Arabidopsis Mutants Lacking Blue Light-Dependent Inhibition of Hypocotyl Elongation

Emmanuel Liscum and Roger P. Hangarter
Department of Plant Biology, The Ohio State University, Columbus, Ohio 43210

We have isolated a new class of photomorphogenic mutants in Arabidopsis. Hypocotyl elongation is not inhibited in the mutant seedlings by continuous blue light but is inhibited by far red light, indicating that these mutations are phenotypically different from the previously isolated long hypocotyl (hy) mutants. Complementation analysis indicated that recessive nuclear mutations at three genetic loci, designated blu1, blu2, and blu3, can result in the blu mutant phenotype and that these mutants are genetically distinct from other long hypocotyl mutants. The BLU genes appear to be important only during seedling development because the blu mutations have little effect on mature plants, whereas hypocotyl elongation and cotyledon expansion are altered in seedlings. The genetic separation of the blue and far red sensitivities of light-induced hypocotyl inhibition in the blu and hy mutants demonstrates that two photosensory systems function in this response.

INTRODUCTION

Light is particularly important to plant growth because it regulates many aspects of plant development and is the energy source for photosynthesis. Examples of light-dependent developmental processes include stem growth inhibition, leaf and root growth, tropic responses, germination, and flower induction. Red, blue, and UV light are especially effective in inducing photomorphogenic responses through the action of the red/far red photoreceptor phytochrome, blue/UV-A photoreceptor(s), and UV-B photoreceptor(s) (Mohr, 1986). However, phytochrome is the only plant photoreceptor pigment to be identified at the genetic and biochemical levels (Quail et al., 1987; Sharrock and Quail, 1989).

In general, blue light responses can be separated into two classes, low fluence responses and high irradiance responses, based on their fluence and exposure time requirements (Mancinelli and Rabino, 1978). Low fluence responses are typically inductive and require only a brief exposure to low fluence rate light for maximal response (Mancinelli and Rabino, 1978). Phototropism is an example of a low fluence blue response. In contrast, high irradiance responses require extended exposures to high fluence rate light for full expression of the response (Mancinelli and Rabino, 1978). The inhibition of hypocotyl elongation is considered a high irradiance response.

Many blue light responses have action spectra that most closely resemble the absorption spectra of riboflavin and some carotenoids (Schmidt, 1980). However, most studies suggest that the blue light receptor has a flavin-type chromophore. For example, inhibitors of carotenoid synthesis had no effect on phototropism in corn (Vierstra and Poff, 1981), and normal blue light responses have been reported in carotenoidless mutants of Euglena (Checcucci et al., 1966), Phycomyces (Presti et al., 1977), and Neurospora (Sargent et al., 1966). In addition, when riboflavin auxotrophs of Phycomyces were provided with the flavin analog roseoflavin, the action spectra of phototropism had spectral shifts corresponding to the absorption properties of roseoflavin compared with riboflavin (Otto et al., 1981).

Several recent studies have identified possible components of blue light signal transduction pathways. Gallagher et al. (1988) described an in vivo blue light treatment of pea stem segments that markedly decreased the in vivo phosphorylation of a plasma membrane-associated protein. Kinetic properties and fluence response relationships for the phosphorylation activity were found to be comparable with the kinetic and fluence response properties for phototropism in pea (Short and Briggs, 1990). A blue light-activated GTP-binding protein, which may be a component in the signal transduction pathway of low fluence blue responses, has been identified in plasma membrane preparations in pea (Warpeha et al., 1990). In addition, a blue light-induced membrane depolarization, which is mediated by a plasma membrane H+-ATPase, has been implicated in the high irradiance reaction leading to inhibition of stem elongation in cucumber (Spalding and Cosgrove, 1989, 1990).

