Fig. 1B, C, E, F), except in stomata-forming meristemoids and vein procambial cells (data not shown, Donnelly et al. 1999). We also examined the effects of the light condition on the cell number of the petiole. During the dark treatment for 42h, the number of mes-

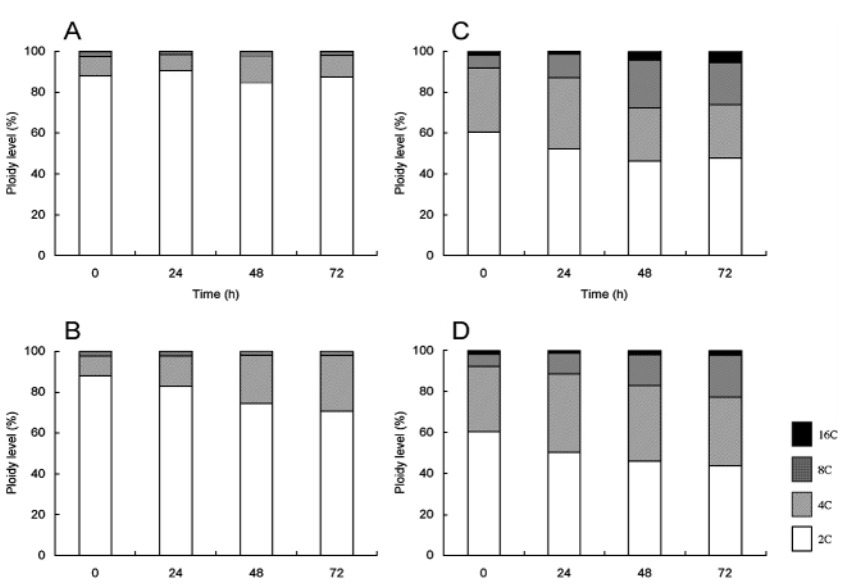

Fig. 2 The effects of darkness (A and C) and white light (50 μmol m^{-2} s^{-1}; B and D) on the DNA content of wild-type seedlings. Fourteen-day-old seedlings were subjected to the pre-treatment, after which leaf blades (A and B) and petioles (C and D) were harvested at the indicated times and analyzed using flow cytometry.

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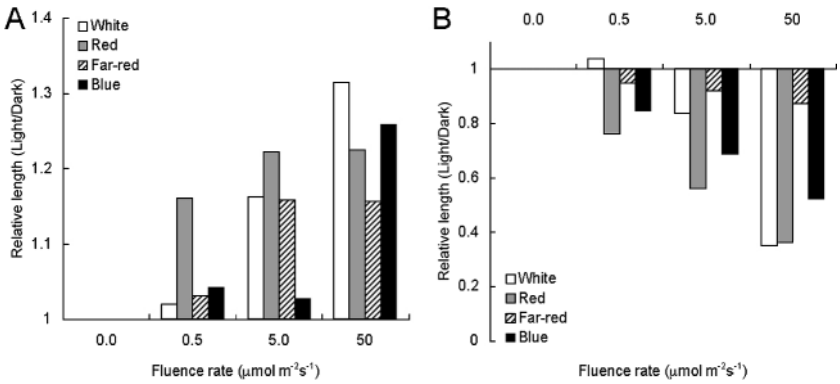

Fig. 3 The relationship between the relative length of leaf blades (A) and petioles (B) and the fluence rate of the various light qualities. Wild-type Col-0 seedlings were grown under continuous white light (50 μmol m^{-2} s^{-1}) for 14 d and then subjected to the pre-treatment. The seedlings were next irradiated with continuous white, red, far-red or blue light for 42 h at the fluence rates indicated, and the lengths of the blades and petioles of the fifth rosette leaves were measured just before and just after the irradiation treatments. In (A), the length of the leaf blade is expressed relative to that of dark-grown plants (5.7 mm, see Table 1). In (B), the elongation of the petiole during the light treatment is expressed relative to that of dark-grown plants (9.1 mm, see Table 1). Each value is the mean of at least three independent experiments, with $n \geq 15$ for each experiment. Significantly different values (Student's *t*-test; $P < 0.05$) were obtained for seedlings grown in darkness and in 50 μmol m^{-2} s^{-1} for all of the light conditions.

ophyll cells was increased slightly compared with before the treatment. Moreover, no significant difference was shown between before and after the white light treatment (data not shown). Taken together, our results show that light-regulated leaf blade expansion and leaf petiole elongation are due mainly to cell enlargement, although light has contrasting effects on leaf blades and petioles.

