INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
Genetic dissection of light-regulated development in *Arabidopsis*

Liscum, Emmanuel, III, Ph.D.

The Ohio State University, 1992
GENETIC DISSECTION OF LIGHT-REGULATED DEVELOPMENT IN ARABIDOPSIS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

Emmanuel Liscum, III, B.S.

* * * * *

The Ohio State University

1992

Dissertation Committee:  
K. R. Davis
R. P. Hangarter
M. L. Tierney

Approved by

Advisor
Department of Plant Biology
To Emmanuel Liscum, Sr.

&

Emmanuel Liscum, Jr.
ACKNOWLEDGEMENTS

I wish to thank my parents for their unshakeable support of my career goals. Thanks to my wife, Janet, for putting up with my retentive nature and compulsive work habits. Thanks to my best friend, Gerard "Vito" Taibi, for being Jer... 'nough said. Go Devils and Habs!

A special thanks to my advisor, Roger Hangarter, for giving me a chance to fulfill my dreams. I'm proud to call the "Rog-meister", friend and mentor. (Every grad student should be so lucky!) Thanks to Mary Tierney and Keith Davis for professional and personal friendship. Infinite thanks to the lab: Jeff Young, Mee-Rye Cha, Emma Knee, Kobuta Kan, and recently Brian Parks. To Jayne Robinson, Jen McMahon, and Sandra & Kurt Weise, thanks for being some of my closest friends. I hope we never lose that. I'll miss you guys!

In a category by himself - David M. Nahra. Thanks Dave (and Deb)!!!!!!! Don't change. RUSH and Roll!!!

And finally to all those rock 'n' rollers who've kept me going (and driven my lab nuts): Black Sabbath, DIO, RUSH, AC/DC, Schenker Bros., Jimi Hendrix, and of course SRV. "The ending is just the beginning. The closer you get to the meaning, the sooner you'll know that you're dreaming" - RJD

iii
VITA

June 30, 1966 .................. Born, Watertown, New York, USA

1988 ............................... B.S., State University of New York at Plattsburgh, Plattsburgh, New York

1988 - 1991 ................... Research and Teaching Associate, Department of Plant Biology, The Ohio State University, Columbus, Ohio

1991 - Present ..................... Presidential Fellow, Graduate School, The Ohio State University, Columbus, Ohio

PUBLICATIONS


FIELDS OF STUDY

Major Field: Plant Biology
Studies in Plant Physiology - Roger P. Hangarter
TABLE OF CONTENTS

DEDICATION .................................................. ii
ACKNOWLEDGEMENTS .......................................... iii
VITA ........................................................ iv
LIST OF TABLES ............................................. viii
LIST OF FIGURES ........................................... ix
INTRODUCTION ............................................... 1

CHAPTER PAGE

I. ISOLATION AND PRELIMINARY CHARACTERIZATION OF Arabidopsis MUTANTS LACKING BLUE LIGHT-DEPENDENT INHIBITION OF HYPOCOTYL ELONGATION .......................... 12

   Materials and Methods .................................. 16
   Results .................................................. 21
   Discussion ............................................. 36

II. GENETIC SEPARATION OF PHOTOTROPISM AND BLUE LIGHT INHIBITION OF STEM ELONGATION .......................... 42

   Materials and Methods .................................. 43
   Results .................................................. 47
   Discussion ............................................. 53

III. LIGHT-STIMULATED APICAL HOOK OPENING IN WILD-TYPE Arabidopsis SEEDLINGS .................................. 57

   Materials and Methods .................................. 58
   Results .................................................. 62
   Discussion ............................................. 74

IV. LIGHT-STIMULATED APICAL HOOK OPENING IN PHOTOMORPHOGENIC MUTANTS OF Arabidopsis thaliana:
   DISSECTION OF THE SIGNALLING PATHWAYS .............. 78

   Materials and Methods .................................. 82
   Results .................................................. 84
   Discussion ............................................. 96
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Genetic analysis of blu mutants</td>
<td>25</td>
</tr>
<tr>
<td>2. Complementation of blu mutants with hy mutants</td>
<td>27</td>
</tr>
<tr>
<td>3. Light-dependent cotyledon expansion in blu mutants</td>
<td>32</td>
</tr>
<tr>
<td>4. Chlorophyll content in cotyledons</td>
<td>34</td>
</tr>
<tr>
<td>5. Growth features of blu mutants</td>
<td>35</td>
</tr>
<tr>
<td>6. Genetic analysis of JK218 and blu mutants</td>
<td>50</td>
</tr>
<tr>
<td>7. Far-red photoreversibility of light-stimulated hook opening</td>
<td>72</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Effect of light quality on the inhibition of hypocotyl elongation in wild-type and blu and hv6 mutants</td>
<td>23</td>
</tr>
<tr>
<td>2. Histograms of hypocotyl lengths for wild-type and blue light-response mutants</td>
<td>24</td>
</tr>
<tr>
<td>3. Comparison of wild-type and mutant seedlings grown under various light conditions</td>
<td>29</td>
</tr>
<tr>
<td>4. Time course of germination of wild-type and blu mutant seeds</td>
<td>31</td>
</tr>
<tr>
<td>5. Comparison of blue light-induced phototropism and inhibition of hypocotyl elongation in wild-type and mutant seedlings</td>
<td>49</td>
</tr>
<tr>
<td>6. Fluence-response relationships for phototropism in wild-type and mutant seedlings</td>
<td>51</td>
</tr>
<tr>
<td>7. Fluence rate-response relationships for inhibition of hypocotyl elongation in wild-type and mutant seedlings</td>
<td>52</td>
</tr>
<tr>
<td>8. Time course of apical hook opening in dark-grown seedlings</td>
<td>63</td>
</tr>
<tr>
<td>9. Representative photographs of apical hook opening and cotyledon unfolding in white light</td>
<td>65</td>
</tr>
<tr>
<td>10. Fluence rate-response curves for blue, red, and far-red light-stimulated hook opening</td>
<td>66</td>
</tr>
<tr>
<td>11. Fluence rate-response curves for blue, red, and far-red light-stimulated cotyledon unfolding</td>
<td>68</td>
</tr>
</tbody>
</table>
12. Reciprocity relationships of the hook opening response ............... 70
13. Kinetics for the escape from far-red photoreversibility of red light-stimulated hook opening .................... 73
14. Representative photographs of de-etiolation responses of the apical hook in wild-type and photomorphogenic mutant seedlings exposed to 14 h of high fluence rate red, far-red, or blue light ............... 86
15. Fluence rate-response curves for red light-stimulated hook opening in wild-type and photomorphogenic mutants .................... 88
16. Fluence rate-response curves for far-red light-stimulated hook opening in wild-type and photomorphogenic mutants .................... 90
17. Fluence rate-response curves for blue light-stimulated hook opening in wild-type and photomorphogenic mutants .................... 91
18. Time course of apical hook opening in wild-type and photomorphogenic mutant seedlings exposed to high fluence rate blue light .................... 93
19. Fluence rate-response curve for blue light-stimulated hook opening in blul hy6 double mutant seedlings .................... 95
INTRODUCTION

Light-regulated development (photomorphogenesis) is common throughout the plant kingdom (Salisbury and Ross, 1985; Kendrick and Kronenberg, 1986). Several light-dependent responses have been extensively studied in plants, including, stem growth inhibition, cotyledon expansion, leaf and root growth, acquisition of photosynthetic competence, control of gene expression and protein activity, and the induction of flowering (see Senger, 1980; Kendrick and Kronenberg, 1986; Furuya, 1986; Attridge, 1990; Quail, 1991; Sage, 1992).

Two of the earliest recognized photomorphogenic responses of higher plants were light-controlled seed germination (Flint, 1934) and phototropic curvature (Johnston, 1934). Flints' observations (1934) on the effects of light on lettuce seed germination were the first to indicate the functioning of a red light-absorbing photoreceptor, while Johnstons' observations (1934) provided evidence for a blue light-absorbing photoreceptor. In the years since those early observations, our knowledge of photoreceptor systems has expanded to include at least three
classes of photoreceptor systems: the red/far-red light (500-800 nm) photoreceptor phytochrome, blue/UV-A light (320-500 nm) photoreceptor(s), and UV-B light (280-320 nm) photoreceptor(s) (Mohr, 1986). However, phytochrome is the only photomorphogenic photoreceptor to have been studied at the molecular genetic and biochemical levels (e.g. Quail et al., 1987; Sharrock and Quail, 1989; Quail, 1991). The nature of the blue and UV photoreceptors has been inferred from descriptive studies (see Senger, 1980, 1984, 1987a, b; Senger and Briggs, 1981; Briggs and Iino, 1983; Galland and Senger, 1988; Attridge, 1990). A brief summary of major advancements, and the current state of photoreceptor research is presented below.

Phytochromes

Since the initial identification and biochemical purification of phytochrome (Butler et al., 1959; Siegelman and Firer, 1964; Siegelman and Hendricks, 1965) much of the research in plant photobiology has been devoted to the study of the biochemistry, genetics, and molecular genetics of phytochrome and phytochrome action (for review see Kendrick and Kronenberg, 1986; Furuya, 1986; Smith and Whitelam, 1990; Quail, 1991; Sage, 1992). Although much of this effort has been directed towards the extensive characterization of phytochrome in dark-grown (etiolated) tissue (see Vierstra and Quail, 1986), physiological studies
comparing etiolated and light-grown plants suggested that there was more than one pool of phytochrome (e.g. Downs et al., 1957; Hillman, 1967; Jabben and Holmes, 1983). Biochemical evidence for the existence of distinct pools of phytochrome came with the development of monoclonal antibodies to phytochrome from etiolated *Avena* tissue (Tokuhisa and Quail, 1983; Shimazaki et al., 1983). Using various monoclonal antibodies against etiolated-pea phytochrome, Abe et al. (1985) were able to partially purify two biochemically distinct phytochromes from light-grown peas, that they designated phytochrome I and phytochrome II. Subsequent studies indicated that phytochrome I represents the predominate phytochrome species in etiolated tissue; while phytochrome II predominates in light-grown tissue (e.g. Abe et al., 1985; Shimazaki and Pratt, 1985; Tokuhisa et al., 1985; Furuya, 1989; Wang et al., 1991). N-terminal amino acid sequencing showed that phytochrome I (otherwise known as Type I phytochrome) and phytochrome II (Type II phytochrome) are divergent at the amino acid level, therefore demonstrating that Type I and Type II phytochromes are encoded by different genes (Abe et al., 1989).

In addition to biochemical evidence for the existence of distinct pools of phytochrome, molecular genetic approaches also indicated that multiple phytochrome genes exist. A phytochrome gene family composed of five diverse members was identified in *Arabidopsis*; three of these genes
(phyA, phyB, and phyC) were isolated by screening cDNA libraries (Sharrock and Quail, 1989), and sequences for two (phyD and phyE) were identified by PCR based cloning methods (see Sage, 1992). The derived amino acid sequences of the Arabidopsis phyA gene and phyA genes isolated from etiolated-tissues of oat (Hershey et al., 1985), rice (Kay et al., 1989a, b), corn (Christensen and Quail, 1989), zucchini (Sharrock et al., 1986), and pea (Sato, 1988) exhibit 64-79% sequence homology. This level of amino acid homology, along with the observation that the phyA gene in Arabidopsis is highly expressed in etiolated tissue but strongly down-regulated by white light, indicates that the phyA gene in Arabidopsis represents Type I, light-labile phytochrome (Sharrock and Quail, 1989).

The phyB, phyC, phyD, and phyE genes exhibit limited sequence homology to phyA genes, and this is mainly limited to the regions in and around the chromophore attachment site (Sharrock and Quail, 1989; see Sage, 1992). In addition, these genes are expressed at low steady state levels in both etiolated and de-etiolated tissue (Sharrock and Quail, 1989; Somers et al., 1991; see Sage, 1992), suggesting that they code for Type II, light-stable phytochromes. The phyB gene from Arabidopsis shows significant amino acid sequence homology with pea phytochrome II peptide fragments (Abe et al., 1989), as does a rice phytochrome gene that is expressed at low levels in rice seedlings independent of
light treatment, as would be expected for a Type II phytochrome (Dehesh et al., 1991). These results provide molecular evidence for phytochrome gene families in both monocots and dicots.