Mutants of Arabidopsis are proving to be particularly useful for investigating signal perception and transduction...
systems in plants (Olsen et al., 1984; Bleecker et al., 1988; Chory et al., 1989b; Wilson et al., 1990). Photomorphogenic mutants are no exception. A class of photomorphogenic mutants, designated *hy*, that have long hypocotyls in high irradiance white light have been isolated in *Arabidopsis* (Koornneef et al., 1980; Chory et al., 1989a). Six complementation groups have been described and five of these have been, or are likely to be, identified as components of phytochrome-regulated perception and signal transduction pathways. Three of them (*hy1*, *hy2*, and *hy6*) lack spectrophotometrically detectable phytochrome but have normal levels of the phytochrome polypeptide as detected by protein gel blotting (Koornneef et al., 1980; Chory et al., 1989a; Parks et al., 1989). The *hy3* and *hy5* mutants are probably response mutants because they have altered red and far red light sensitivity but have wild-type levels of phytochrome (Koornneef et al., 1980; Chory et al., 1989a; Parks et al., 1989).

*Arabidopsis* is the only higher plant for which blue light-response mutants have been identified. One of these, the *hy4* mutant line, shows reduced hypocotyl inhibition in blue light while maintaining normal phytochrome levels and red/far red responses (Koornneef et al., 1980). Unfortunately, no further characterization of this mutant has been made. The other blue light-response mutants that have been isolated are phototropism mutants and include reduced response mutants (Khurana et al., 1989), “null” phototropism mutants, and presumptive photoreceptor mutants (Khurana and Poff, 1989).

This paper presents the initial characterization of four mutant lines that fail to show blue light-dependent inhibition of hypocotyl elongation. Phytochrome-mediated inhibition of hypocotyl growth by far red light appears to be normal in these mutants. These mutants are genetically distinct from all of the long hypocotyl mutants previously isolated (Koornneef et al., 1980; Chory et al., 1989a) and represent three new loci involved in photomorphogenesis in seedlings of higher plants.

**RESULTS**

Isolation and Genetic Characterization of Blue Light-Response Mutants

To identify genes involved in blue light perception and signal transduction, we searched for mutants that exhibit altered blue light-regulated inhibition of hypocotyl elongation. Our approach was similar to one used in the isolation of the *hy* mutants of *Arabidopsis*, in which plants with long hypocotyls were selected from *M2* populations grown under high-intensity white light conditions that are inhibitory to hypocotyl elongation in wild-type plants (Riede and Hirose, 1964; Koornneef et al., 1980; Chory et al., 1989a). However, we used light only from the blue region of the electromagnetic spectrum to increase the probability of isolating blue light-specific response mutants. Under our blue light conditions, hypocotyl elongation in wild-type plants was 70% inhibited with respect to wild-type plants grown in the dark, as shown in Figure 1.

By using this selection scheme, we isolated 10 blue light-specific elongation mutants of *Arabidopsis* from 14 different populations of ethyl methanesulfonate-mutagenized *M2* seeds. Hypocotyl elongation in four of these mutants was not inhibited by blue light and resembled hypocotyl elongation in dark-grown seedlings (Figure 1). We designated this previously unidentified class of mutants as *blu*, for blue light uninhibited. Hypocotyl elongation in the remaining six mutants was intermediate between dark-grown and blue light-grown wild-type seedlings. We tentatively designated this class of mutants as *blu*, for blue light intermediate. The histogram plots in Figure 2 of hypocotyl lengths of *M3* progeny homozygous for a *blu* and an intermediate mutant illustrate that these phenotypic classes are separable from wild-type plants when grown under blue light. However, we have not yet conducted further characterization of the intermediate class of mutants to determine whether any of them are leaky alleles of the *blu* mutants.

To determine the genetic basis for the *blu* phenotype, each mutant line was crossed to a wild-type plant and the hypocotyl phenotype was scored in F1 and F2 seedlings grown under continuous blue light. A summary of the genetic analysis is shown in Table 1. Each mutant line segregated three short hypocotyl plants to one long hypocotyl plant in the F2 generation. Reciprocal crosses indicated that each line represents a single recessive nuclear mutation. In addition, complementation analysis indicated that the four mutant lines represent three separate genetic loci: *blu1*, *blu2*, and *blu3*. Two of the mutations, *blu3-1* and *blu3-2*, are allelic.