Analysis of the ploidy level of leaf blades and petioles grown in light and dark conditions

Using flow cytometry, we examined whether endoreduplication is involved in the growth regulation of leaf blades and petioles, since endoreduplication in hypocotyls is controlled differentially in light and darkness (Gendreau et al. 1998). Fig. 2 shows the ploidy levels of leaf blades and petioles grown in darkness or white light (120 μmol m^{-2} s^{-1}). The ploidy profiles of the leaf blades remained constant during the dark treatment (Fig. 2A). In white light, the proportion of 2C nuclei decreased, with a corresponding increase in the 4C nuclei, and the levels of 8C and 16C nuclei were unchanged (Fig. 2C). In leaves treated with white light (120 μmol m^{-2} s^{-1}) for 72 h, the leaf blades expanded by 3.9 ± 0.9 mm (mean ± SD; $n \geq 15$), but the leaf blades did not expand during dark treatment. These results suggest that the light dependency of leaf blade expansion is correlated with an enhanced endoreduplication cycle. In contrast, in leaf petioles, only small differences in the levels of 4C to 16C nuclei were observed between plants grown in light and dark conditions (Fig. 2B, D). The leaf petioles elongated by 3.5 ± 0.9 mm (mean ± SD; $n \geq 15$) when exposed to white light (120 μmol m^{-2} s^{-1}) for 72 h, and by 12.8 ± 1.0 mm (mean ± SD; $n \geq 15$) when held in darkness for 72 h. These data indicate that in the petiole, endoreduplication proceeds independently of the light conditions and the extent of growth.

The effects of wavelength on the growth of leaf blades and petioles

Next, we examined the effects of different light environments on leaf growth. The data are expressed relative to the lengths of the dark treatment rather than actual values, since the basal growth of the mutant lines used varied. As shown in Fig. 3, continuous white, red, far-red and blue light enhanced the growth of the leaf blade, which increased with the light intensity. In contrast, continuous white, red, far-red and blue light inhibited leaf petiole elongation; this effect also increased with the light intensity (Fig. 3). These results suggest that leaf photomorphogenesis during the shade-avoidance response is regulated not only by the ratio of red to far-red light, but also by the fluence rate of each monochromatic wavelength. The fluence response curves of the sources of monochromatic light used differed. With red light irradiation, a promotion of leaf blade elongation was evident, even at 0.5 μmol m^{-2} s^{-1} (Fig. 3A), whereas no enhanced growth of the leaf blade was observed under blue light of intensities of <5.0 μmol m^{-2} s^{-1} (Fig. 1A). This suggests that the mechanisms that promote the growth of leaf blades in red and blue light differ. Leaf petiole elongation was strongly inhibited under red and blue light irradiation, but was only weakly inhibited under far-red light (Fig. 3B). These results suggest that far-red light has a mild effect on leaf petiole elongation. Low-intensity (0.5 μmol m^{-2} s^{-1}) white light did not inhibit the elongation of the leaf petiole (Fig. 3B), a phenomenon which may be due to the low proportion of red and blue light emitted from white fluorescent tubes.

Regulation of leaf blade expansion and petiole elongation by phytochrome and cryptochrome

As shown in Fig. 3, the mechanisms of promotion of the growth of leaf blades and petioles by different light qualities

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Table 3 The relative lengths of leaf blade cultured in darkness, red light or blue light conditions

Strain (ecotype)	Relative length [a] (%)			SD		n [c]
	Dark	Red [b]	Blue [b]	Red [b]	Blue [b]	
Wild type (L*er*)	100	140.8	144.6	3.3	6.7	3
Wild type (Col)	100	127.0	125.1	7.3	10.6	5
phyA (L*er*)	100	**130.1**	129.4	3.6	8.4	4
phyB (L*er*)	100	**115.0**	136.0	6.6	7.3	4
phyD (L*er*)	100	141.2	141.6	9.2	12.5	3
phyA phyB (L*er*)	100	**114.7**	143.8	10.0	10.2	6
phyB phyD (L*er*)	100	**108.5**	141.5	4.6	9.1	5
cry1 (L*er*)	100	131.0	**128.5**	7.8	7.0	5
cry1cry2 (L*er*)	100	149.1	**124.6**	8.0	6.2	3
phot1phot2 (Col)	100	127.7	127.7	2.1	6.9	5
cry1cry2phot1phot2 #33 [d]	100	143.2	**117.3**	12.7	4.1	4
cry1cry2phot1phot2 #57 [d]	100	129.1	**116.0**	10.6	6.0	5
cry1cry2phot1phot2 #210 [d]	100	146.2	**120.8**	6.0	5.3	3
cry1cry2phot1phot2 #257 [d]	100	140.3	**120.0**	15.2	6.5	3

Twelve-day old seedlings were subjected to the pre-treatment, and the lengths of third rosette leaf blades were measured after seedlings were incubated in darkness, red light and blue light conditions for 42 h.
[a] Relative value of the lengths of leaf blade of each genotype in darkness.
[b] 50 μmol m^{-2} s^{-1}.
[c] The number of experiments (15 leaves were examined number for each experiment).
[d] See Materials and Methods.
Values in bold are significantly different from wild type (Student's *t*-test; $P < 0.05$).