Until recently only correlative physiological evidence linked Type I and Type II phytochromes with various photomorphogenic responses (for review, see Smith and Whitelam, 1990), however analysis of photomorphogenic mutants and transgenic plants provides a genetic and molecular genetic approach to studying the physiological roles of Type I and Type II phytochromes in higher plants (for review, see Adamse et al., 1988b; Tomizawa et al., 1990; Smith and Whitelam, 1990; Kendrick and Nagatani, 1991). For example, studies of the aurea mutant of tomato, which lacks light-labile Type I phytochrome apoprotein (Koornneef et al. 1985; Parks et al. 1987), demonstrated that this mutant lacks the far-red high irradiance-dependent inhibition of hypocotyl growth (Adamse et al. 1988a), but responds normally to end-of-day far-red treatments (Adamse et al. 1988c). These results suggest that Type I phytochrome mediates far-red-dependent hypocotyl inhibition in etiolated seedlings, while light-stable, Type II phytochrome mediates the enhanced stem growth observed in de-etiolated plants after end-of-day far-red treatment. These conclusions are also supported by studies of the hy3 mutant of Arabidopsis and the lh mutant of cucumber. These
mutants are deficient in phytochrome B apoprotein, the predominant Type II phytochrome, but retain wild-type amounts of the phytochrome A apoprotein (hyβ3: Nagatani et al. 1991; Somers et al. 1991; lh: Lopez-Juez et al. 1992). In contrast to the phytochrome A-deficient aurea mutant, these mutants retain sensitivity to far-red light with respect to hypocotyl inhibition (hyβ3: Koornneef et al. 1980; lh: Adamse et al. 1987, 1988d; Peters et al. 1991), but lack sensitivity to end-of-day far-red (hyβ3: Goto et al. 1991; Nagatani et al. 1991; lh: Adamse et al. 1988d; Lopez-Juez et al. 1990, 1992). Recently it was shown that the overexpression of a heterologous phytochrome A protein in tobacco causes a persistent high irradiance-dependent hypocotyl inhibition by far-red light (McCormac et al. 1992). However, these phytochrome A-overexpressing plants responded to end-of-day far-red treatment similar to wild-type controls (McCormac et al. 1992). All of these studies provide evidence for the role of Type I phytochrome (phytochrome A) in the far-red light high irradiance-dependent hypocotyl growth inhibition response, and Type II phytochrome (at least phytochrome B) in the sensitivity to end-of-day far-red light.

Blue/UV-A photoreceptor(s)

Although blue/UV-A responses are found in halobacteria, fungi, mammals, and plants (Senger, 1980, 1984), no blue/UV-
A light-absorbing photoreceptor has been biochemically identified. However, several studies suggest that flavins or flavoproteins are involved in blue/UV-A light photoperception in fungi and plants (for review, see Schmidt and Butler, 1976; Schmidt, 1980; Senger, 1980, 1984; Galland and Senger, 1988a). For example, action spectra of phototropism in wild-type *Phycomyces* exhibit a small peak that corresponds to a transitional energy state of riboflavin (Delbruck et al., 1976). In fact, the absolute extinction coefficient of the receptor pigment for phototropism in *Phycomyces* matches the extinction coefficient of riboflavin (Delbruck et al., 1976). In addition, when a riboflavin auxotroph was provided with the riboflavin analog roseoflavin, which has significantly different absorption properties compared to riboflavin, corresponding shifts in the phototropism action spectrum were observed (Otto et al., 1981). In addition, selected photobehavioral mutants of *Phycomyces* have been shown to have reduced levels of flavins (Hohl et al., 1992a). Furthermore, Vierstra and Poff (1981a) demonstrated that phototropism was inhibited in corn seedlings treated with the flavin antagonist phenylacetic acid, suggesting that flavins act as blue/UV-A light receptor chromophores in plants as well. In contrast, when seedlings were treated with Nurflurazon, an inhibitor of carotenoid biosynthesis, the phototropic response was not abrogated, indicating that
this class of pigments with absorption maxima in the 400-500 nm range-500 nm range are probably not acting as blue/UV-A light photoreceptor chromophores in this response (Vierstra et al., 1981b).

Systems involved in blue light-induction of flavonoid biosynthesis, phosphorylation of a specific plasma membrane protein, and binding of GTP to a plasma membrane bound G-protein have recently yielded compelling evidence for flavin-based blue light photoperception in higher plants. In the case of flavonoid biosynthesis, Ensminger and Schafer (1992) have shown that light treatment induces higher levels of chalcone synthase and flavonoids in parsley cells that have been fed riboflavin, compared to control cells that haven’t received exogenous flavin. This flavin sensitized reaction apparently requires uptake of flavin and association with flavin binding sites on the plasma membrane. Warpeha et al. (1992) showed that the blue light-dependent binding of GTPyS to a plasma membrane-bound G-protein in pea can be inhibited by the flavin antagonist KI. Concentrations of less than 10 μM KI were required for inhibition of the GTP binding reaction, suggesting that a flavin excited triplet state is necessary for this reaction. Short et al. (1992) have shown that KI can also inhibit the blue light-dependent phosphorylation of a 120 kD plasma membrane protein in pea that has been hypothesized to be involved in phototropism. Inhibition of the phosphorylation reaction by KI requires
concentrations of at least 20 mM, suggesting that a singlet state flavin is required for this reaction. Correlative genetic evidence for the involvement of a flavin chromophore-based photoreceptor for phototropism in higher plants has been provided by a study of a presumptive phototropism photoreceptor mutant of Arabidopsis. This mutant exhibits a shift in fluence dependence for phototropic response (Khurana and Poff, 1989; Konjevic et al., 1992), and also shows a similar shift in quantum efficiency for the blue light-induced protein phosphorylation reaction mentioned above (Reymond et al., 1992).

Pterins have also been considered as possible chromophores for blue/UV-A light photoreceptors in plants because of their chemical and photophysical properties (Galland and Senger, 1998b). Indeed, both flavins and pterins appear to act during photoperception in microorganisms (Jorns et al., 1990), fungi (Hohl et al., 1992a, b), and lower plants (Brodhum and Hader, 1990). However, it has not yet been demonstrated that pterins participate in blue/UV-A photoperception in higher plants.

Although significant progress is being made in characterizing the properties of specific photoreceptors, it is still often difficult to determine which photoreceptor system(s) are controlling various responses. For example, it can be difficult to study the blue and ultraviolet light
responses independently, since the action spectra of the blue and UV light responses overlap. Furthermore, since phytochrome absorbs light in the blue spectral range it has also been difficult to exclude the possibility that phytochrome itself is involved in many blue light-driven photomorphogenic responses in plants. In addition, even though there are numerous developmental and biochemical events which have been shown to be regulated by UV, blue, and red/far-red light, the number of signal transduction pathways involved is unknown. In fact, no signal transduction component downstream of a photoreceptor has been biochemically identified. One approach to dissecting the complex networks controlling physiological and biochemical responses is to isolate mutants altered in components of various signalling pathways. For example, plants containing mutations that eliminate certain parts of a photomorphogenic response, while leaving others intact, would provide instrumental tools for understanding these complex processes.

Several studies have described in detail the advantages of using the cruciferous plant Arabidopsis thaliana for obtaining and characterizing specific mutants (for reviews see, Estelle and Somerville, 1986; Meyerowitz, 1987, 1989). Among its advantages are, its short generation time (4-6 weeks), small size, prolific seed production, small genome (100,000 Kb) with little repetitive DNA, ease of generating
mutagenized populations, and susceptibility to transformation using Agrobacterium. In addition, mutants of Arabidopsis are proving to be useful for investigating signal perception and transduction processes in plants (e.g. Olsen et al., 1984; Bleecker et al., 1988; Wilson et al., 1990; Chory et al., 1991). Also significant is the fact that others have isolated photomorphogenic mutants of Arabidopsis by selective screening, demonstrating the feasibility of isolating mutants for specific photosensory systems (e.g. Koornneef et al., 1980; Chory et al., 1989a; Khurana and Poff, 1989). The objectives of this dissertation were:

1. To isolate and characterize mutants that are altered in the inhibition of hypocotyl elongation caused by continued exposure to blue light.

2. To use these new mutants, as well as previously isolated photomorphogenic mutants, to determine what photoperception systems mediate various responses, such as the inhibition of hypocotyl elongation and de-etioluteion of the apical hook.

3. To use available mutants to investigate the genetic relatedness of blue light-stimulated responses that depend on changes in cell elongation patterns for phenotypic expression, such as the inhibition of hypocotyl elongation, phototropism, and apical hook opening.
CHAPTER I

ISOLATION AND PRELIMINARY CHARACTERIZATION OF Arabidopsis mutants lacking blue light-dependent inhibition of hypocotyl elongation

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 ultraviolet light are especially effective in inducing photomorphogenic responses through the action of the red/far red photoreceptor phytochrome, blue/UV-A photoreceptor(s), and ultraviolet (UV-B) photoreceptor(s) (Mohr, 1986). However, phytochrome is the only plant photoreceptor pigment to be identified at the genetic and biochemical level (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 which 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 carotenoid-less 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 to 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 vitro phosphorylation of a plasma membrane-associated protein. Kinetic properties and fluence response relationships for the phosphorylation activity were found to be comparable to 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 plasmamembrane 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, which 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 (hyl, 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 chapter 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.

MATERIALS AND METHODS

Plant Materials, Growth Conditions and Light Sources

*Arabidopsis thaliana* ecotype Columbia homozygous for the recessive *gll* mutation (Koornneef et al., 1982) was the parental strain used for the isolation of blue light-response mutants. The *hv6* mutant was also in the Columbia background (Chory et al. 1989a). The other *hv* 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 soilless 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 s⁻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 s⁻¹) for induction of germination was obtained by filtering light from cool-white fluorescent lamps (F48T12-CW-1500) through one layer of Rhom and Haas red plexiglass No. 2444 (3.18 mm thick, Dayton Plastics, Columbus, OH), one layer of yellow Roscolux no. 10, and 1 cm of a 1.5% (w/v) CuSO₄·7H₂O solution. Blue light used for isolation of mutants was 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, Columbus, OH). The CuSO₄·7H₂O solution was cooled by running cold tap H₂O 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 H$_2$O 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 P$_{fr}$ 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 a LI-1800 portable spectroradiometer (LiCor, Inc., Lincoln, NE).

Isolation of Mutants and Genetic Analysis

Ethyl methanesulfonate (EMS)-mutagenized M$_2$ seeds were obtained from Lehle Seeds (Tucson, AZ). Approximately 3000 - 3500 M$_2$ seeds were screened from each of 14 independent populations. After the cold and red light germination-induction treatment, M$_2$ seeds were transferred to 56 ± 2 μmol m$^{-2}$ s$^{-1}$ of blue light given from directly above. Seedlings were scored for hypocotyl length after 4 days of growth in continuous blue light. A total of 47 putative long hypocotyl mutants were recovered using this selection. Thirty-one putative mutants survived to maturity after transfer to soilless growth medium and were allowed to self pollinate. The resulting M$_3$ 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 blul and hy6 were obtained from F2 progeny of a cross between blul 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. Prior to using these F3 seeds for the experiment in Figure 3, several individuals were grown under blue and far red light to confirm that the seedlings showed both blul and hy6 phenotypes.

Hypocotyl lengths were measured to the nearest millimeter on 5-day-old seedlings with opened cotyledons and no seed coat attached so that late germinating seeds were not included in the measurements. During the segregation analysis with the blu mutants, hypocotyls were considered long if they were longer than 6 mm (Figure 2).

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 (10X) and traced
on paper. The paper tracings were cut out, weighed, and the areas 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 $65 \pm 5 \mu$mol m$^{-2}$ s$^{-1}$. 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 ($300 \mu$mol m$^{-2}$ s$^{-1}$). 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 ethanol to extract chlorophylls (McCourt et al., 1987) and anthocyanins. The sample was centrifuged in a microfuge for 5 min at 10,000 rpm and the supernatant removed. The pellet was re-extracted in 50 µL 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 (1986) and were normalized to the fresh weight used in each sample.

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 which are inhibitory to hypocotyl elongation in wild-type plants (Redei and Hirono, 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.