Each of the *blu* mutant lines was crossed to each of the six *hy* mutant lines (Koornneef et al., 1980; Chory et al., 1989a) to test the genetic relationship between the *blu* and *hy* mutants. A summary of the complementation analysis between *blu* and *hy* mutants is shown in Table 2. All of the F1 progeny from these crosses had short hypocotyls when grown in continuous blue light, indicating that the *blu* mutant phenotype is genetically separable from that of the *hy* mutants and represents a new class of photomorphogenic mutants.

**Characterization of Light Effects on *blu* Mutant Seedlings**

Different regions of the electromagnetic spectrum vary greatly in the efficiency with which they inhibit hypocotyl elongation in wild-type plants, with blue and far red light being most effective (Kranz, 1977; Koornneef et al., 1980;
Figure 1. Effect of Light Quality on the Inhibition of Hypocotyl Elongation in Wild Type and blu and hy6 Mutants of Arabidopsis.

Light was provided from above. Hypocotyl lengths (in millimeters) were measured after 5 days of growth. The hy6 mutant is phytochrome deficient (Chory et al., 1989a). Each bar represents the mean measurement for 50 to 75 seedlings. Error bars indicate the standard error. Fluence rates were as follows: white, 45 ± 3 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \); blue, 56 ± 2 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \); far red, 30 ± 2 \( \mu \text{mol m}^{-2} \text{sec}^{-1} \). WT, wild type.

J.C. Young and R.P. Hangarter, unpublished results. However, white light, which consists of all the visible wavelengths and represents more natural conditions, is more effective than blue or far red alone. Therefore, we investigated the response of blu seedlings to blue light, far red light, and white light.

As shown in Figure 1, hypocotyl elongation in seedlings of all four blu mutants grown in the dark or in continuous far red light was similar to wild-type seedlings grown under the same conditions. However, in continuous white light, blu1, blu3-1, and blu3-2 seedlings were approximately twice as long as wild type, whereas blu2 seedlings were only slightly longer than wild type. The most striking differences between the blu and wild-type seedlings are seen when seedlings are grown in blue light. In continuous blue light, wild-type hypocotyls were 70% shorter than their dark controls, whereas the blu mutants were uninhibited. In contrast, hypocotyls in the phytochrome-deficient mutant hy6 were inhibited by blue light but in far red light grew as long as etiolated controls. Like the blu mutants, hy6 was longer than wild type in white light.

Figure 3 is a representative photograph of wild type, blu1, and hy6 under the different light conditions. Figure 3 also shows the phenotype of double mutants homozygous for blu1 and hy6. The double mutants were essentially blind to blue and far red light, and even after 5 days of growth in continuous white light they had a typical etiolated phenotype. Although the double mutants eventually developed and reproduced, the plants were weak and their seed production was low. When enough seed of the double mutants become available, their phenotype will be characterized in detail.

After 5 days of growth, hypocotyl elongation varied only slightly among the different blu mutant lines in a given light condition (Figure 1). For example, blu1 and blu3-1 elongated the same amount for a given light condition. The mean hypocotyl length of blu2 was 1 to 2 mm shorter than blu1 and blu3-1 in all light conditions. However, the germination experiment shown in Figure 4 indicates that the small differences in hypocotyl length may reflect the fact that radicle emergence occurred 6 to 12 hr later in blu2 than in the other blu mutants. Seeds germinated without the red light pretreatment showed the same time course, but the germination percentages were lower for all the genotypes, indicating that the slower germination in the blu mutants is not light dependent (data not shown). Moreover, the blu phenotype was similar whether or not the seeds received the red light treatment, although germination was more uniform in the red light-treated seed.

Light-induced cotyledon expansion in the blu mutants was different from wild type, as illustrated in Table 3. In blue light, cotyledon expansion was reduced by about 60% in all of the blu mutants. In white light, cotyledon expansion was reduced by about 30% in blu1 and blu2 seedlings, whereas blu3-1 and blu3-2 cotyledons expanded similarly to wild-type cotyledons. Under far red
Blue light was provided from above at a fluence rate of 56 ± 2 μmol m⁻² sec⁻¹. Hypocotyl lengths (in millimeters) were measured after 5 days of growth. Line 16b12 represents a typical blu type mutant, and line blu3-1 represents a typical blu mutant. WT, wild type.