Table 4 The relative lengths of leaf petiole cultured in darkness, red light or blue light conditions

Strain (ecotype)	Relative length [a] (%)			SD		n [c]
	Dark	Red [b]	Blue [b]	Red [b]	Blue [b]	
Wild type (L*er*)	100	56.0	56.0	4.9	6.2	3
Wild type (Col)	100	67.3	60.9	2.8	10.1	5
phyA (L*er*)	100	**66.8**	75.2	4.1	11.8	4
phyB (L*er*)	100	**124.2**	67.2	10.0	10.2	4
phyD (L*er*)	100	63.8	59.8	11.7	12.2	3
phyA phyB (L*er*)	100	**105.3**	69.5	17.1	13.0	6
phyB phyD (L*er*)	100	**105.1**	54.6	13.6	5.2	5
cry1 (L*er*)	100	55.0	**77.7**	11.0	8.7	5
cry1cry2 (L*er*)	100	**70.4**	**101.9**	4.6	5.7	3
phot1phot2 (Col)	100	62.1	66.8	4.9	2.9	5
cry1cry2phot1phot2 #33 [d]	100	56.3	**93.8**	4.7	7.7	4
cry1cry2phot1phot2 #57 [d]	100	50.8	**89.2**	3.3	12.9	5
cry1cry2phot1phot2 #210 [d]	100	59.2	**101.2**	4.7	6.5	3
cry1cry2phot1phot2 #257 [d]	100	62.7	**113.4**	5.9	5.7	3

Plants were grown as described in Table 3, after which the lengths of third rosette leaf petiole were measured.
[a] Relative value of the lengths of leaf petiole of each genotype in darkness.
[b] 50 μmol m^{-2} s^{-1}.
[c] The number of experiments (15 leaves were examined number for each experiment).
[d] See Materials and Methods.
Values in bold are significantly different from wild type (Student's *t*-test; $P < 0.05$).

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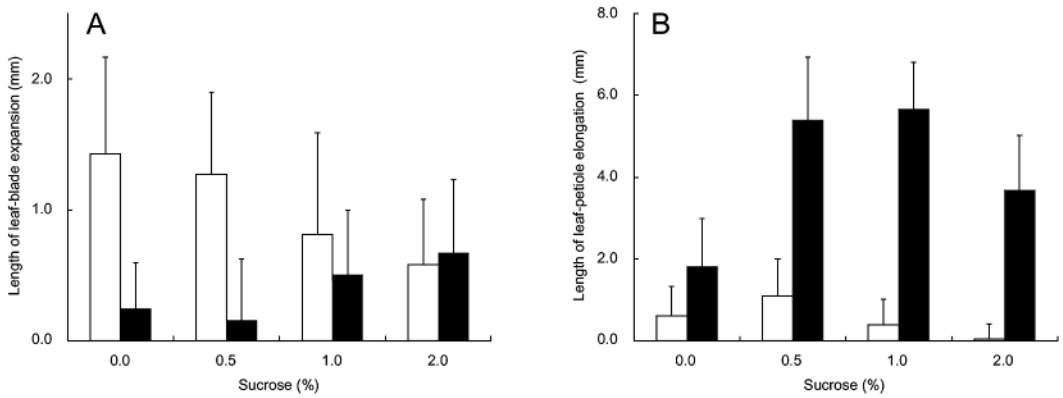

Fig. 4 The effects of exogenous sucrose on the growth of the leaf blade and petiole. In (A) and (B), the fifth rosette leaves of 14-day-old seedlings were excised at the basal end of the petioles, and the excised leaves were transferred to new media containing different concentrations of sucrose. The excised leaves were subjected to the pre-treatment before incubation in white light (50 μmol m^{-2} s^{-1}; open bars) or darkness (solid bars) for 42 h. The leaf blades (A) and petioles (B) were measured before and after these light treatments. The data shown are the mean elongated lengths (±SD), with $n \geq 15$ for each measurement.

appeared to differ. We next examined the effects of light signals on the growth responses of leaf blades and petioles of the photoreceptor mutants, *phyA*, *phyB*, *phyD*, *cry1*, *cry2*, *phot1*, *phot2* and combinations of these lines, by growing the lines under continuous red and blue light conditions. In Tables 3 and 4, the lengths of the leaf blades and petioles of each genotype are shown as percentages relative to the lengths of dark-grown leaf blades and petioles.