Using this selection scheme, we isolated 10 blue light-specific elongation mutants of Arabidopsis from 14 different populations of ethyl methanesulfonate (EMS)-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 and blue light-grown wild-type seedlings. We tentatively designated this class of mutants as bli, 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
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 (mm) 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 μmol m\(^{-2}\) s\(^{-1}\); blue, 56 ± 2 μmol m\(^{-2}\) s\(^{-1}\); far red, 30 ± 2 μmol m\(^{-2}\) s\(^{-1}\).
Figure 2. Histograms of hypocotyl lengths for wild-type and blue light-response mutants of Arabidopsis. Blue light was provided from above at a fluence rate of 56 ± 2 μmol m⁻² s⁻¹. Hypocotyl lengths (mm) were measured after 5 days of growth. Line 16bl2 represents a typical bli type mutant and line blu3-1 represents a typical blu mutant.
Table 1. Genetic analysis of blu mutants.

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Short</th>
<th>Long</th>
<th>$X^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$blu1/blu1 \times BLU1/BLU1^c$</td>
<td>F1 34</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$BLU1/blu1 \times BLU1/blu1$</td>
<td>F2 374</td>
<td>118</td>
<td>0.271</td>
</tr>
<tr>
<td>$blu2/blu2 \times BLU2/BLU2$</td>
<td>F1 37</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$BLU2/blu2 \times BLU2/blu2$</td>
<td>F2 238</td>
<td>83</td>
<td>0.126</td>
</tr>
<tr>
<td>$blu3-1, blu3-1 \times BLU3-1/BLU3-1^c$</td>
<td>F1 46</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$BLU3-1/blu3-1 \times BLU3-1/blu3-1$</td>
<td>F2 450</td>
<td>161</td>
<td>0.594</td>
</tr>
<tr>
<td>$blu1/blu1 \times blu2/blu2$</td>
<td>F1 18</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$blu1/blu1 \times blu3-1/blu3-1$</td>
<td>F1 27</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$blu1/blu1 \times blu3-2/blu3-2$</td>
<td>F1 16</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$blu2/blu2 \times blu3-1/blu3-1$</td>
<td>F1 39</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$blu2/blu2 \times blu3-2/blu3-2$</td>
<td>F1 16</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$blu3-1, blu3-1 \times blu3-2/blu3-2$</td>
<td>F1 0</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

*Wild-type have short hypocotyls ($\leq$ 6 mm), mutants have long hypocotyls ($> 6$mm).

P > 0.05.

Data represent mutant (♂):wild-type (♀) crosses; similar results were obtained wild-type (♂):mutant (♀) crosses.

$X^2$ expected ratio. 3 wild-type:1 mutant.

Similar results were obtained with blu3-2.
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; 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
Table 2. Complementation of blu mutants with hy mutants\(^a\).

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

\(^a\) Wild-type have short hypocotyls (≤ 6 mm), mutants have long hypocotyls (> 6 mm).

\(^b\) Data represent hy (♂):blu (♀) crosses.

\(^c\) Similar results were obtained with blu3-2.
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 continuous far red light was similar to wild-type seedlings grown under the same conditions. However, in continuous white light blu1, blu2, and blu3-1 seedlings were approximately twice as long as wild type, while blu3-2 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 while 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.

A representative photograph of wild-type, blu1, and hy6 under the different light conditions is illustrated by Figure 3. In addition, Figure 3 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
Figure 3. Comparison of wild-type and mutant seedlings grown under various light conditions. Seedlings were photographed when 5 days old. Light conditions were as described in Figure 1.
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, blul 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 blul and blu2 seedlings, while
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.
Table 3. Light-dependent cotyledon expansion in *blu* mutants.

<table>
<thead>
<tr>
<th>Light</th>
<th>Wild-type</th>
<th><em>blu1</em></th>
<th><em>blu2</em></th>
<th><em>blu3-1</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cotyledon Area (mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>white</td>
<td>4.1 ± 0.3</td>
<td>3.1 ± 0.4</td>
<td>3.0 ± 0.2</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>blue</td>
<td>4.6 ± 0.3</td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>far red</td>
<td>2.4 ± 0.1</td>
<td>2.5 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>3.3 ± 0.1</td>
</tr>
</tbody>
</table>

*a* Actinic light-conditions were as described in Figure 1.

*b* Similar results were obtained with *blu3-2*.

*c* Data represent mean ± SE of 10 cotyledon pairs. Cotyledon area of dark-grown seedlings averaged 0.34 ± 0.01 mm² for all genotypes.
blu3-1 and blu3-2 cotyledons expanded similarly to wild-type cotyledons. Under far red light, cotyledon expansion was reduced slightly in blu2 seedlings, but expansion was enhanced in blu3-1 and blu3-2 when compared with wild-type cotyledons.

To determine if 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 blu1 and wild-type cotyledons, indicating that chloroplast development was unaffected by the blu mutations.

Growth Features of Mature blu Mutant Plants

To determine whether the blu mutations exhibit pleiotropic effects in mature plants, a number of growth characteristics were examined in plants grown under white light. The data in Table 5 show that the morphology of 21- and 35-day-old blu mutants and wild-type plants was similar. Although chloroplast development is controlled partly by blue light (Richter and Ottersbach, 1990), total leaf chlorophyll content was similar in the blu mutants and wild-type plants grown in white light. The chlorophyll a/b ratio was between 2.6 and 2.8 in all of the plants. However, the accumulation of anthocyanin pigments, which is regulated by high irradiance blue and ultraviolet light (Mancinelli and
Table 4. Chlorophyll content in cotyledons.

<table>
<thead>
<tr>
<th>Light(^a)</th>
<th>Wild-type</th>
<th></th>
<th></th>
<th></th>
<th>blu(^b)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Chlorophyll(^c)</td>
<td>Chl (a/b) ratio</td>
<td>Total Chlorophyll(^c)</td>
<td>Chl (a/b) ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dark</td>
<td>0.00</td>
<td>1.17</td>
<td>0.00</td>
<td>1.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>white</td>
<td>0.25</td>
<td>2.30</td>
<td>0.19</td>
<td>2.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>blue</td>
<td>0.33</td>
<td>2.39</td>
<td>0.48</td>
<td>2.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>far red</td>
<td>0.01</td>
<td>2.71</td>
<td>0.01</td>
<td>2.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Actinic light-conditions were as described in Figure 1.

\(^b\) Similar results were obtained with the other blu mutants.

\(^c\) Total chlorophyll is expressed as \(\mu\text{mol} \cdot \mu\text{g} \text{ total protein}^{-1}\).
Table 5. Growth features of *blu* mutants$^a$.

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th><em>blu</em>1</th>
<th><em>blu</em>2</th>
<th><em>blu</em>3-$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>21 days</strong></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 ratio$^c$</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>Petiole-blade ratio$^c$</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)$^d$</td>
<td>1.70 ± 0.06</td>
<td>1.80 ± 0.05</td>
<td>1.97 ± 0.07</td>
<td>1.48 ± 0.08</td>
</tr>
<tr>
<td>Anthocyanin induction$^e$</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><strong>35 days</strong></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 dominance$^f$</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>

$^a$ Seeds were grown on agar plates for 7 days in continuous white light (45 ± 3 μmol·m$^{-2}$·s$^{-1}$) and then transferred to soil and grown in continuous white light at a fluence rate of 65 ± 5 μmol·m$^{-2}$·s$^{-1}$. Wild-type data represent mean ± SE from six plants. *Blu* mutant data represent mean ± SE from 10 plants.

$^b$ Similar results were observed with *blu*3-2.

$^c$ Determined from measurements on the longest rosette leaf of each plant.

$^d$ Chlorophyll *ab* ratios were consistent (2.6-2.8) between genotypes.

$^e$ Data represent the ratio of anthocyanin content in induced versus uninduced plants (see Methods for details).

$^f$ Data represent the number of out-grown side shoots in the rosette.
Rabino, 1978; Ohl et al., 1989), was enhanced threefold in blu1 plants and reduced threefold in blu2 plants exposed to high irradiance white light. Anthocyanin induction was normal in blu3-1 and blu3-2. Although anthocyanin induction in blu1 and blu2 was altered, it was the only consistently large difference observed among the various parameters measured between adult wild-type and blu plants. These results, when taken together, indicate that the blu mutations, which were identified at the seedling level, exert relatively benign effects during adult growth stages.

DISCUSSION

The common phenotype of the blu class of photomorphogenic mutants is the lack of blue light-dependent inhibition of hypocotyl elongation. It appears that the BLU genes that control the blue light-induced inhibition of hypocotyl growth have little to do with growth of adult plants, suggesting that these genes are primarily involved in seedling development in Arabidopsis. This is different from the phytochrome-related hy mutants which show pleiotropic affects in adult plants (Koornneef et al., 1980; Chory et al., 1989a). The blu mutants, which are not inhibited in hypocotyl elongation by blue light, are therefore different from the previously isolated blue light-response mutant, hy4, which is inhibited in hypocotyl
elongation 35% to 40% by blue light (Koornneef et al., 1980). Furthermore, heterozygotes obtained from crosses between blu and hy4 mutants have short hypocotyls, demonstrating genetic separation of the mutant phenotypes.

Because phytochrome can absorb blue and near UV light, it has been difficult to exclude the possibility that phytochrome itself is involved in the blue light absorption responsible for high irradiance-dependent responses (Hartmann, 1966; Wildermann et al., 1978). However, until now experiments testing this possibility relied on simultaneous exposures to different levels of blue and red or far red light to modulate the phytochrome photoequilibrium. The availability of mutants altered in specific blue light responses allows more direct approaches to testing the role of phytochrome in the high irradiance responses. Under the conditions we used to isolate them, the blu mutants lack blue 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; 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 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 ultraviolet 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, 1984). Because hypocotyl elongation was not inhibited in the blu mutants when blue light was given from above but differential growth occurred when they 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,
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 demonstrate 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.
CHAPTER II

GENETIC SEPARATION OF PHOTOTROPISM AND BLUE LIGHT INHIBITION OF STEM ELONGATION

Regulation of cell elongation is an intrinsic step in blue light-dependent inhibition of stem elongation and induction of phototropic curvature in higher plants (Cosgrove, 1986; Firn, 1986). It has been suggested that blue light inhibits longitudinal stem growth and induces phototropism through the same photoinhibition events at the cellular level (Blaauw, 1918) or that these responses share components in their signal transduction pathways (Elliot and Shen-Miller, 1976; Franssen et al., 1981). However, other studies indicate that cell elongation is controlled by discrete signal transduction systems in these two responses (Cosgrove, 1985; Macleod et al., 1985; Rich et al., 1985). Most of the data regarding these hypotheses are based on physiological experiments that were designed to distinguish between what are normally superimposed responses.

Several blue light response mutants have been found in Arabidopsis thaliana that provide a means of genetically dissecting the relationships between phototropism and
hypocotyl elongation. In particular, mutants have been isolated that lack blue light-dependent inhibition of hypocotyl growth, but curve in response to unilateral blue light (Liscum and Hangarter, 1991). This chapter presents results from a detailed investigation of blue light-induced phototropic and longitudinal growth responses of these hypocotyl elongation mutants as well as two phototropism mutants. The results show that blue light induces phototropism and inhibition of hypocotyl elongation through genetically distinct pathways.

MATERIALS AND METHODS

Plant Material

Hypocotyl elongation mutants (blul, blu2, and blu3) of Arabidopsis thaliana ecotype Columbia were described previously by Liscum and Hangarter (1991). Phototropism mutants (JK218 and JK224) of Arabidopsis thaliana ecotype Estland were described previously by Khurana and Poff (1989). Specifically, the blu mutants lack blue light-dependent inhibition of hypocotyl elongation, JK218 seedlings lack phototropic curvature, and JK224 seedlings require 20- to 30-fold more actinic light to induce first positive phototropism than do wild-type seedlings.

Genetic analysis
For genetic analysis, seeds were handled as described by Liscum and Hangarter (1991). Surface-sterilized seeds were planted on agar medium and incubated at 4°C in the dark for 2-3 days. The seeds were then given 30 min of red light at 23°C, followed by 23.5 h in the dark, and then transferred to blue light (56 ± 2 μmol m⁻² s⁻¹) given from directly above. After 4 d of growth in continuous blue light, the angle of the actinic light was changed to 55° from vertical for an additional 24 h to induce phototropic curvature. After this treatment, hypocotyls could be visually scored for both length and curvature for segregation analysis. When appropriate, seedlings were transferred to pots and grown to seed as described previously (Liscum and Hangarter, 1991).