To determine whether the blu mutant phenotype was related to differences in photosynthetic capacity of the cotyledons, chlorophyll contents were measured. Table 4 shows that within each light condition, chlorophyll content and the chlorophyll a/b ratios were the same in blu7 and wild-type cotyledons, indicating that chloroplast development was unaffected by the blu mutations.

### DISCUSSION

The common phenotype of the blu class of photomorphogenic mutants is the lack of blue light-dependent inhibition

### Table 1. Genetic Analysis of blu Mutants

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Short a</th>
<th>Long a</th>
<th>χ² b</th>
</tr>
</thead>
<tbody>
<tr>
<td>blu1/blu1 x BLU1/BLU1 c</td>
<td>F1 34</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>BLU1/blu1 x BLU1/blu1</td>
<td>F2 374</td>
<td>118</td>
<td>0.271 dad</td>
</tr>
<tr>
<td>blu2/blu2 x BLU2/BLU2 c</td>
<td>F1 37</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>BLU2/blu2 x BLU2/blu2</td>
<td>F2 238</td>
<td>83</td>
<td>0.126 a</td>
</tr>
<tr>
<td>blu3-1/blu3-1 x BLU3-1/BLU3-1 c</td>
<td>F1 46</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>BLU3-1/blu3-1 x BLU3-1/blu3-1</td>
<td>F2 450</td>
<td>161</td>
<td>0.594 a</td>
</tr>
<tr>
<td>blu1/blu1 x blu2/blu2</td>
<td>F1 18</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>blu1/blu1 x blu3-1/blu3-1</td>
<td>F1 27</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>blu1/blu1 x blu3-2/blu3-2</td>
<td>F1 16</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>blu2/blu2 x blu3-1/blu3-1</td>
<td>F1 39</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>blu2/blu2 x blu3-2/blu3-2</td>
<td>F1 16</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>blu3-1/blu3-1 x blu3-2/blu3-2</td>
<td>F1 0</td>
<td>14 -</td>
<td></td>
</tr>
</tbody>
</table>

*Wild type have short hypocotyls (≤6 mm); mutants have long hypocotyls (>6 mm).
*P > 0.05.
*Data represent mutant (♀):wild-type (♂) crosses; similar results were obtained with wild-type (♀):mutant (♂) crosses.
*χ² expected ratio, 3 wild type:1 mutant.
*Similar results were obtained with blu3-2.
Table 2. Complementation of blu Mutants with hy Mutants

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Short</th>
<th>Long</th>
</tr>
</thead>
<tbody>
<tr>
<td>blu1/blu1 × hy1/hy1</td>
<td>F1 13 0</td>
<td></td>
</tr>
<tr>
<td>blu1/blu1 × hy2/hy2</td>
<td>F1 41 0</td>
<td></td>
</tr>
<tr>
<td>blu1/blu1 × hy3/hy3</td>
<td>F1 24 0</td>
<td></td>
</tr>
<tr>
<td>blu1/blu1 × hy4/hy4</td>
<td>F1 49 0</td>
<td></td>
</tr>
<tr>
<td>blu1/blu1 × hy5/hy5</td>
<td>F1 74 0</td>
<td></td>
</tr>
<tr>
<td>blu1/blu1 × hy6/hy6</td>
<td>F1 11 0</td>
<td></td>
</tr>
<tr>
<td>blu2/blu2 × hy1/hy1</td>
<td>F1 25 0</td>
<td></td>
</tr>
<tr>
<td>blu2/blu2 × hy2/hy2</td>
<td>F1 17 0</td>
<td></td>
</tr>
<tr>
<td>blu2/blu2 × hy3/hy3</td>
<td>F1 14 0</td>
<td></td>
</tr>
<tr>
<td>blu2/blu2 × hy4/hy4</td>
<td>F1 21 0</td>
<td></td>
</tr>
<tr>
<td>blu2/blu2 × hy5/hy5</td>
<td>F1 19 0</td>
<td></td>
</tr>
<tr>
<td>blu2/blu2 × hy6/hy6</td>
<td>F1 81 0</td>
<td></td>
</tr>
<tr>
<td>blu3-1/blu3-1 × hy1/hy1</td>
<td>F1 47 0</td>
<td></td>
</tr>
<tr>
<td>blu3-1/blu3-1 × hy2/hy2</td>
<td>F1 30 0</td>
<td></td>
</tr>
<tr>
<td>blu3-1/blu3-1 × hy3/hy3</td>
<td>F1 56 0</td>
<td></td>
</tr>
<tr>
<td>blu3-1/blu3-1 × hy4/hy4</td>
<td>F1 18 0</td>
<td></td>
</tr>
<tr>
<td>blu3-1/blu3-1 × hy5/hy5</td>
<td>F1 70 0</td>
<td></td>
</tr>
<tr>
<td>blu3-1/blu3-1 × hy6/hy6</td>
<td>F1 33 0</td>
<td></td>
</tr>
</tbody>
</table>