Among the single mutants examined, the leaf blade expansion in red light was weakest in the *phyB* mutant, and similar results were also obtained in the *phyAphyB* and *phyBphyD* double mutants (Table 3. There were no significant differences in the leaf blade expansion of the cryptochrome mutants, the phototropin mutants and the wild type in red light. In contrast, in blue light, the *cry1* and *cry1cry2* mutants showed very similar decreases in the relative leaf blade expansion, whereas no differences were observed between the wild type and the *phot1phot2* mutant (Table 3). Furthermore, in blue light, the leaf blade expansion of all of the phytochrome mutants was similar to that of the wild type. These results suggest that phyB and cry1 regulate the promotion of leaf blade expansion in red and blue light, respectively.

The leaf petiole elongation in red light was weakest in the *phyB* mutant among the single mutants examined. These results suggest that phyB plays a critical role in the red light-mediated inhibition of petiole elongation. In blue light, the relative lengths of the petioles of the *cry1* mutant and the *cry1cry2* double mutant were 77.7 and 101.9%, respectively (Table 4). The *cry1cry2* double mutant showed the weakest leaf petiole growth response of the photoreceptor mutants examined. In contrast, there was little difference in the growth responses of leaf blades of the *phot1phot2* double mutant and the wild type. Furthermore, the *cry1cry2phot1phot2* quadruple mutants showed responses similar to that of the *cry1cry2* double mutant (Table 4). These results indicate that cry1 and cry2, the major blue light photoreceptors, regulate petiole elongation in blue light. In addition, in the *phyB* mutant, blue light enhanced leaf blade expansion and inhibited petiole elongation, suggesting that cryptochrome, as well as phytochromes, regulates leaf blade expansion and petiole elongation in shady conditions.

The effects of sucrose on the growth of the leaf blade and petiole in response to white light and darkness

Although leaf photomorphogenesis was found to depend on photoreceptors, as described above, the promotion of leaf blade growth by blue light was only observed under the strong irradiation (50 μmol m^{-2} s^{-1}) conditions in which photosynthesis can occur (Fig. 3). It is possible that photosynthesis regulates growth and development through photo-assimilated sugars, since sugars act as signals in the regulation of these phenomena (Gibson and Graham 1999). When seedlings were grown in sucrose-free medium and then transferred to fresh medium containing 2% sucrose, the growth of the leaf blade and petiole was enhanced in both white light and darkness, as compared with seedlings transferred to 0% sucrose (data not shown). This result suggests that sucrose promotes the growth of both the leaf blade and the petiole, irrespective of the light conditions, despite the fact that photoreceptor signals act differentially in the regulation of the growth of the leaf blade and petiole. To eliminate effects of the complex reactions of whole-plant systems, we examined the effect of sucrose concentrations of 0–2% on the growth of excised leaves in white light and darkness (see Materials and Methods). With increasing sucrose concentrations, leaf blade expansion was inhibited in white light but enhanced in darkness (Fig. 4A). The greatest elongation of leaf petioles occurred under conditions of approximately 0.5% sucrose in white light and 1.0% sucrose in darkness (Fig. 4B). Excessive sucrose concentrations might have an inhibitory effect, as high sucrose concentrations have been shown to inhibit cotyledon expansion and leaf develop-

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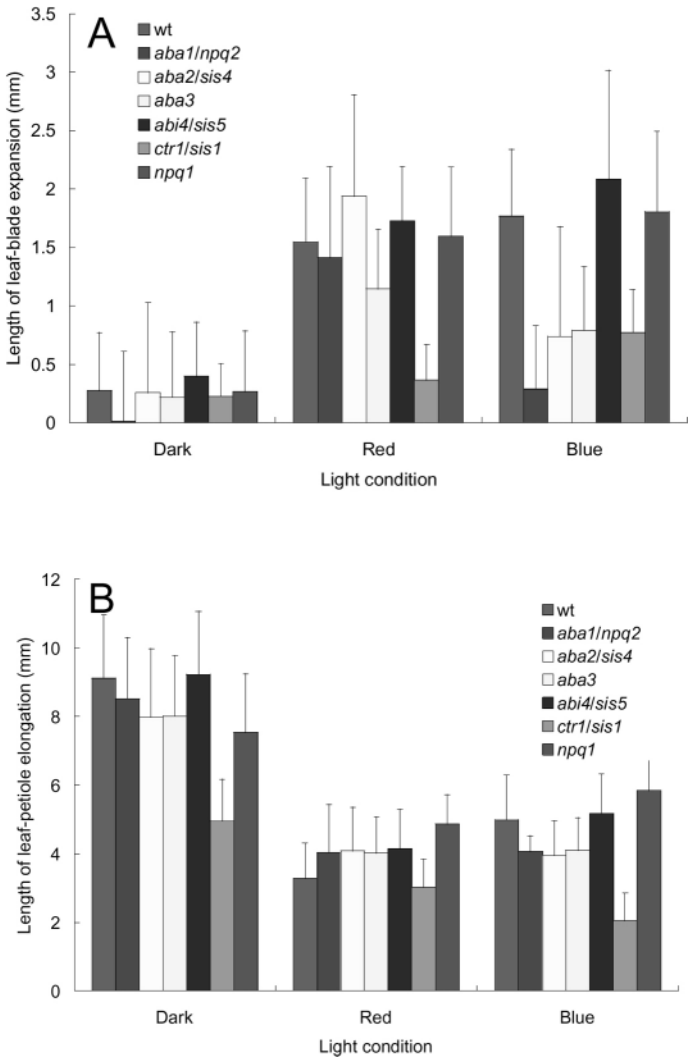