Allelism was tested by crossing the blu mutants (♂) to JK218 (♀) and patterns of inheritance were determined from the F2 generation. Double mutants homozygous for a given blu mutation and JK218 were selected in blue light as F2 seedlings that grew tall and straight under the conditions described above. Double mutant lines for each genetic combination were grown to maturity and F3 seeds were collected. Samples from the F3 populations were grown under the inductive conditions to confirm that the seedlings showed both mutant phenotypes.
Measurement of phototropism and inhibition of hypocotyl elongation

For phototropism experiments, seeds were handled as described for the genetic analysis except that they were sown on 1.0 mM KN03 containing 1.0% (w/v) agar in micro-titer wells (0.3 ml). Phototropic curvature was measured as degrees from vertical as described by Janoudi and Poff (1990).

For hypocotyl elongation experiments, seeds were handled as described by Liscum and Hangarter (1991) except that un-supplemented, half-strength Murashige and Skoog nutrients (Murashige and Skoog, 1962) were used. After the cold and red light treatments to induce germination, seeds were transferred to darkness at 23°C for 23.5 h, followed immediately by 2 d of continuous blue light given from directly above at the indicated fluence rates. At the end of the blue light treatment, the seedlings were placed onto transparent tape. Using a projector, lengths of enlarged images (10X) of the taped seedlings were measured with a resolution of ± 0.05 mm using SigmaScan (Jandel Scientific, Sausalito, CA).

Light Sources

Red light (15 μmol m⁻² s⁻¹) for induction of germination was obtained by filtering light from cool-white fluorescent lamps (F48T12-CW-1500) and four incandescent bulbs (GE 100W)
through one layer of Rohm and Haas red Plexiglass no. 2444 (3.18 mm thick), one layer of yellow Roscolux no. 10, and 1 cm of a 1.5% (w/v) CuSO₄·7H₂O solution. This filter combination cut off wavelengths below 580 nm and had a peak intensity at 658 nm. The 660:730 nm light ratio was 1.4. Light for potted plants was provided continuously at 65 ± 5 μmol m⁻² s⁻¹ from cool-white fluorescent lamps (F96T12-CW).

For genetic analysis, blue light was obtained by filtering light from four halogen flood lamps (GE 150 W Quartzline) through 5-cm of 1.5% CuSO₄·7H₂O and a layer of Rohm and Haas blue Plexiglass no. 2045 (3.18-mm thick). The resulting spectral output had a peak intensity at 480 nm and a 100 nm half-bandwidth. The lights were mounted in a row on a frame that could be rotated to change the angle of irradiation. The CuSO₄·7H₂O solution was cooled by running cold tap water through copper tubing submerged in the solution.

Unilateral blue light for phototropism experiments was obtained by filtering light from a 750-W (GE) projector lamp through 3-cm of 1.5% (w/v) CuSO₄·7H₂O and a 450-nm interference filter with a half-bandwidth of 10 nm (Ealing Electro-Optics, Inc., Holliston, MA). The fluence rate was changed with neutral density filters, and fluence was varied by changing exposure time.

Blue light for hypocotyl elongation experiments was obtained by filtering light from a halogen flood lamp (GE
150W Quartzline) through 6.5-cm of water-cooled 1.5% CuSO₄·7H₂O and a 450-nm interference filter. The fluence rate was varied by using a voltage rheostat and by changing the distance between the plant material and light source. Fluence rates for all experiments were measured with a LI-189 quantum photometer or a LI-1800 portable spectroradiometer (LiCor, Inc., Lincoln, NE).

RESULTS

The phenotypes of wild-type and mutant Arabidopsis seedlings after 4 d of continuous blue light from above followed by 24 h of unilateral blue light are shown in Figure 5. Under these conditions, hypocotyl elongation was inhibited and phototropic curvature occurred in wild-type seedlings. Hypocotyl elongation in the blul mutant was not inhibited, but phototropic curvature was similar to that in wild-type seedlings. In contrast to wild-type and blul, JK218 seedlings showed no measurable curvature in the direction of the phototropic stimulus. However, inhibition of hypocotyl elongation in JK218 was similar to that in wild-type seedlings. Although not shown, the responses of blu2, blu3-1 and blu3-2 were similar to those shown for blul in Fig. 5. JK218-blul double mutants showed both parental phenotypes. Thus, in the double mutants, hypocotyl
elongation was not inhibited and the seedlings did not bend towards blue light.

Crosses between the blu mutants and JK218 resulted in complementation of the mutant phenotypes such that F1 progeny had short, curved hypocotyls similar to wild-type seedlings after the blue light treatment (Table 6). Segregation of F2 progeny into wild-type, blu, JK218, and blu-JK218 double mutant phenotypes was as expected for two independently segregating, recessive nuclear genes.

The results in Figure 5 and Table 6 were obtained using saturating levels of light for both the longitudinal growth inhibition and phototropic response. To determine if the responses were normal under limiting light conditions, fluence-response relationships for phototropism (Fig. 6) and fluence rate-response relationships for inhibition of hypocotyl elongation (Fig. 7) were examined. The fluence and time thresholds for phototropism (Fig. 6) were the same in blu1 and Columbia wild-type seedlings and are consistent with previous results reported for Estland wild-type seedlings (Janoudi and Poff, 1990). Moreover, the magnitude of the response was the same in wild-type and blu1 seedlings. Similar results were found with blu2, blu3-1, and blu3-2 (data not shown). In contrast to blu1 and wild-type seedlings, JK218 and JK218-blu1 double mutant seedlings did not curve in response to unilateral blue light at any fluence tested. Similar results were found with JK218-
Figure 5. Comparison of blue light-induced phototropism and inhibition of hypocotyl elongation in wild-type and mutant Arabidopsis seedlings. Seedlings were grown under blue light (56 ± 2 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) given from above for 4 d, followed by 24 h of illumination at 55° from vertical as indicated by the arrow. The photograph shows representative seedlings. WT, Columbia wild type; dbl, double mutant homozygous for blul and JK218.
Table 6. Genetic analysis of JK218 and blu mutants

<table>
<thead>
<tr>
<th>Cross (♂ x ♀)</th>
<th>Hypocotyl Phenotype</th>
<th>F1</th>
<th>F2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>F2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>F2&lt;sup&gt;c&lt;/sup&gt;</th>
<th>F2&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>blu1 x JK218</td>
<td>Short/Curved</td>
<td>10</td>
<td>164</td>
<td>51</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Long/Curved</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Short/Straight</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>blu3 x JK218</td>
<td>Short/Curved</td>
<td>14</td>
<td>182</td>
<td>49</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Long/Curved</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>blu3-1 x JK218</td>
<td>Short/Curved</td>
<td>45</td>
<td>146</td>
<td>44</td>
<td>46</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Long/Curved</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> F2's were obtained from F1 self fertilizations.

<sup>b</sup> χ² expected ratio 15:1 (P > 0.05). wild type (short/curved), blu (long/curved), and JK218 (short/straight) : double mutant (long/straight).

<sup>c</sup> Similar results were obtained for blu3-2.
Figure 6. Fluence-response relationships for phototropism in wild-type and mutant Arabidopsis seedlings. The top panel shows the angle of phototropic curvature in wild-type seedlings after single pulses of 450 nm light at the indicated fluence rates (μmol m⁻² s⁻¹). The data are from experiments with Columbia wild type (genetic background of the blu mutants), but similar results were obtained with Estland wild type (genetic background of JK218 and JK224). The bottom panel shows the angle of phototropic curvature in mutant seedlings after exposure to single pulses of 450-nm light. Data points represent the mean ± SE of 70-90 seedlings.
Figure 7. Fluence rate-response relationships for inhibition of hypocotyl elongation in wild-type and mutant Arabidopsis seedlings. Inhibition in Estland wild type, JK218, and JK224 seedlings is shown in the top panel. Inhibition in Columbia wild type, blul, and blul-JK218 double mutant seedlings is shown in the bottom panel. Each data point represents the mean of 25-50 seedlings exposed to continuous 450 nm light for 2 d. Vertical error bars represent the combined SE for the dark- and light-grown seedlings.
blu2, JK218-blu3-1, and JK218-blu3-2 double mutants (data not shown).

Wild-type Columbia and Estland seedlings had similar fluence rate-response relationships for inhibition of longitudinal hypocotyl elongation (Fig. 7). Elongation in the wild-type seedlings was inhibited by about 73% at 10 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), which is about the maximum level of inhibition caused by saturating amounts of white light (Liscum and Hangarter, 1991). Hypocotyl inhibition in the phototropism null mutant JK218 was indistinguishable from that of wild-type seedlings. The degree of inhibition of hypocotyl elongation in JK224, which is a mutant that exhibits a shift in the fluence dependence for first positive phototropism (Khurana and Poff, 1985), was similar to that of the wild type. In contrast to JK218 and JK224, hypocotyl inhibition in blu1 and blu1-JK218 double mutant seedlings saturated at 1 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) with a maximal response of only 37%. Similar results were obtained with double mutants between JK218 and the other blu mutants (data not shown).

**DISCUSSION**

The results presented here show that blue light-induced inhibition of hypocotyl elongation and phototropism are mediated by genetically separate signal transduction systems. These systems require at least five distinct gene
products coded for by BLU1, BLU2, BLU3, and the wild-type
genes for the mutations in strains JK218 and JK224.
Unfortunately, these results are not sufficient to indicate
where in the signal transduction chains these genes
function. However, because hypocotyl elongation and
phototropism are ultimately dependent on changes in basic
processes that affect cell elongation patterns, these
responses might be expected to share functions near the end
of their signal transduction chains. However, the mutations
examined here affect one of the responses, but not both.
Thus, if the responses share functions, the gene products
affected by these mutations probably function relatively
early in signal transduction.

At this point, it is not possible to determine with
certainty if the responses are controlled by a photoreceptor
with diverging signal transduction pathways before
influencing cell elongation, or if the responses are
controlled by different photoreceptors. However, the JK224
mutant, in which the light threshold for first positive
phototropism is shifted, has recently been suggested to be a
photoreceptor mutant (Khurana and Poff, 1985; Konjevic et
al., 1992). Therefore, the observation made here, that blue
light-induced inhibition of hypocotyl elongation is
unaffected in JK224, supports the involvement of different
blue light photoreceptors in these different responses.
In addition to the genetic data presented here, several physiological observations suggest that blue light-dependent inhibition of stem elongation and phototropism are distinct processes. For example, pea epicotyls are far more sensitive to blue light for induction of phototropism than for inhibition of epicotyl elongation (Baskin, 1986; Laskowski and Briggs, 1988). In addition, blue light-induced inhibition of stem elongation occurs within 15-150 s in etiolated seedlings of Sinapis, Cucumis, Pisum, Cucurbita, Helianthus, and Phaseolus species, whereas the onset of phototropism usually takes orders of magnitude longer (Cosgrove, 1981; 1982). In a detailed study with cucumber, longitudinal growth inhibition occurred within 30 s of blue light exposure, but phototropism did not occur until 4.5 h after the start of irradiation (Cosgrove, 1985). Because inhibition of stem elongation occurs more rapidly than phototropism in several different species (Cosgrove, 1981; 1982; 1985), the same probably holds true for Arabidopsis. Although the time course for inhibition of hypocotyl elongation has not been determined in Arabidopsis, the onset of phototropism was found to occur 10-20 min after blue light irradiation in Arabidopsis (Orbović and Poff, 1991). Kinetic separation alone, however, is not sufficient evidence that the responses are independent. For example, the slower phototropic response may be initiated by earlier effects on elongation of the stem cells.
The results obtained here with the blue light-response mutants are consistent with the conclusions of many physiological experiments, that the responses occur through distinct signal transduction systems. Moreover, the altered responses in the mutant plants demonstrate that phototropism and inhibition of stem elongation are controlled by genetically distinct products and possibly different photoreceptor pigments.
CHAPTER III

LIGHT-STIMULATED APICAL HOOK OPENING IN WILD-TYPE

Arabidopsis SEEDLINGS

One of the most important developmental programs initiated in a plant is the switch from etiolated to de- etiolated growth. Growth responses affected by this developmental switch include stem growth inhibition, apical hook opening, cotyledon unfolding and expansion, leaf and root growth, and the acquisition of photosynthetic competence. One of the most morphologically striking of these is the apical hook opening response (Withrow et al., 1953). In dark-grown epigeal plants, the cotyledons and the most apical portion of the hypocotyl fold back toward the long axis of the hypocotyl to form a "U" shaped structure: the apical hook. Upon exposure to light, the hook opens, cotyledons enlarge and unfold, and chlorophyll is synthesized. Light-stimulated hook opening has generally been considered a phytochrome-mediated response, since exposure to red light causes hook opening (Withrow et al., 1957; Lane and Kasperbauer, 1965; Caubergs and De Greef, 1975; Powell and Morgan, 1980). However, in at least two
species, blue light-stimulated hook opening has been observed that does not appear to be mediated by phytochrome (Mohr and Noble, 1960; Kujawski and Truscott, 1974; Silk, 1980).