a Wild type have short hypocotyls (≤6 mm); mutants have long hypocotyls (>6 mm).

b Data represent hy (3):blu (9) crosses.
c Similar results were obtained with blu3-2.

due light-mediated inhibition of hypocotyl elongation but exhibit normal far red inhibition, which is consistent with the involvement of two distinct photoreceptor systems. Similarly, the hy4 mutant line has reduced sensitivity to blue light but retains red/far red sensitivity (Koornneef et al., 1980). Furthermore, the phytochrome-deficient hy mutants lack red/far red-dependent inhibition but show no change in blue light responsiveness (Koornneef et al., 1980; Chory et al., 1989a). As shown here, double mutants homozygous for blu1 and the phytochrome-deficient hy6 were insensitive to blue, far red, and white light. Together, these studies with the blu and hy mutants demonstrate unequivocally that two genetically distinct photosensory systems function in the high irradiance-induced inhibition of hypocotyl elongation and confirm physiological observations that suggest the existence of a separate blue light perception system (Holmes and Schafer, 1981; Cosgrove, 1982; Gaba et al., 1984; Laskowski and Briggs, 1989;
Figure 4. Time Course of Germination of Wild-Type and blu Mutant Seeds.

Seeds were given a standard cold and red light treatment (see Methods) and then incubated in the dark. A seed was considered to have germinated when the radicle emerged from the seed coat. The data are presented as the percentage of the seeds that had germinated at each time point. WT, wild type.

Warpeha and Kaufman, 1989). Moreover, the results with the blu mutants indicate that blue light-induced phytochrome photoconversion plays a relatively minor role in suppression of hypocotyl elongation in Arabidopsis.

Sarkar and Song (1982) have suggested that blue light may act through phytochrome transduction pathways by direct energy transfer from a blue light-absorbing flavin molecule to phytochrome. If blue and red/far red red responses do share transduction pathways, the isolation of four mutant lines (the three blu mutants and hy4) altered in the blue light component of the inhibition of hypocotyl elongation but retaining phytochrome control suggests that at least four steps are unique to the blue light response system. Furthermore, no single gene mutants have yet been isolated that lack both blue- and red/far red-dependent inhibition of hypocotyl elongation. Thus, it is unlikely that high-irradiance blue and red/far red response systems have many signal transduction steps in common.