Fig. 5 The growth of leaf blades and petioles in ABA-deficient mutants in different light conditions. Seedlings were grown under continuous white light (50 μmol m^{-2} s^{-1}) for 14 d and then subjected to the pre-treatment. The seedlings were incubated in darkness, red light (50 μmol m^{-2} s^{-1}) or blue light (50 μmol m^{-2} s^{-1}), for 42 h, and the lengths of the blades (A) and petioles (B) of the fifth rosette leaves were measured just before and just after these treatments. The data shown are the mean elongated lengths (±SD), with $n \geq 15$ for each genotype.

ment (Laby et al. 2000). Since the effect of sucrose on the growth of both leaf blades and petioles differed in white light and darkness (Fig. 4), the responses of leaf blades and petioles to sucrose appear to be regulated by the light conditions.

The growth of leaf blades of ABA-deficient mutants is not promoted in blue light

Recent genetic studies have indicated that sugar signals have extensive connections with phytohormone pathways (Finkelstein and Gibson 2002, Leon and Sheen 2003). Endogenous ABA mediates sugar signaling, and ethylene plays an antagonistic role in sugar signaling (Finkelstein and Gibson 2002, Leon and Sheen 2003). Therefore, we analyzed the effects of phytohormone signaling pathways on leaf photomorphogenesis using ABA-deficient mutants (*aba1*, *aba2* and *aba3*), an ABA-insensitive mutant (*abi4*) and a constitutive ethylene response mutant (*ctr1*) that have been shown to be sucrose and glucose insensitive (Finkelstein 1994, Finkelstein and Lynch 2000, Laby et al. 2000, Gibson et al. 2001, Cheng et al. 2002). Fig. 5 shows the growth of leaf blades and petioles of these mutants in response to dark, red light and blue light conditions. In blue light, leaf blades of the *aba1*, *aba2* and *aba3* mutants expanded much less than those of the wild type, but no differences were evident in darkness or red light (Fig. 5A). In addition, there were no differences in the leaf blade expansion of the wild type and the *abi4* mutant. These results suggest that in blue light, a portion of the growth promotion of the leaf blade is dependent on endogenous ABA. Furthermore, this ABA signaling pathway is independent of ABI4. Consistent with this finding, it has been reported that rosette leaves of transgenic plants carrying the *ABI4::GUS* construct have no detectable GUS activity (Arroyo et al. 2003). There were no significant differences in the leaf petiole elongation of the wild-type, *aba1*, *aba2*, *aba3* and *abi4* plants, irrespective of the light conditions. These results suggest that endogenous ABA is not required for the portion of leaf petiole elongation that responds to the light environment.

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In the *ctr1* mutant, as in the *aba* mutants, less leaf blade expansion occurred in blue light. However, less leaf blade expansion was also observed in red light in this mutant. The leaf petiole elongation of *ctr1* was strongly inhibited in both darkness and blue light, but was not affected in red light. Thus, the *ctr1* mutation appears to have a negative effect on the growth of the leaf blade and the petiole, except for the leaf petiole elongation in red light, suggesting that ethylene signal transduction is inactive in the leaf petiole in red light.

Although our results suggest an important role for ABA synthesis in the regulation of leaf blade photomorphogenesis, the *aba1* mutant has been reported to be allelic to the *npq2* mutant, which accumulates large amounts of zeaxanthin (Niyogi et al. 1998). Because zeaxanthin absorbs blue wavelengths and quenches excess light energy (Muller et al. 2001), it is possible that photosynthesis might not utilize blue light effectively in the *aba1/npq2* mutant. If this is the case, the *npq1* mutant, which accumulates abnormally low levels of zeaxanthin (Muller et al. 2001), would be hypersensitive to blue light. However, examination of the *npq1* mutant revealed no differences in the lengths of the leaf blade and petiole of the mutant and the wild type in these light conditions (Fig. 5). This result suggests that zeaxanthin is not involved in the regulation of leaf growth under these conditions, whereas endogenous ABA promotes leaf blade expansion in blue light.