*Arabidopsis* has proven to be a valuable model system for studying light-dependent processes in higher plants (e.g. Koornneef et al., 1980; Khurana and Poff, 1989; Chory et al., 1989; Boylan and Quail, 1991; Deng et al., 1991; Liscum and Hangarter, 1991; Nagatani et al., 1991; Whitelam and Smith, 1991; Liscum et al., 1992; Young et al., 1992).

In this paper, the effects of light on apical hook responses in wild-type seedlings of *Arabidopsis* have been examined. These studies provide the first detailed analysis of light-stimulated hook opening in *Arabidopsis* and indicate that multiple photosensory systems are involved in red, blue, and far-red light-stimulated apical hook opening and cotyledon unfolding in etiolated *Arabidopsis* seedlings.

**MATERIALS AND METHODS**

Plant Materials and Growth Conditions

Wild-type *Arabidopsis thaliana* (L.) Heynh. ecotypes Columbia, homozygous for the recessive *gl1* mutation (Koornneef et al., 1982), and Landsberg *erecta* (Redei, 1962) were used. Seeds were surface sterilized for 20 min in 1.5% (w/v) sodium hypochlorite, rinsed several times with sterile
H₂O, and planted in polystyrene Petri dishes containing growth medium consisting of 0.5x Murashige and Skoog salts (Murashige and Skoog, 1962) and 0.8% (w/v) agar. Seeds were incubated for 2 to 3 days at 4 ± 1°C, then exposed to red light for 30 min to induce uniform germination (Liscum et al., 1992). The red light-treated seeds were incubated in darkness at 23 ± 2°C for an additional 71.5 h and then moved to the various light treatments for the indicated times.

Light Sources

White light was provided by a 1:1 mixture of cool white fluorescent lamps (F40CW; Philips Lighting Co., Salina, KS, USA) and warm white fluorescent lamps (F40CW; General Electric Co., Cleveland, OH, USA). The red light source for induction of germination was as described by Liscum et al. (1992).

Blue light for fluence rate-response and reciprocity experiments was obtained by filtering light from four halogen flood lamps (150 W Quartzline; General Electric Co.) through 7 cm of 1.5% (w/v) CuSO₄·7H₂O and Rohm and Haas blue Plexiglass no. 2045 (3.18 mm thick; Dayton Plastics, Columbus, OH, USA). For pulse experiments, one halogen lamp was used with 3 cm of 1.5% CuSO₄·7H₂O, 3 cm of H₂O, and blue Plexiglass. The resulting spectral output had peak intensity at 480 nm and a 100 nm half-bandwidth.
Red light for fluence rate-response and reciprocity experiments was obtained by filtering light from four halogen flood lamps through 3.5 cm of 1.5% CuSO₄·7H₂O and one layer of Rohm and Haas red Plexiglass no. 2444 (3.18 mm thick; Dayton Plastics, Columbus, OH, USA). This filter combination cut off wavelengths below 580 nm and had peak intensity at 630 nm. For pulse experiments, light from one halogen lamp was filtered through 3 cm of 1.5% CuSO₄·7H₂O, 3 cm of H₂O, and Rohm and Haas red Plexiglass no. 2423 (3.18 mm thick; Dayton Plastics, Columbus, OH, USA). This filter combination cut off wavelengths below 580 nm and had peak intensity at 644 nm. Less than 15% of the total irradiance from these sources was from wavelengths greater than 700 nm.

Far-red light for fluence rate-response experiments was obtained by filtering light from one halogen flood lamp through 5 cm of H₂O and one layer of far-red Plexiglass no. FRF 700 (3.18 mm thick; Westlake Plastics Co., Lenni, PA, USA). The fluence rate for this light source 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 tested here. For pulse experiments, light from one halogen lamp was filtered through 3 cm of H₂O and a 734 nm interference filter with a half-bandwidth of 10 nm.

Fluence rates were adjusted by changing voltage, distance between the seedlings and the light source, and/or
using neutral density filters such that the spectral quality remained constant. Fluence rates at the level of the seedlings were measured with a LI-189 quantum photometer or a LI-1800 portable spectroradiometer (LiCor, Inc., Lincoln, NE, USA). In all experiments that involved long light exposures, the H$_2$O and CuSO$_4$ solutions that were used as light filters were cooled by running cold tap water through copper tubing submerged in the solutions.

Measurement of Hook Opening and Cotyledon Unfolding

At the end of the treatments, the seedlings were carefully pulled from the agar and placed onto transparent tape. Images of the seedlings were projected from a photographic enlarger (3.5 x magnification) and the angle of the apical hook was measured to the nearest degree (see inset in Fig. 8). Light-stimulated hook opening was calculated by subtracting the angle of the apical hook of seedlings that were kept in darkness from the angle observed after light treatment. Although the apical hook was treated as a simple angle in this work, it should be noted that the morphological changes that occur during hook opening are more complex.

Cotyledon unfolding is expressed as the percentage of seedlings with cotyledons that were at least visibly curved outward away from the axis of the seedling as a result of differential cell enlargement and division on the adaxial
and abaxial sides of the cotyledon. For example, the seedling shown for the 3.5 h treatment in Fig. 9 would be scored as having cotyledons unfolding because the outward curvature is apparent. In contrast, the seedling shown for the 2 h treatment would be scored as closed because, although the cotyledon tips are not tightly appressed, they do not show outward curvature. Using this procedure, cotyledons that are just beginning to unfold are lumped together with those that have fully opened. Unless otherwise indicated, each data point represents the mean response from a minimum pooled sample size of 75 seedlings from at least three replicate experiments.

RESULTS

Apical Hook Opening and Cotyledon Unfolding in Darkness

Etiolated, wild-type seedlings of *Arabidopsis* exhibited appreciable apical hook opening in darkness as shown in Figure 8, but cotyledon unfolding did not occur within the 5 d growth period examined here. The kinetics for apical hook opening in darkness were similar for the two ecotypes used here, except that Landsberg *erecta* exhibited a slightly slower initial rate of opening. The majority of seedlings had opened hooks and the cotyledons were unfolded after 2 weeks growth in darkness (data not shown).
Figure 8. Time course of apical hook opening in dark-grown Arabidopsis seedlings. The vertical error bars represent the SEs. Curves were fit by regression analysis. The inset demonstrates how hook angles were determined from projected images. o, Columbia; o, Landsberg erecta.
Apical hook opening and cotyledon unfolding occurred sequentially when 3-d-old, etiolated wild-type Arabidopsis seedlings were transferred to fluorescent white light (Fig. 9). Hook opening was noticeable after about 2 h in white light. By 3.5 h, the hooks were nearly completely opened and the cotyledons were beginning to unfold. After 6 h of continuous white light, the hooks were opened completely and the cotyledons had unfolded in most of the seedlings. De- etiolation of the apical hook was completed after about 12-h of continuous irradiation. Similar kinetics for hook and incandescent white light (Kujawski and Truscott, 1974) opening have been reported for etiolated Cuscuta seedlings after transfer to fluorescent (Lane and Kasperbauer, 1965).

Figure 10 shows the fluence rate-responses for apical hook opening in wild-type Arabidopsis seedlings exposed to 14 h of blue, red, and far-red light. In blue light, hook opening exhibited a log-linear response with a threshold of about 0.01 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and saturation at about 10 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The threshold for far-red light was also around 0.01 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) but saturation occurred at about 3 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The hooks were completely open at saturation in blue and far-red light. Red light-stimulated hook opening exhibited a log-linear response with a threshold below 0.01 \( \mu \text{mol m}^{-2} \text{s}^{-1} \).
Figure 9. Representative photographs of apical hook opening and cotyledon unfolding in *Arabidopsis* in white light. Three-d-old, dark-grown seedlings were transferred to white fluorescent light (50 ± 5 μmol m⁻² s⁻¹). Representative seedlings were then photographed after the indicated times.
Figure 10. Fluence rate-response curves for blue, red, and far-red light-stimulated hook opening in Arabidopsis seedlings. Three-d-old, dark-grown seedlings were transferred to continuous blue, red, or far-red light provided from above. After 14 h the angle of the apical hook was measured. In this experiment the apical hooks of dark controls were opened to 114±2° for Columbia (o) and 100±3° for Landsberg erecta (o). Error bars represent the SE for the light-treated seedlings. For some data points the SE was less than the size of the symbol. Curves were drawn by inspection. Note that the magnitude of the response to red light is less than for the other colors.
s\textsuperscript{-1} and saturation at about 1 \( \mu \text{mol m}^2 \text{s}^{-1} \). The fluence rate dependence for red light-stimulated hook opening in Arabidopsis is in the range of those observed for other species (Withrow et al., 1957; Lane and Kasperbauer, 1965; Caubergs and De Greef, 1975; Powell and Morgan, 1980).

In blue and far-red light, the threshold for cotyledon unfolding is at a fluence rate just below saturation for hook opening (Fig. 10 and 11). In addition, although the hooks didn’t open completely in red light at any fluence rate tested (Fig. 10), cotyledon unfolding occurred on more than 50% of the seedlings at fluence rates greater than 1 \( \mu \text{mol m}^2 \text{s}^{-1} \) (Fig. 11). This indicates that, although hook opening may be related to cotyledon unfolding, it is not a prerequisite for cotyledon unfolding and the two processes may therefore have divergent signal transduction pathways.

Although Columbia and Landsberg erecta represent polymorphic genetic backgrounds (Chang et al., 1988; Nam et al., 1989), the dark-grown pattern and de-etiolation responses of the apical hook appear to be conserved (Fig. 8, 10, and 11). Thus, only Columbia was used in the remaining experiments.

Reciprocity and Photoreversibility of the Light-Stimulated Hook Opening Response

To further characterize the photobiology of hook opening, reciprocity relationships were examined in red and
Figure 11. Fluence rate-response curves for blue, red, and far-red light-stimulated cotyledon unfolding in *Arabidopsis* seedlings. Seedlings were handled as described in Fig. 10 and cotyledon separation was visually scored as described in Materials and Methods. Error bars represent the percent SE. Curves were drawn by inspection. For some data points the SE was less than the size of the symbol. •, Columbia; ○, Landsberg *erecta*. 
blue light (Fig. 12). Red light-stimulated hook opening was essentially reciprocal over at least 4 orders of exposure time. Thus, short pulses of red light were nearly as effective for activating the hook opening response as continuous irradiation. These results are consistent with the red light-stimulated hook opening response being an inductive phytochrome response as observed in other species (Withrow et al., 1957; Lane and Kasperbauer, 1965; Caubergs and De Greef, 1975; Powell and Morgan, 1980).

Because blue light stimulated hook opening over a wider range of fluence rates than did red light (Fig. 10), reciprocity relationships were examined with low and high fluence blue light. Apical hook opening in low fluence blue light showed reciprocity over at least 3 orders of exposure time (Fig. 12). However, reciprocity failed in high fluence blue light. These data suggest that two different signal transduction systems operate in blue light-stimulated hook opening: a "low fluence requiring system" that is dependent only on the number of incident photons, and a "high fluence requiring system" that is dependent upon exposure time and fluence rate. The former is typical of an inductive response, while the latter is typical of a high irradiance response (Kronenberg and Kendrick, 1986).