In addition to the inhibition of stem growth, blue and red/far red light regulate many other processes in the normal development of dicotyledonous plants, including chlorophyll and anthocyanin biosynthesis, chloroplast development, apical hook opening, and cotyledon expansion (Attridge, 1990). The results from our blu mutant lines suggest that cotyledon expansion and inhibition of stem elongation may be genetically coupled. The lack of blue light-dependent hypocotyl growth inhibition exhibited by the blu mutants was accompanied by a 60% reduction of cotyledon expansion. Furthermore, cotyledon expansion in blu1 and blu2 was greater in white light, which contains some red and far red light, than in blue light, suggesting that phytochrome is involved in the expression of the full developmental response. The induction of anthocyanin biosynthesis by high-irradiance light seems to be affected in blu1 and blu2. In parsley, the UV light-induced activation of chalcone synthase was found to be modulated by a blue light-derived signal (Ohl et al., 1989). It is possible that the blu1 and blu2 mutations may be related to a similar modulation system in Arabidopsis. However, linkage analysis between the long hypocotyl phenotype and anthocyanin induction has to be completed before this possibility can be tested.

Several other blue light-sensitive photomorphogenic responses appear to be normal in the blu mutants. For example, opening of the apical hook appears qualitatively normal, but we need to examine fluence response relationships to determine whether the light conditions used in this study were saturating a low fluence response that might be altered in the mutants. Chlorophyll accumulation is apparently not affected by the blu mutations in seedlings or mature plants, indicating that the blue light response system controlling chloroplast development is different from the system controlling hypocotyl elongation. Interestingly, during the characterization of the blu mutants, we noticed that all of the blu mutant lines responded to phototropic stimuli (data not shown). As already mentioned, phototropism is a low fluence response, and the inhibition of hypocotyl elongation is a high irradiance response. However, both responses are dependent on changes in cell elongation and have similar blue light action spectra (Senger, 1980). Because hypocotyl elongation was not inhibited in the blu mutants when blue light was given from above but differential growth occurred when they

### Table 3. Light-Dependent Cotyledon Expansion in blu Mutants

<table>
<thead>
<tr>
<th>Light</th>
<th>Cotyledon Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild Type</td>
</tr>
<tr>
<td>White</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>Blue</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>Far red</td>
<td>24 ± 1</td>
</tr>
</tbody>
</table>

* Actinic light conditions were as described in Figure 1.
* Data represent mean ± se of 10 cotyledon pairs. Cotyledon area of dark-grown seedlings averaged 0.34 ± 0.01 mm² for all genotypes.
* Similar results were obtained with blu3-2.
Table 4. Chlorophyll Content in Cotyledons

<table>
<thead>
<tr>
<th>Light</th>
<th>Wild Type</th>
<th>blu1a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Chlorophyll</td>
<td>Chlorophyll a/b Ratio</td>
</tr>
<tr>
<td>Dark</td>
<td>0.79</td>
<td>1.17</td>
</tr>
<tr>
<td>White</td>
<td>61.98</td>
<td>2.30</td>
</tr>
<tr>
<td>Blue</td>
<td>82.98</td>
<td>2.39</td>
</tr>
<tr>
<td>Far red</td>
<td>3.25</td>
<td>2.71</td>
</tr>
</tbody>
</table>

a Actinic light conditions were as described in Figure 1.
b Total chlorophyll is expressed as micromoles per microgram of total protein.
c Similar results were obtained with the other blu mutants.

were exposed to blue light from the side (data not shown), the two responses are probably independent, consistent with the demonstration that the onset of these responses can be kinetically separated in cucumber hypocotyls (Cosgrove, 1985). A detailed investigation of the phototropic response in the blu mutants is in progress.

The complexity of photomorphogenic responses in the dicot seedling is astounding (Attridge, 1990). Isolation of mutants that have attenuated or nullified photomorphogenic responses demonstrates the potential for identifying, at the molecular level, components of light perception/transduction pathways leading to those responses. The blu mutants represent a new class of genes that will aid in the dissection of the complex network of light-regulated responses.