Discussion

The differential growth of the leaf blade and petiole is regulated by phytochrome and cryptochrome

Plastic and organ-specific regulation of growth allows plants to exhibit acclimation against changing light environments for efficient photosynthesis. Analysis of the shade-avoidance syndrome has suggested that the different modes of growth of leaf blades and petioles are regulated by phytochromes (Nagatani et al. 1991, Reed et al. 1993, Devlin et al. 1999, Franklin et al. 2003a). However, our analysis of the growth of wild-type leaves in response to monochromatic wavelengths, including red, far-red and blue light, demonstrated that leaf blade expansion is promoted and leaf petiole elongation is inhibited with increasing light intensities, for all the wavelengths examined. These results suggest that the leaf shade-avoidance syndrome is induced not only by a low ratio of red to far-red light, but also by blue light. We have also consistently shown, through genetic analyses of a variety of photoreceptor mutants, that both phytochromes and cryptochromes are redundantly involved in the control of leaf growth. The promotion of cotyledon expansion and the inhibition of hypocotyl elongation are regulated primarily by phyB in red light and cry1 in blue light (Blum et al. 1994, Neff and Van Volkenburgh 1994). Therefore, the expansion of photosynthetic organs (leaf blade and cotyledon) and the elongation of supportive organs (leaf petiole and hypocotyl) are regulated by similar mechanisms.

However, we have also observed that the relative importance of phytochrome species in leaf growth differs from that in other organs; specifically, the growth regulation of both leaf blades and petioles in red light driven primarily by phyB is stronger than in far-red light, which is governed by phyA. The opposite is true for the process of de-etiolation in other organs (Neff and Chory 1998). The primary blue light photoreceptors important in leaf growth are also different from those that are important in other organs. Many photomorphogenic responses in de-etiolated seedlings, such as the inhibition of hypocotyl elongation and cotyledon opening, are not dependent on the two phototropins, whereas cotyledon expansion is decreased in a phototropin-deficient background (Ohgishi et al. 2004). However, phot1 and phot2 appear not to have major roles in leaf morphogenesis under our experimental conditions. To our knowledge, this is the first report on the primary photoreceptors required for the differential growth of leaf blades and petioles.

The regulation of leaf petiole elongation in response to various light conditions differs from that of hypocotyl elongation

Although the inhibition of the elongation of leaf petioles by light irradiation appears superficially similar to that observed in de-etiolated hypocotyls, we showed that the effect of light quality on the growth of leaf petioles is different from its effect on de-etiolated hypocotyls. Red light, a wavelength that inhibits leaf petiole elongation, plays a more important role in petioles than does far-red light, in contrast to its role in the inhibition of hypocotyl elongation (Neff and Chory 1998). The reason for the differences in regulation may be that the high transmittance of far-red light is used by etiolated seedlings to distinguish the above-ground portion of the seedling, and red light is an indication of appropriate light conditions for photosynthesis in leaves. Another difference between the photomorphogenesis of hypocotyls and leaf petioles is in the endoreduplication cycle. Given the significant differences in the leaf petiole elongation in light and darkness, the very subtle difference in ploidy levels in seedlings grown in white light and darkness was surprising, since in hypocotyls of seedlings grown in darkness the increase in ploidy levels is accompanied by cell enlargement (Gendreau et al. 1998). There are at least two possible explanations for this difference. First, endoreduplication may be necessary for the promotion of leaf petiole growth, but cell growth itself may be inhibited by light in petioles. The greater overall DNA content in leaf petioles, as compared with leaf blades, is consistent with this idea. Secondly, the growth promotion of leaf petioles in darkness may be independent of the DNA content. Interestingly, trichome morphogenesis has been proposed as being mediated by both endoreduplication-dependent and -independent processes (Schnittger et al. 2003). Although cell expansion is positively and negatively controlled by light signals in both leaf blades and petioles, the mechanisms that control cell expansion probably include endoreduplication-dependent and -independent pathways downstream of light signals.

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Sucrose-mediated leaf growth in response to light conditions

In blue light, little growth of wild-type leaf blades was observed, even at 5.0 mmol m^{-2} s^{-1}, whereas leaf blade expansion was evident in red light at 5.0 mmol m^{-2} s^{-1} and in blue light at 50 mmol m^{-2} s^{-1} (Fig. 1), suggesting that the low fluence rate of blue light is ineffective in promoting leaf blade expansion. Moreover, we showed that media containing appropriate amounts of sucrose promote the growth of leaf blades and petioles in white light and darkness (Fig. 4). Thus, the promotive effects of strong blue light on leaf blade growth may be mediated, at least in part, by the photosynthesis of sucrose.