If the red light-stimulated hook opening response is an inductive phytochrome response it should be far-red reversible, as is red light-stimulated hook opening in other
Figure 12. Reciprocity relationships of the hook opening response. Three-d-old, dark-grown Columbia seedlings were exposed to red or blue light given from directly above at various combinations of fluence rates and exposure times to achieve the indicated fluence. The fluence of red light used in these experiments was equal to a 14 h exposure at a fluence rate that stimulated the hooks to open by about 20°. For blue light, the low and high fluence treatments used were photon equivalents of 14 h exposures to low and high fluence rates, respectively, that stimulated the hooks to open by about 18°. The longest light exposure was 14 h and the shortest was 24 s. When required, seedlings were transferred to the dark so that all seedlings were allowed to grow for 14 h after the beginning of light exposure. The apical hooks for dark controls were opened to 114 ± 2°. Error bars represent the SE for the light-treated seedlings. Curves were drawn by inspection.
species (Withrow et al., 1957; Lane and Kasperbauer, 1965; Caubergs and De Greef, 1975; Powell and Morgan, 1980). Similarly, the low fluence blue light-stimulated hook opening response may also operate via a photoreversible phytochrome system (Bertsch, 1963; Downs and Siegelman, 1963; Wellmann, 1971, 1974).

Table 7 demonstrates that when a red pulse sufficient to cause 30° of opening, or blue light pulse sufficient to cause 18° of opening were followed by a far-red light pulse of an equivalent fluence to the red and blue pulses, the affect of the inductive pulse was negated. These results suggest that hook opening in red light and low fluence blue light is induced via a photoreversible phytochrome signal transduction system. In addition, the results in Table 7 suggest that hook opening in continuous far-red light (Fig. 10) may be a dependent upon a phytochrome high irradiance response system, since pulses of far-red light did not induce hook opening.

The kinetics for the loss of photoreversibility are shown in Figure 13. The effectiveness of far-red light for reversing red light-stimulated hook opening in wild-type seedlings of Arabidopsis decreased as the dark-time between red and far-red light exposure increased up to 20 min. It took 12 to 13 minutes for 50% of the red light-induced signal to escape from far-red photoreversibility, and at
Table 7. Far-Red Photoreversibility of Light-Stimulated Hook Opening.

Hook opening was stimulated in three-d-old, dark-grown Columbia seedlings by 5 min of red light (116 μmol m\(^{-2}\) s\(^{-1}\)) or 10 min of blue light (78 μmol m\(^{-2}\) s\(^{-1}\)). Far-red light, of a fluence equal to the inductive treatments, was given 30 s after the inductive treatments. Control seedlings were exposed to far-red light alone at the same fluence given for the red or blue pulses. Hook opening was allowed to develop in the dark for a total of 14 h after the start of light exposure. Data represent the mean ± SE for light-stimulated samples minus the value for dark controls (114 ± 2°). The number in parentheses equals the pooled sample size from a minimum of three independent experiments. R, red; FR, far-red; B, blue.

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>Light-stimulated hook opening</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>31 ± 1° (150)</td>
</tr>
<tr>
<td>FR</td>
<td>0 ± 2° (206)</td>
</tr>
<tr>
<td>R-FR</td>
<td>1 ± 2° (147)</td>
</tr>
<tr>
<td>B</td>
<td>18 ± 2° (230)</td>
</tr>
<tr>
<td>FR</td>
<td>2 ± 2° (144)</td>
</tr>
<tr>
<td>B-FR</td>
<td>1 ± 2° (178)</td>
</tr>
</tbody>
</table>
Figure 13. Kinetics for the escape from far-red photoreversibility of red light-stimulated hook opening. Three-d-old, dark-grown Columbia seedlings were given 5 min of red light (116 µmol m\(^{-2}\) s\(^{-1}\)) followed, after various times in the dark, by 15 min of far-red light (39 µmol m\(^{-2}\) s\(^{-1}\)). Hook opening was allowed to develop in the dark for a total of 14 h after the start of light exposure. The shortest delay time between red and far-red treatments was 30 s and resulted in complete reversibility of the red light induction. Data are expressed relative to this treatment. Error bars represent the SE. Curves were drawn by inspection.
intervening dark periods longer than 20 min
photoreversibility reached its minimum.

DISCUSSION

The effect of light quality and quantity on light-stimulated apical hook opening and cotyledon unfolding were investigated in etiolated Arabidopsis seedlings. Fluence rate dependencies showed that red light was less effective than far-red or blue light for stimulating hook opening. In addition, red light-stimulation of hook opening showed reciprocity and was far-red reversible. It took about 15 min of dark time after red light treatment to lose 50% of the far-red reversibility. Other species show similar effects of red and far-red light (Klein et al., 1957; Withrow et al., 1957; Lane and Kasperbauer, 1965; Caubergs and De Greef, 1975; Porath and Atsman, 1977; Powell and Morgan, 1980). Stimulation of hook opening in Arabidopsis by low fluence blue light also showed reciprocity and exhibited far-red photoreversibility. The far-red reversibility of the inductive effects of red light and low fluence blue light indicate that phytochrome is involved in light-stimulated hook opening in Arabidopsis.

In addition to the inductive effects of red light and low fluence blue light, hook opening in Arabidopsis also appears to be mediated by high irradiance response systems
as indicated by the responses to far-red and high fluence blue light. For example, in contrast to the effects of low fluence blue light on hook opening, the response to high fluence blue light does not obey reciprocity (Fig. 12). To our knowledge, blue light-stimulation of hook opening has not been observed in most species. Notable exceptions are *Cuscuta* (Kujawski and Truscott, 1974) and lettuce (Mohr and Noble, 1960; Silk, 1980). However, because cotyledons do not develop in *Cuscuta*, the hook and hook opening response are not necessarily analogous to hypocotyl and epicotyl hooks and hook responses of dicotyledonous plants. In addition, while blue light stimulates hook opening in etiolated *Arabidopsis* seedlings, apical hooks in lettuce must be induced to close by a red light treatment before blue light will stimulate opening. The affects of far-red light in *Arabidopsis* also appear to be atypical since far-red light-stimulated hook opening has only been reported for *Cuscuta* (Lane and Kasperbauer, 1965; Kujawski and Truscott, 1974), *Cucumis* (Porath and Atsman, 1977; Porath et al., 1980), and after red light-induced hook closure in lettuce (Mohr and Noble, 1960).

The fluence rate-response curves for red, blue, and far-red light-stimulated hook opening (Fig. 10) are very similar to those generated for the inhibition of hypocotyl elongation in wild-type *Arabidopsis* (Young et al., 1992). These results suggest that light-stimulated hook opening and
inhibition of hypocotyl elongation may have common signal transduction systems. This may be expected since the apical hook is a region of the hypocotyl that has undergone differential cell elongation during the development of the seed to give rise to the "U" shaped hook structure, however, in other studies apical hook opening and inhibition of hypocotyl elongation did not appear to be closely related photobiological processes (Mohr and Noble, 1960; Janes et al., 1976; Porath et al., 1980).

Although the threshold for light-stimulated unfolding of the cotyledons was slightly lower for red light than blue or far-red light, unfolding occurred at fluence rates that were at least 100-fold greater than those required for the stimulation of hook opening in all light conditions tested. The requirement for relatively high fluence rates (> 1 μmol m⁻² s⁻¹) suggests that cotyledon unfolding is a high irradiance response. This possibility is currently being investigated in several photomorphogenic mutants.

The results presented here indicate that the apical hook opening response in wild-type Arabidopsis is controlled by multiple signal transduction pathways, including low fluence and high irradiance phytochrome systems and a blue light-sensitive high irradiance photosensory system. Phytochrome probably acts as the photoreceptor for red light and low fluence blue light stimulation of hook opening, since a terminal far-red light pulse can negate the effects
of red and low fluence blue light pulses. Presumably, the far-red high irradiance response acts via a phytochrome photoreceptor, but the nature of the high irradiance blue light photoreceptor is unknown.
CHAPTER IV

LIGHT-STIMULATED APICAL HOOK OPENING IN PHOTOMORPHOGENIC MUTANTS OF Arabidopsis thaliana: DISSECTION OF THE SIGNALLING PATHWAYS

The transition from etiolated to de- etiolated growth represents a crucial step in the establishment of a seedling. Dramatic changes in morphology, such as stem growth inhibition, apical hook opening, cotyledon expansion and unfolding, and leaf and root growth, are observed during this developmental transition. Also significant are the metabolic changes that occur as pigments are synthesized and photosynthetic competence is acquired. These changes in the developmental program of the seedling are initiated by the absorption of light by phytochromes, blue light photoreceptors, UV-A photoreceptor(s), UV-B photoreceptor(s), and possibly others. Although phytochrome is the only photomorphogenic-photoreceptor to be identified at the genetic and biochemical levels (Sharrock and Quail, 1989; for review see Quail, 1991), several recent genetic and biochemical studies have demonstrated the function of blue light photoreceptors (Konjević et al., 1992; Liscum et
al. 1992; Short et al., 1992; Warpeha et al., 1992), UV-A photoreceptor(s) (Galland and Senger, 1988; Young et al., 1992), and UV-B photoreceptor(s) (Ensminger and Schäfer, 1992) in various light-regulated processes.

Many questions about photoperception and signal transduction systems in plants are being addressed with physiological and genetic studies of photomorphogenic mutants of plants such as Arabidopsis (e.g. Nagatani et al., 1991; Somers et al., 1991; Whitelam and Smith, 1991; Chory, 1992; Liscum et al., 1992; Young et al., 1992; for review see Okada and Shimura, 1992). Of specific interest to de-etiolation processes, are the two classes of long hypocotyl mutants, the hy (Redei and Horono, 1964; Koornneef et al., 1980; Chory et al., 1989a) and blu mutants (Liscum and Hangarter, 1991), and a class of mutants that show characteristics of de-etiolated seedlings when grown in complete darkness, the det (Chory et al., 1989b, 1991) and cop (Deng et al., 1991) mutants. Because the det and cop mutants exhibit de-etiolation without the input of a light stimulus, they can not provide direct information about the photoperception events occurring during the transition from etiolated to de-etiolated growth. However, the hy and blu mutants lack responses to specific wavelengths of light (Koornneef et al., 1980; Liscum and Hangarter, 1991; Young and Hangarter, 1992; Young et al., 1992), and provide a means of dissecting the various photoperception systems.
Six homozygous recessive \textit{hv} mutations have been described. The \textit{hvl}, \textit{hv2}, and \textit{hv6} mutants represent lesions that result in deficiencies in active phytochrome (Koornneef et al., 1980; Chory et al., 1989a; Parks et al., 1989) despite the fact that these mutants contain wild-type levels of light-labile (Type I) and the major light-stable (Type II) phytochrome apoproteins (Chory et al., 1989a; Parks et al., 1989; Nagatani et al., 1991). In addition, genetic evidence indicates that these mutations are unlinked to the \textit{phyA}, Type I and the \textit{phyB}, Type II phytochromes (Chang et al., 1988; Koornneef, 1990; Chory, 1992). Apparently the lesions in \textit{hvl} and \textit{hv2} affect the synthesis of the phytochrome tetrapyrrole chromophore, since functional phytochrome can be produced by providing these mutants biliverdin IX\textsubscript{a} (Parks and Quail, 1991), the immediate precursor of the phytochrome chromophore, phytochromobilin (Elich et al., 1989; Terry and Lagarias, 1991; Cornejo et al., 1992). The \textit{hv6} mutant has been proposed to be chromophore mutant, defective in either biosynthesis or attachment to the phytochrome apoprotein (Chory, 1989a; Chory, 1992). The \textit{hv3} mutant is also phytochrome deficient, however, the lesion apparently resides in the coding region of the \textit{phyB} gene since \textit{hv3} mutations are genetically linked to \textit{phyB} (Chory, 1992), and \textit{hv3} mutants lack the phytochrome B apoprotein (Nagatani et al., 1991; Somers et al., 1991). Because, the light-labile phytochrome (phytochrome A) is
present and functional in hy3 (Nagatani et al., 1991; Somers et al., 1991), it can be used to study the function of Type I phytochrome in the absence of the major Type II phytochrome. The hy5 mutant exhibits drastic reductions in red and far-red light-dependent inhibition of hypocotyl elongation, however, it retains normal levels of functional, Type I phytochrome (Koornneef et al., 1980; Chory et al., 1989a; Parks et al., 1989) and Type II phytochrome (Nagatani et al., 1991; Somers et al., 1991). Although the molecular basis for the phenotype of hy5 is not known, it has been concluded that hy5 represents a lesion in a signal transduction component other than phytochrome itself (Koornneef et al., 1980; Chory, 1992). The hy4 mutant (Koornneef et al., 1980) and blul, blu2, and blu3 mutants (Liscum and Hangarter, 1991), represent strains that are defective specifically in blue light-dependent hypocotyl growth inhibition. However, the molecular basis for these deficiencies are not known at present.