METHODS

Plant Materials, Growth Conditions, and Light Sources

Arabidopsis thaliana ecotype Columbia homozygous for the recessive gl1 mutation (Koornneef et al., 1982) was the parental strain used for the isolation of blue light-response mutants. The hy6 mutant was also in the Columbia background (Chory et al., 1989a). The other hy mutants were in the Landsberg erecta background (Koornneef et al., 1980). Seeds were surface sterilized for 20 min in 30% (v/v) commercial bleach, rinsed several times with sterile H2O, and planted in Petri dishes containing growth medium consisting of Murashige and Skoog salts (Murashige and Skoog, 1962), 2% (w/v) sucrose, and 0.8% (w/v) agar. Seeds were incubated on this medium in the dark for 2 to 3 days at 4 ± 1°C. The cold-conditioned seeds were exposed to saturating red light for 30 min to induce germination (Cone and Kendrick, 1985), then transferred to the dark for 23.5 hr at 23 ± 2°C. The dishes were then moved to the various light conditions. When appropriate, seedlings (5 to 7 days old) were transferred (four seedlings/10-cm pot) to a soil-less growth medium (Cornell-Mix:sand, 3:1) saturated with nutrient solution (Estelle and Somerville, 1987) and grown to maturity under constant illumination (65 ± 5 μmol m⁻² sec⁻¹) at 23 ± 2°C. Potted plants were watered daily with distilled H₂O and once a week with nutrient solution (Estelle and Somerville, 1987).

Light for potted plants and white light treatments was provided from cool-white fluorescent lamps (F96T12-CW). Red light (10 μmol m⁻² sec⁻¹) for induction of germination was obtained by filtering light from cool-white fluorescent lamps (F48T12-CW).

Table 5. Growth Features of blu Mutants

<table>
<thead>
<tr>
<th>Time</th>
<th>Wild Type</th>
<th>blu1</th>
<th>blu2</th>
<th>blu3-1a</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosette diameter (cm)</td>
<td>3.2 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>Blade length/width ratiob</td>
<td>1.3 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Petioleblade ratiob</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Chlorophyll content (mmol g⁻¹, fresh weight)c</td>
<td>426 ± 15</td>
<td>451 ± 12</td>
<td>493 ± 18</td>
<td>371 ± 20</td>
</tr>
<tr>
<td>Anthocyanin inductiond</td>
<td>6.7 - 6.8</td>
<td>19.9 - 20.0</td>
<td>2.0 - 2.1</td>
<td>5.1 - 5.2</td>
</tr>
<tr>
<td>35 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of main stem (cm)</td>
<td>21.4 ± 1.0</td>
<td>22.8 ± 0.9</td>
<td>17.4 ± 1.5</td>
<td>19.8 ± 1.5</td>
</tr>
<tr>
<td>Apical dominancee</td>
<td>1.3 ± 0.2</td>
<td>2.1 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

Seeds were grown on agar plates for 7 days in continuous white light (45 ± 3 μmol m⁻² sec⁻¹) and then transferred to soil and grown in continuous white light at a fluence rate of 65 ± 5 μmol m⁻² sec⁻¹. Wild-type data represent mean ± se from six plants. blu mutant data represent mean ± se from 10 plants.

a Similar results were observed with blu3-2.
b Determined from measurements on the longest rosette leaf of each plant.
c Chlorophyll a/b ratios were consistent (2.6 to 2.8) between genotypes.
d Data represent the ratio of anthocyanin content in induced versus uninduced plants (see Methods for details).
e Data represent the number of out-grown side shoots in the rosette.
from Lehle Seeds (Tucson, AZ). Approximately 3000 to 3500 MP
Ethyl methanesulfonate-mutagenized MO seeds were obtained

Isolation of Mutants and Genetic Analysis

seeds were transferred to 56 * 2 pmol m-' sec-' of blue light

plants were obtained by filtering light from four halogen flood lamps (GE 150W Quartzline) through 5 cm of 1.5% CuSO4.7H2O and one layer of Rohm and Haas blue plexiglass no. 2045 (3.18 mm thick, Dayton Plastics). The CuSO4.7H2O solution was cooled by running cold tap water through copper tubing submerged in the solution. The resulting spectral output had a peak intensity at 480 nm and a 100 nm half-bandwidth. Blue light for characterization of mutants was obtained by filtering light from one halogen flood lamp through 3 cm of water-cooled 1.5% CuSO4.7H2O and a wide-band blue interference filter (5 cm²). The filtered light had a peak intensity at 470 nm and a 100 nm half-bandwidth. Far red light was obtained by filtering light from one halogen flood lamp through 3 cm of H2O and one layer of far red plexiglass no. FRF 700 (3.18 mm thick, Westlake Plastics Co., Lenni, PA). The fluence rate for far red light was measured for wavelengths between 710 and 750 nm because far red-absorbing phytochrome absorbs maximally at 730 nm. Wavelengths above 750 nm were assumed to be inactive in the responses we tested. Fluence rates were adjusted by changing the lamp voltage or by changing the distance between plant material and the light source. Fluence rates at the level of seedlings were measured with an LI-1800 portable spectroradiometer (LiCor, Inc., Lincoln, NE).