Although this sucrose-dependent growth promotion could be due to enhanced metabolic states, we identified a connection between the light environment and sugar responses in leaf blades. The leaf blades of ABA-deficient mutants were unable to expand in blue light, but expanded normally in red light (Fig. 5). The rate of ethylene evolution by the *aba2* mutant is greater than that of the wild type, and the reduced growth of *aba2* mutant leaves is partially restored by introducing the ethylene perception mutation *etr1* (LeNoble et al. 2004). We also showed that in the *ctr1* mutant, in which constitutively active ethylene signaling suppresses sugar signaling (Gibson et al. 2001, Cheng et al. 2002), leaf blade expansion and leaf petiole elongation are decreased under blue light irradiation. Similarly, ethylene overproduction in the *eto2* mutant results in a compact stature and, conversely, ethylene-insensitive mutants such as *ein2* and *etr1* have larger leaves than wild type in low light intensities (Vandenbussche et al. 2003). Thus, it appears that the promotive effects of sucrose on leaf expansion are mediated by ABA-related sugar signaling, which is suppressed by the ethylene signal. The expression of certain ethylene biosynthesis genes is highly induced in leaf blades in response to low light intensity, and in the *phyB* mutant (Vandenbussche et al. 2003). In addition, light that contains sufficient intensities of blue and far-red light, and low levels of red light, mimics open shade conditions (Stoutjesdijk 1974). These facts suggest that the ABA-mediated growth of leaf blades in blue light acts as an antagonist to the ethylene signal during the adaptation to open shade conditions, or that phyB signals suppress ethylene synthesis in conditions of sufficient red light. Moreover, since light enhances the effects of sucrose on the growth of the leaf petiole but inhibits the growth of the leaf blade (Fig. 4), photoreceptors and sugar might have contrasting roles in the parallel regulatory pathway of leaf growth. Alternatively, involvement of endogenous ABA in photomorphogenesis of leaves might be mediated by a cryptochrome signal, considering the fact that characteristic phenotypes of ABA-deficient mutants were recognized under blue light. Further analyses on the relationship among hormonal action, photoreceptor signaling and sugar signaling are required.

In the present study, we have shown that light controls the differential growth and development of leaves, at multiple levels, by means of various photoreceptors, the sugar responses and leaf petiole-specific regulation. The control mechanisms of the differential regulation of leaf blade and petiole photomorphogenesis are of great interest.

Materials and Methods

Plant materials

The phytochrome and cryptochrome mutants studied were *phyA-201* (Nagatani et al. 1993), *phyB-1* (Koornneef et al. 1980, Nagatani et al. 1991), *phyD-1* (Aukerman et al. 1997, Devlin et al. 1999), *hy4-1* (carrying a mutation in the CRY1 gene; Koornneef et al. 1980, Ahmad and Cashmore 1993) and *fha-1* (carrying a mutation in the CRY2 gene; Koornneef et al. 1980, Guo et al. 1998), all of which are in the Landsberg *erecta* (L*er*) background. The *phyAphyB* double mutant, obtained by crossing *phyA-201* with *phyB-1*, was provided by Dr. F. Hamazato (Hitachi, Tokyo, Japan). The *phyBphyD* double mutant, obtained by crossing *phyB-1* with *phyD-1*, was provided by Dr. P. Devlin (Kings College London, U.K.). The *cry1cry2* double mutant was created by crossing *hy4-2.23n* in the L*er* background (Koornneef et al. 1980, Ahmad and Cashmore 1993) with *fha-1*. The ecotype of the *phot1-5phot2-1* double mutant (provided by Dr. T. Kinoshita, Kyushu University, Fukuoka, Japan) was Columbia (Col; Huala et al. 1997, Kagawa et al. 2001, Kinoshita et al. 2001). Because the *cry1cry2-phot1phot2* quadruple mutants were created by crossing L*er* background mutants [*hy4-2.23n*, *fha-1* and *phot1-101* (Sakai et al. 2001)] with a Wassilewskija (Ws) background mutant [*phot2-5*, formerly designated *npl1-1*/*cav1-5* (Sakai et al. 2001)], four lines (#33, #57, #210 and #257; Ohgishi et al. 2004) were investigated with a view to eliminating potential phenotypic variations arising from the hybridization of different ecotypes. The ABA-deficient mutants studied, *aba1-6* [formerly isolated as *npq2-1* (Niyogi et al. 1998)], *aba2-1* and *aba3-1* (Leon-Kloosterziel et al. 1996), are in the Col background. The ABA-insensitive mutant *abi4-11* (Nambara et al. 2002) and the zeaxanthin-deficient mutant *npq1-2* (Niyogi et al. 1998) were also in the Col background. Transgenic *A. thaliana* (Col) carrying the *cyc1At::GUS* reporter construct was provided by Dr. J. L. Celenza (Boston University, MA, U.S.A.).