Previously it was shown that apical hook opening in wild-type Arabidopsis is stimulated by red, far-red, and blue light (Liscum and Hangarter, 1992). Stimulation of hook opening by red light and low fluence blue light was inductive, far-red reversible, and obeyed reciprocity as are characteristic of many low fluence-dependent phytochrome-mediated responses (for review see Kendrick and Kronenberg, 1986; Furuya, 1987). Far-red light and high fluence blue
light appeared to stimulate hook opening through high irradiance response systems during long term light treatments. It was concluded that the responses in far-red light are probably mediated by a phytochrome high irradiance system, while the response to high fluence blue light may be dependent upon a separate blue light-specific photosensory system (Liscum and Hangarter, 1992). In this chapter, apical hook opening was examined in the six hy and three blu mutants in order to more clearly determine the photoperception and transduction systems that control this critical de-etiolation response.

MATERIALS AND METHODS

Plant materials and growth conditions

Wild-type *Arabidopsis thaliana* (L.) Heynh. ecotypes Columbia and Landsberg erecta (Redei, 1962), and mutant strains homozygous for blu1, blu2, blu3-1 (Liscum and Hangarter, 1991), hy1, hy2, hy3, hy4, hy5 (Koornneef et al., 1980), and hy6 (Chory et al., 1989a) were used. Double mutants homozygous for blu1 and hy6 (Liscum and Hangarter, 1991), were also used. The blu mutants and hy6 were in the Columbia background, while the other hy mutants were in the Landsberg erecta background. Seed handling and growth conditions were as described by Liscum and Hangarter (1992). After cold and red light treatments to induce uniform
germination (Liscum et al., 1992), seeds were transferred to darkness at 23 ± 2 °C for 71.5 h and then moved to the various light conditions for the indicated times.

Light sources

Blue light was obtained by filtering light from four halogen flood lamps (150 W Quartzline; General Electric Co., Cleveland, OH, USA) through 7 cm of 1.5% (w/v) CuSO₄·7H₂O and one layer of Rohm and Haas blue Plexiglass no. 2045 (3.18 mm thick; Dayton Plastics, Columbus, OH, USA). The resulting spectral output had peak intensity at 480 nm and a 100 nm half-bandwidth. Red light was obtained by filtering light from four halogen flood lamps through 3.5 cm of 1.5% CuSO₄·7H₂O and one layer of Rohm and Haas red Plexiglass no. 2444 (3.18 mm thick; Dayton Plastics, Columbus, OH, USA). This filter combination cut off wavelengths below 580 nm and had peak intensity at 630 nm. Less than 15% of the total irradiance from this light source was from wavelengths greater than 700 nm. Far-red light was obtained by filtering light from one halogen flood lamp through 5 cm of H₂O and one layer of far-red Plexiglass no. FRF 700 (3.18 mm thick; Westlake Plastics Co., Lenni, PA, USA). The fluence rate of this light source 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 tested here.
Fluence rates for all light sources were adjusted by changing voltage, distance between the seedlings and the light source, and/or using neutral density filters such that the spectral quality remained constant. Fluence rates at the level of the seedlings were measured with a LI-189 quantum photometer or a LI-1800 portable spectroradiometer (LiCor, Inc., Lincoln, NE, USA). The H₂O and CuSO₄ solutions that were used as light filters were cooled by running cold tap water through copper tubing submerged in the solutions.

Measurement of hook opening

At the end of the treatments, seedlings were carefully pulled from the agar and placed onto transparent tape. Projected images of the seedlings (3.5x magnification) were traced and the angle of the apical hook was measured to the nearest degree. Light-stimulated hook opening was calculated by subtracting the angle of the apical hook of dark control seedlings from the angle observed in light-treated seedlings. Unless otherwise indicated each data point represents the mean response from a minimum pooled sample size of 75 seedlings from three replicate experiments for blue light treatments and two replicate experiments for red and far-red light treatments.

RESULTS
Phenotypes of apical hooks after exposure to blue, red, and far-red light

Representative photographs of wild-type and mutant Arabidopsis seedlings after 14 h of saturating levels of blue, red, and far-red light are shown in Fig. 14. In, wild-type seedlings the apical hook opened completely and cotyledons unfolded fully blue and far-red light, while only partial hook opening occurred in red light. Several different phenotypes were observed among the different photomorphogenic mutants. The hv6 mutant, which is deficient in phytochrome chromophore, showed little or no hook opening in red and far-red light, but hook opening in blue light was similar to wild-type. The other phytochrome chromophore-deficient mutants hv1 and hv2 were phenotypically similar to hv6 (data not shown). In contrast, the phyB-deficient hv3 mutant was phenotypically similar to wild-type under all light conditions. The blu mutants (data not shown for blu2 and blu3) and the hv4 mutant exhibited wild-type hook opening in red and far-red light, but reduced hook opening in blue light.
Figure 14. Representative photographs of de-etiolation responses of the apical hook in wild-type and photomorphogenic mutant seedlings of Arabidopsis exposed to 14 h of high fluence rate red, far-red, or blue light. The fluence rate of each light source was 30 μmol m$^{-2}$ s$^{-1}$. WT, wild-type Columbia; D, dark; R, red light; FR, far-red light; B, blue light.
Extreme cases of deficient apical hook opening are represented by hy5 and blul.hy6 double mutant seedlings. For example, only partial hook opening occurred in hy5 seedlings under any light condition, while no recognizable hook opening occurred in blul.hy6 double mutants. The light-grown double mutant seedlings were also chlorophyll-deficient similar to their dark-grown siblings. This etiolated phenotype was previously reported for double mutants grown in continuous light for 5 d (Liscum and Hangarter, 1991).

Red and far-red light-stimulated hook opening in mutant seedlings

Fluence rate-responses for red light-stimulated apical hook opening differed among the photomorphogenic mutants, as shown in Fig. 15. The fluence rate-response curve for hy3 was indistinguishable from wild-type at all fluence rates tested, in agreement with the phenotypes shown in Fig. 14. Hook opening was reduced by about 50% of wild-type levels in hy5 at all fluence rates tested. The phytochrome chromophore-deficient mutants, hy6, hyl, and hy2 also exhibited greatly reduced hook opening in red light (data not shown for hyl and hy2). At saturating fluence rates the hooks in the chromophore-deficient mutants opened to only about 25% of the wild-type response. Hook opening in hy4 and blu2, was reduced in magnitude at fluence rates of red
Figure 15. Fluence rate-response curves for red light-stimulated hook opening in wild-type and photomorphogenic mutants of Arabidopsis. Seedlings were exposed to 14 h of continuous red light at the indicated fluence rates. The apical hook angle of dark controls were as follows: 114 ± 2° for wild-type Columbia, 100 ± 3° for wild-type Landsberg erecta, 83 ± 4° for hy3, 96 ± 4° for hy4, 111 ± 3° for hy5, 111 ± 3° for hy6, 114 ± 2° for blu1, 120 ± 3° for blu2, and 114 ± 4° for blu3-1. These values represent the mean ± SE when all dark controls (from all experiments) for a given genotype are pooled. Each data point represents the hook angle of light-treated seedlings minus the appropriate dark control value, as described in Materials and Methods. Error bars represent the SE for the light-treated seedlings. For some data points the SE was less than the size of the symbol. Each mutant is shown plotted with the wild-type of its genetic background. Curves were drawn by inspection. WT, wild-type.
light below 1 μmol m\(^{-2}\) s\(^{-1}\), but similar to wild-type above 1 μmol m\(^{-2}\) s\(^{-1}\). Two other blue light-response mutants, blu1 and blu3, showed wild-type response to red light. The altered response to red light in hv4 and blu2 was only revealed under limiting light conditions and therefore not apparent in Fig. 14 where saturating light was used.

In general, the trends observed for red light-stimulated hook opening in mutant seedlings were similar in far-red light (Fig. 16). As with red light-stimulated hook opening, far-red light-stimulated hook opening in hv3 was like wild-type. In fact, hv3 was slightly more responsive to far-red light than wild-type. In far-red light, the apical hooks in hv5 seedlings opened to a maximum of 10°, whereas wild-type apical hooks opened to about 60°. As with red light, hv6 seedlings showed minimal light-stimulated hook opening (5 to 10°) in far-red light. The fluence rate-responses of hv1 and hv2 were similar to hv6 (data not shown). All of the blue light-response mutants (hv4, blu1, blu2, and blu3) were similar to wild-type seedlings in far-red light.

Blue light-stimulated hook opening in mutant seedlings

The fluence rate-response relationships for blue light-stimulated hook opening varied among the different photomorphogenic mutants (Fig. 17). A wild-type hook opening response was seen in hv3, while hv6 showed a
Figure 16. Fluence rate-response curves for far-red light-stimulated hook opening in wild-type and photomorphogenic mutants of *Arabidopsis*. Seedlings were exposed to 14 h of continuous far-red light at the indicated fluence rates. The apical hook angle of dark controls and other methods of data analysis used in this experiment were as in Fig. 15. WT, wild-type.
Figure 17. Fluence rate-response curves for blue light-stimulated hook opening in wild-type and photomorphogenic mutants of *Arabidopsis*. Seedlings were exposed to 14 h of continuous blue light at the indicated fluence rates. The apical hook angle of dark controls and other methods of data analysis used in this experiment were as in Fig. 15. WT, wild-type.
reduction in blue light-stimulated hook opening at fluence rates below 1 μmol m⁻² s⁻¹. The threshold for the response in hv6 was shifted from about 0.01 μmol m⁻² s⁻¹ to about 0.1 μmol m⁻² s⁻¹. Above 1 μmol m⁻² s⁻¹ hook opening in hv6 was similar to wild-type. The hook opening responses of hyl and hv2 were similar to that observed for hv6 (data not shown). The fluence rate-response curve for hv5 paralleled the wild-type curve but showed lower sensitivity to blue light over the range of fluence rates tested. All of the blue light-response mutants exhibited reduced hook opening in blue light at fluence rates greater than 1 μmol m⁻² s⁻¹. The hv4 mutant was also less sensitive to blue light below 1 μmol m⁻² s⁻¹, whereas the blu mutants responded like wild-type at these fluence rates.

Blue light-stimulated hook opening in wild-type Arabidopsis was previously shown to have properties of both a low fluence and high irradiance response system (Liscum and Hangarter, 1992). In low fluence blue light, hook opening operates through an inductive, far-red reversible photosensor, while the response in high fluence blue light is dependent upon a high irradiance response photosystem. Therefore, the effect of exposure time on blue light-stimulated hook opening was examined to determine if the blue light-response mutants were specifically lacking the time dependent, high irradiance response system. Fig. 18 demonstrates that an exposure to 8 to 10 h of a saturating
Figure 18. Time course of apical hook opening in wild-type and photomorphogenic mutant seedlings of *Arabidopsis* exposed to high fluence rate blue light. Data represent the mean ± SE for a minimum of 40 seedlings. The fluence rate of blue light was 23 μmol m⁻² s⁻¹. Wild-type Columbia was used in this experiment since it represents the genetic background of the *hy6* and *blu3-1* mutants. Curves were drawn by inspection. •, wild-type; ○, *blu3-1*; ▼, *hy6*. 
high fluence rate of blue light (23 \(\mu\text{mol} \ \text{m}^{-2} \ \text{s}^{-1}\)) was required to stimulate maximal hook opening response in wild-type seedlings. However, the hook opening response in \textit{blu3-1} was saturated after as little as 2 h exposure to blue light. As a positive control, \textit{hy6} seedlings were included in this experiment since they exhibit normal hook opening above 1 \(\mu\text{mol} \ \text{m}^{-2} \ \text{s}^{-1}\). The response kinetics for \textit{hy6} seedlings were similar to those observed for wild-type (Fig. 18).