Isolation of Mutants and Genetic Analysis

Ethyl methanesulfonate-mutagenized M2 seeds were obtained from Lehle Seeds (Tucson, AZ). Approximately 3000 to 3500 M2 seeds were screened from each of 14 independent populations. After the cold and red light germination-induction treatment, M2 seeds were transferred to 56 ± 2 μmol m² sec⁻¹ of blue light given from directly above. Seedlings were scored for hypocotyl length after 4 days of growth in continuous blue light. Forty-seven putative long hypocotyl mutants were recovered using this selection. Thirty-one putative mutants survived to maturity after transfer to soil-less growth medium and were allowed to self-pollinate. The resulting M3 seeds were rescreened for the long hypocotyl phenotype in blue light. Ten nonlethal, fertile, blue light-response mutants were recovered from these M3 populations. The four mutants with the longest hypocotyls were analyzed further in the M4 generation. Each of these four mutants was crossed to wild type to determine patterns of inheritance. Allelism was tested by crossing the blue light-response mutants to each other and to the six hy mutants (Koornneef et al., 1980; Chory et al., 1989a). Double mutants homozygous for blu1 and hy6 were obtained from F2 progeny of a cross between blu1 and hy6. Selection was done under white light where the double mutants grew as long as dark controls. A number of the double mutants survived to maturity and set seed. Before using these F3 seeds for the experiment in Figure 3, several individual seeds were grown under blue and far red light to confirm that the seedlings showed both blu1 and hy6 phenotypes.

Measurement of Cotyledon Expansion

Cotyledon pairs were excised from 5-day-old seedlings and placed onto transparent tape. Images of the cotyledons were projected from a photographic enlarger (magnification ×10) and traced on paper. The paper tracings were cut out and weighed, and the areas were calculated from the weight.

Extraction and Analysis of Chlorophyll and Anthocyanin Pigments

For chlorophyll determination in seedlings, cotyledon pairs were excised from 5-day-old seedlings grown under the indicated light conditions. For chlorophyll determinations in leaves, plants were grown in continuous white light at a fluence rate of 65 ± 5 μmol m⁻² sec⁻¹. Anthocyanin pigments were induced to form by transferring 19-day-old plants to the dark for 24 hr, followed by 24 hr of high-intensity white light (300 μmol m⁻² sec⁻¹). Control plants were left in the normal growth conditions for the entire 21 days. Leaves (0.1 to 0.5 g, fresh weight) or cotyledons (10 pairs) were ground in 200 μL of ethanol to extract chlorophylls (McCourt et al., 1987) and anthocyanins. The sample was centrifuged in a microcentrifuge for 5 min at 10,000 rpm and the supernatant removed. The pellet was re-extracted in 50 μL of ethanol, the supernatants were combined, and acetone was added to give a final acetone concentration of 80% (v/v). Chlorophyll contents were determined in the final extract as described by Graan and Ort (1984).

To determine the anthocyanin content, the acetone and ethanol in the final extract were evaporated at reduced pressure at 65°C. The remaining aqueous phase was extracted with chloroform (2:1) to remove chlorophyll. The aqueous phase was acidified with 20 μL of concentrated HCl (Chory et al., 1989a) and brought to a final volume of 2 mL with H2O. Quantification of anthocyanin pigments followed the methods of Feinbaum and Ausubel (1988) and were normalized to the fresh weight used in each sample.

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