Growth conditions

Seeds were sterilized in a solution of NaClO (Tsuge et al. 1996), and incubated for 3 d in sterile distilled water at 4°C in darkness. The seeds were then sown on MS0 plates (Tsuge et al. 1996) and grown under standard culture conditions of continuous white fluorescent light (photon flux density, 55 μmol m^{-2} s^{-1}) at 22°C. For the analysis of leaf elongation, seedlings were grown for 12 d and the third rosette leaf was measured; alternatively, they were grown for 14 d, at which time the fifth rosette leaf was measured. Before exposure to specific light conditions, plants were treated with far-red light for 15 min and then placed in complete darkness for 6 h. The age of the plants was defined as the days after sowing.

Light sources

White light was provided by cool white fluorescent tubes (FL20SSW/18; Toshiba Co. Ltd., Tokyo, Japan). Red, far-red and blue lights were provided by light-emitting diodes (LEDs) at maximum wavelengths of 660 nm (181360; Eyela Co. Ltd., Tokyo, Japan), 735 nm (181390; Eyela Co. Ltd., Tokyo, Japan) and 470 nm (181380; Eyela Co. Ltd., Tokyo, Japan), respectively.

Measurements of leaves and cells

All measurements of the lengths of leaf blades and petioles were performed just before and just after treatment with specific light conditions. For leaf measurement, the leaves were excised at the basal por-

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tion of the leaf petiole and placed on a glass slide; then, images of the leaves were scanned, and the lengths of the leaf blades and petioles were determined using the program Image J (http://rsb.info.nih.gov/ij/, NIH, MD, U.S.A.). For anatomical studies, leaves were fixed in FAA solution and cleared with chloral hydrate solution (Tsuge et al. 1996). Samples were photographed under Nomarski optics and the cell size was determined using Image J.

Sucrose treatment

The fifth rosette leaves of 14-day-old plants were excised at the basal portion of the petiole. The petioles of excised leaves were inserted vertically into the medium of MS0 plates supplemented with various concentrations of sucrose. Just before white light or dark treatment, the plants were treated with far-red light for 15 min. The lengths of the leaf blade and petiole were determined just before the far-red light treatment and just after the white light or dark treatment of 48 h.

Detection of GUS activity

Histochemical detection of GUS activity was performed using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide solution, as described previously (Donnelly et al. 1999). The leaves were then cleared with chloral hydrate and photographed under Nomarski optics, as described previously (Tsuge et al. 1996).

Flow-cytometric analysis

Before and after the white light or dark treatments, the rosette leaves were divided into the leaf blade and petiole, and chopped with a razor blade in 1 ml of extraction buffer containing 2 mM magnesium chloride, 10 mM Tris (hydroxymethyl) aminomethane, 1% (v/w) β-mercaptoethanol, 40 mg ml^{-1} polyvinylpyrrolidone, 0.1 mg ml^{-1} RNase A and 0.1% (v/w) Triton X-100 at pH 8.0 (Galbraith et al. 1983). The suspension was filtered through a nylon filter (20 μm) and incubated at 37°C for 10 min; following this, 100 μl of propidium iodide solution was added [1 mg ml^{-1} propidium iodide (Sigma, MO, U.S.A.) and 12.5 mg ml^{-1} of 1,4-diazabicyclo(2.2.2)octane (Sigma) in phosphate-buffered saline (pH 7.4)], and the mix was incubated at 4°C for 1 h. At least 5,000 nuclei per sample were analyzed using the EPICS XL System II (Coulter Co., Miami, FL, U.S.A.). Each result presented the mean value of three independent measurements (involving at least 20 leaves).

Acknowledgments

The authors thank Dr. Akira Nagatani at Kyoto University for the gifts of the *phyA201*, *phyB-1* and *phyA201phyB-1* mutants, Dr. Garry C. Whitelam at the University of Leicester and Dr. Paul Devlin at King's College London, U.K. for the gifts of the *phyD-1* and *phyB-1phyD-1* mutants, Dr. Eiji Nambara of RIKEN for the gifts of the *aba2-1*, *aba3-1* and *abi4-11* mutants, and Dr. Masamitsu Wada of Tokyo Metropolitan University and Dr. Takatoshi Kagawa of the University of Tsukuba for the gifts of the *phot1-5phot2-1* mutants. This study was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture of Japan; by funds from the Bio-Design Program of the Ministry of Agriculture, Forestry, and Fisheries, Japan; and was also carried out as a part of the Ground-Based Research Announcement for Space Utilization promoted by the Japan Space Forum.

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(Received October 4, 2004; Accepted November 5, 2004)

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