Because the phytochrome chromophore-deficient mutants (\textit{hy1}, \textit{hy2}, and \textit{hy6}) exhibited reduced hook opening in low fluence rate blue light (< 1 \(\mu\text{mol} \ \text{m}^{-2} \ \text{s}^{-1}\)), while the blue light-response mutants (\textit{blu1}, \textit{blu2}, \textit{blu3}, and \textit{hy4}) had reduced opening in high fluence rate blue light (> 1 \(\mu\text{mol} \ \text{m}^{-2} \ \text{s}^{-1}\)) plants with mutations from both classes of mutants should result in severely reduced blue light-stimulated hook opening across a range of fluence rates. Fig. 19 shows the fluence rate-response relationships for \textit{blu1 hy6} double mutants exposed to 14 h of blue light. Indeed, the double mutants lacked hook opening at all fluence rates examined.
Figure 19. Fluence rate-response curve for blue light-stimulated hook opening in blu1 hy6 double mutant seedlings. Seedlings were treated as described in Fig. 17. Apical hook angles of dark controls were 114 ± 2 for wild-type and 99 ± 8 for blu1 hy6 seedlings. Data represent the mean ± SE for a minimum of 25 seedlings. Wild-type Columbia (genetic background of blu1 and hy6) is presented for comparison. Curves were drawn by inspection.
Contributions of phytochrome in red and far-red light-stimulated apical hook opening

The phytochrome chromophore-deficient mutants (hy1, hy2, and hy6) exhibited severely reduced hook opening in red and far-red light (Fig. 14, 15, and 16), demonstrating that phytochrome acts as the primary, if not sole, photoreceptor for this response in red and far-red light. However, hy3, a phytochrome B-deficient mutant, exhibited wild-type hook opening response in red and far-red light (Fig. 14, 15, and 16). These results indicate that phytochrome B is not the photosensor for red and far-red light-stimulated hook opening. Unfortunately, it remains to be determined which of the other four phytochromes in Arabidopsis (Sharrock and Quail, 1989; Quail, 1991) are operating in this de-etiolation response. However, Type I phytochrome (phytochrome A) is perhaps the most likely candidate since it has been implicated as the major phytochrome operating in other de-etiolation responses (Smith and Whitelam, 1990). However, until a collection of mutants that lack each of the individual phytochromes is available, the function of phytochromes C, D, and E cannot be discounted.
Contribution of phytochrome and blue light-absorbing photosensor(s) in blue light-stimulated apical hook opening

In wild-type *Arabidopsis* it was found that blue light-stimulated apical hook opening involves a "low fluence-dependent system" that operates through an inductive, far-red reversible photosensor, and a separate "high fluence-dependent system" (Liscum and Hangarter, 1992). The experiments reported here with the blue light-response mutants (*hy4, blu1, blu2*, and *blu3*) and the phytochrome chromophore-deficient mutants (*hy1, hy2*, and *hy6*) confirmed the results observed with wild-type seedlings and further defined the photosensors involved in blue light-stimulated hook opening. The hook opening response in *blu1* and *blu3* was like wild-type in red and far-red light (Fig. 14, 15, and 16) and to fluence rates of blue light below 1 μmol m$^{-2}$ s$^{-1}$, but was reduced at fluence rates of blue light above 1 μmol m$^{-2}$ s$^{-1}$ (Fig. 14 and 17). Moreover, time course data (Fig. 18) indicate that these mutants lack the time dependent, high irradiance-dependent component of the blue light-stimulated hook opening response observed in wild-type seedlings. In contrast, the phytochrome chromophore-deficient mutant *hy6* showed wild-type response to blue light above 1 μmol m$^{-2}$ s$^{-1}$ (Fig. 14 and 17) and a wild-type time dependence at a saturating fluence rate of blue light (Fig. 18). However, *hy6* exhibited reduced response below 1 μmol m$^{-2}$ s$^{-1}$, including a higher fluence rate threshold for the
response (Fig. 17). These data indicate that blue light stimulates apical hook opening through the action of a blue light-absorbing photosensor(s) and phytochrome(s).

The function of two distinct photosensory systems in blue light-stimulated hook opening is most clearly demonstrated with blu1 hv6 double mutants (Fig. 19). Since complimentary hook opening responses were observed in the single mutant gene parents (Fig. 17), but little or no hook opening was observed in the double mutants across the range of fluence rates tested (Fig. 19), the two photosensory systems probably act in an additive manner.

Other components of the signal transduction pathways involved in light-stimulated apical hook opening

In addition to defining which photoreceptors perceive the light signal that elicits the hook opening response in Arabidopsis, the study of mutants for which no gene product is known or implied (hv4, hv5, blu1, blu2, and blu3) helps to further define in what signal transduction pathways these genes may function, as well as how divergent various pathways may be.

The blue light-response mutants hv4 and blu2 exhibited reduced hook opening in both red and blue light (Fig. 15 and 17). The results with hv4 are especially compelling, since the hook opening response is reduced in both red light and low fluence rate blue light (Fig. 15 and 17), as well as
high fluence rate blue light (Fig. 17). However, the hook opening response in blu2 was not altered in low fluence rate blue light, suggesting that the altered response of blu2 in red light may represent an some type of artifact. The results with hy4 indicate that both the low fluence phytochrome- and high irradiance blue light photosensor-dependent pathways are affected, and suggest that HY4 may provide a convergence point between these pathways.

Since the molecular identity of HY4 is unknown, it is also possible that the HY4 gene codes for a component of a blue light photoperception system, and if so the results from this study would imply that phytochrome can interact directly with a blue light photoreceptor. However, for this to occur, multiple blue light photoreceptors must function in the apical hook opening response since blu1, blu2, and blu3 exhibit wild-type or near wild-type response in red, far-red, and low fluence blue light (Fig. 15, 16, and 17).

One of the most poorly understood and least studied photomorphogenic mutants is hy5. Although little is known about how the wild-type HY5 gene may function, a previous study of double mutant combinations of hy5 with the phytochrome-deficient hy1 mutant, and the blue light-response mutant hy4, implied that HY5 represents a component of a signal transduction pathway that operates independently of phytochrome and blue light-photosensory systems (Koornneef et al., 1980). More recently, Chory (1992)
proposed two models to explain the action of HY5 based on the analysis of double mutants between hy5 and two mutant downstream regulators of photomorphogenesis, det1 and det2. In one model, HY5 was placed on a unique signal transduction pathway (Chory, 1992). This model could also be predicted based on the data of Koornneef et al., (1980). A second model, based partially on the possibility that the hy5 and det1 alleles used in the study may have been leaky, placed HY5 downstream of the DET1 gene product in a phytochrome-dependent signal transduction pathway, with DET1 providing a convergence point between phytochrome- and blue light photosensory system-dependent pathways (Chory, 1992). This model can also account for the near additive phenotypes of hy1 hy5 and hy4 hy5 double mutants observed by Koornneef et al. (1980). For example, although phytochrome is missing but the blue light-absorbing system is intact in a hy1 hy5 double mutant, an additive phenotype would be expected since functional HY5 is also required for blue light-photosensory system-dependent signal transduction. The same argument can be made when the blue light-photosensory system is non-functional but phytochrome remains functional, as occurs with hy4 hy5 double mutants.

The observations presented here that hy5 exhibited reduced hook opening across the entire range of fluence rates tested in blue, red, and far-red light (Fig. 14, 15, 16, and 17) strongly supports Chorys' model (Chory, 1992).
that places HY5 downstream of a convergence point between phytochrome- and blue light-photosensory system-dependent transduction pathways. It is unlikely that HY5 is in a unique signal transduction pathway operating independently of phytochrome and blue light-absorbing photoreceptors since blu1 hv6 double mutants didn’t retain hook opening response (Fig. 19), as would be predicted by this alternate model when HY5 is active.

In summary, comparison of the fluence rate- and spectral-dependencies for light-stimulated hook opening and cotyledon separation in the hy and blu mutants of Arabidopsis show that the responses are dependent upon both phytochrome and blue light-absorbing photosensory systems. The phytochrome B chromoprotein apparently doesn’t act as a primary photoreceptor for hook opening in Arabidopsis. The phytochrome and blue light-absorbing systems probably act largely in an additive manner to control apical hook opening response in wild-type seedlings under natural conditions. However, results with hy4 and hy5 suggest that the pathways operating through these photoreceptor systems may have some common components. Taken together, the results presented here indicate that de-etiolation of the apical hook in Arabidopsis seedlings involves a number of photoreceptor systems whose signals are coordinated through a complex web of signal transduction systems.
CONCLUSIONS

Many aspects of seedling development are regulated by red/far-red, blue, and UV light. For example, when seedlings are exposed to light, stem growth is inhibited, cotyledons expand, leaves and roots develop, and photosynthetic competence is acquired. Although the red-far-red photoreceptor phytochrome has been investigated at the genetic and biochemical levels, much of our understanding of blue and UV light photosensory systems has been inferred from descriptive studies. The goal of this dissertation research was to genetically dissect light-regulated developmental responses in higher plants using Arabidopsis as a model system. Plants containing mutations that eliminate specific photomorphogenic responses while leaving other responses intact represent important tools for understanding the physiology of these complex processes.

Several mutant Arabidopsis strains were isolated that have altered hypocotyl growth under blue light. These mutants were shown to belong to a new class of photomorphogenic mutants that we have designated blu for blue light uninhibited. Comparative studies with the blu mutants and a phytochrome-deficient mutant (hy6)

102
unequivocally demonstrated that a blue light photosensory system functions independently of the phytochrome system during the inhibition of hypocotyl elongation. Cotyledon expansion and apical hook opening under blue light are also altered in the blu mutants indicating that these responses are linked processes. However, several blue light responses are unaffected by the blu mutations, and thus, must be controlled by separate blue light sensory systems. One of the more interesting of these is phototropism. Suppression of cell elongation is the underlying cause for both blue light inhibition of hypocotyl elongation and phototropism. However, the blu mutants, which have altered blue light-regulated hypocotyl growth, have normal phototropism. Furthermore, a phototropic null mutant and a presumptive phototropism photoreceptor mutant have normal blue light-inhibited hypocotyl growth. Thus the processes of blue light-dependent inhibition of hypocotyl elongation and phototropism exhibit at least some genetically distinct components; possibly photoreceptors.

A detailed study of apical hook opening in wild-type, phytochrome-related mutants (hy1, hy2, hy3, hy5, hy6), and blue light-response mutants (blu1, blu2, blu3, hy4) demonstrated that this response is controlled by both the phytochrome- and blue light-photosensory systems. This is contrary to previous studies that had suggested that phytochrome was the only photosensory system operating in
this response. Analysis of fluence rate-response curves demonstrated that the photon dependencies for apical hook opening in blue, red, and far-red light are similar to those for the inhibition of hypocotyl elongation. This indicates that the photosensory systems controlling apical hook opening are the same as those controlling hypocotyl growth in visible light. Results with the phytochrome B-deficient mutant hy3 provided the first evidence that this phytochrome species is not involved in apical hook opening. The hy5 mutant, which was previously described as a phytochrome response mutant, shows alterations in both blue light- and phytochrome photosensory system-dependent regions of the blue light fluence rate-response curve. Therefore, the HY5 gene product represents a shared component of the two photosensory systems.

These studies have provided significant insight into the complex network of signalling pathways required for normal light-regulated development in higher plants. Although the developmental responses examined here represent only a subset of the total number of responses required for transition from a pattern of etiolated to de-etiolated growth, further investigation of the blu mutants and other photomorphogenic mutants will undoubtedly lead to further advancements in our understanding of plant development.
LIST OF REFERENCES


Blaauw, A.H. (1918) Licht und Wachstum. III. (Die Erklärung des Phototropismus). Mededelingen van de Landbouwhogeschool de Wageningen 15: 89-204


in etiolated and green plants deduced from characterization of *Arabidopsis thaliana* mutants. Plant Cell 1: 867-880


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


fragment length polymorphism linkage map of Arabidopsis thaliana. Plant Cell 1: 699-705


Parks, B.M., Shanklin, J., Koornneef, M, Kendrick, R.E., Quail, P.H. (1989) Immunochemically detectable phytochrome is present at normal levels but is photochemically nonfunctional in the hyl and hy2 long hypocotyl mutants of Arabidopsis thaliana. Plant Mol. Biol. 12: 425-437


enzyme-linked immunosorbent assay with monoclonal antibodies. Planta 159: 534-544


Wang, Y.-C., Stewart, S.J., Cordonnier, M.-M., Pratt, L.H. (1991) Avena sativa L. contains three phytochromes, only one of which is abundant in etiolated tissue. Planta 184: 96-104


