

ABSTRACT

PHOTOTROPISM AND GRAVITROPISM IN TRANSGENIC LINES OF *ARABIDOPSIS* ALTERED IN THE PHYTOCHROME PATHWAY

by Jane A. Hopkins

Roots of *Arabidopsis thaliana* grow toward gravity, positive gravitropism. In addition, these roots exhibit negative phototropism relative to blue light and positive phototropism relative to red light. Our studies investigated the importance of phytochromes, the red-light photoreceptors, for root and shoot gravitropism and phototropism. We used two transgenic lines, one which was deficient in phytochrome in the roots (M0062/UASBVR) and the other was deficient in phytochrome in the cotyledons (CAB3::pBVR). The transgenic lines were grown in either light or dark conditions to determine whether roots directly perceive light signals or if the signal is perceived in the shoot and then transmitted to the root. Kinetics of tropistic curvature and growth were assayed by standard methods or with a computer-based feedback system. We found that the perception of red light occurs directly in the root and that signaling also may occur from root to shoot in gravitropism.

Phototropism and Gravitropism in Transgenic Lines of *Arabidopsis* Altered
in the Phytochrome Pathway

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1. Introduction

1.1. General Introduction

Plants actively respond to many factors in their environment. One type of response is termed a tropism, defined as the ability of a plant to respond to external stimuli in a directional manner (Kiss, 2000). There are several types of tropisms corresponding to specific stimuli, such as phototropism, gravitropism and thigmotropism, which refer to the response to light, gravity, and touch, respectively. Studies in our laboratory have focused on understanding the tropistic responses of the model plant *Arabidopsis thaliana*, in which roots exhibit positive gravitropism, growing toward the gravity vector, while shoots exhibit negative gravitropism. In addition, shoots will grow toward blue light, exhibiting positive phototropism (Fig. 1), while roots, in general, display negative phototropism by growing away from the light source. These different tropisms are interconnected and interrelated. While it is fairly straightforward to study the effects of gravity on roots without the effects of light, it is nearly impossible to study the effect of light on roots without gravity playing a role, and this has led to the development of space experiments to study plants in microgravity (Millar et al., 2010).

In addition, it has recently been shown that *Arabidopsis* roots respond differently to varying wavelengths of light. In general, roots will grow away from blue light (negative phototropism; Hubert and Funke, 1937; Okada and Shimura, 1992) and grow toward red light (positive phototropism; Ruppel et al., 2001). Phytochromes, plant pigment-protein complexes, have been shown to play an important role in root phototropic and gravitropic responses (Kiss et al., 2003).

Tropisms can be divided into three main temporal processes: perception, signal transduction, and response (Kiss, 2000). Phototropins are plant photoreceptors involved in the perception of blue light, and phytochromes are the pigments involved in the initial perception of red light (Christie, 2007; Montgomery, 2008). In addition, phytochromes are located in the shoot; however, they are also present in the root (Kiss et al., 2003).

Some researchers have suggested that light signals are primarily perceived in the shoots, and then transduced to other parts of the plant, like roots, for the response (Hall et al., 2001). Knowing that phytochromes are present in the roots, the question I aim to answer through this research is whether red-light signals are perceived in the shoot and transduced to the root, or if the phytochromes in the root directly perceive red-light signals.

Phytochrome responses to a light signal have been shown to be localized to specific plant organs or tissues such as roots or shoots (Jiao et al., 2005; Montgomery, 2008). The experiments for this thesis utilized two transgenic lines deficient in the production of biliverdin IXa reductase (BVR), a mammalian enzyme and precursor of phytochrome (Fig. 2) (Warnasooriya and Montgomery, 2009). In these transgenic lines, phytochromes are nearly non-functioning since bilin is necessary for phytochrome photosensory activity and phytochromes are less able to respond to red and far-red-light when the synthesis of this bilin is inhibited (Lagarias et al., 1997). Biliverdin is a precursor of phytochromobilin and is reduced by BVR (Fig. 2). These expression-specific lines provided the opportunity to study the spatial-specific roles phytochromes can have in the photoregulatory system (Warnasooriya and Montgomery, 2009). We were able to investigate whether light signals are perceived in the shoot and transferred to the root for a response, or if the signal is perceived in the root as well.

1.2. Tropisms

As mentioned in Section 1.1, tropisms can be divided into three temporal processes, which include perception, signal transduction and response (Kiss, 2000). There are two main hypotheses for the mechanism by which gravity is first perceived: the “starch-statolith hypothesis” which describes the involvement of settling plastids in specialized columella cells and the “protoplast-pressure hypothesis” which states that plastids do not perceive the stimuli by settling, rather the entire weight of the cytoplasm is involved in perception (Kiss, 2000). Of these two hypotheses, the starch statolith hypothesis is more broadly accepted because amyloplasts are usually found in gravitropic

organs and low-starch or starchless mutants have an attenuated response to gravity (Kiss et al., 1989; Kiss, 2000).

The starch-statolith hypothesis states that amyloplasts, organelles densely filled with starch, will settle relative to gravity and those statoliths are involved in perception. When the cells are displaced from an upright position (for example rotated 90° from the vertical to horizontal), the amyloplasts move through the cytoplasm and cytoskeleton and settle on the lower side of the cell where they may either physically press against or chemically interact with the plasmolemma (Hillman and Wilkins, 1982).

According to the protoplast-pressure hypothesis, the entire protoplast settles in response to gravity, and causes differential tension between the cell wall and plasma membrane which activates gravireceptors identifying the bottom and top of the cell (Staves, 1997). It has also been hypothesized that plants have multiple mechanisms by which they respond to gravity and perhaps the explanation is really a combination of the two hypotheses, the “statolith-pressure hypothesis” (Kiss, 2000).

The interaction with the plasmolemma is then hypothesized to lead to the response phase of gravitropism in which auxin, a plant phytohormone, and the auxin-dependent transduction pathways are involved (Firn et al., 2000). The response phase occurs primarily in the zone of elongation, just behind the root cap. (Kiss, 2000). Therefore, there must be some transfer of signal from the sensing columella cells to the responding cells of the elongation zone.

It has been hypothesized that a combination of auxin and pH are involved via acid growth response due to the alkalization of cytoplasm and acidification of the apoplast within minutes of gravistimulation (Fasano et al., 2001). Studies done comparing the rate of differential surface acidification between the root cap and elongation zone were shown to be similar to the known rates of auxin transporter distribution (Monshausen and Sievers, 2002) in those zones, thus suggesting that auxin distribution may be regulated by the pH changes in root columella cells which are induced by gravistimulation (Fasano et al., 2001).

In plants, light is not only an important energy source for photosynthesis, but it is also necessary for many growth and developmental processes such as seed germination, branching and the circadian rhythm (Christie, 2007). Phototropism is the directional

growth response to light, and like gravitropism, includes a perception, transduction and response phase. The perception phase first begins with photoreceptors, molecules present throughout the plant which allow it to detect the intensity and directionality of the light (Fankhauser and Staiger, 2002). The three main photoreceptors which have been identified are phytochromes, which are involved in the perception of red and far-red light, and cryptochromes and phototropins which are associated with blue and ultraviolet-A wavelengths (Christie, 2007). These photoreceptors will be discussed more in the following section. For now, it is important to understand that these are the molecules that start a cascade of signaling events when plants respond to a light source. Differing light exposures (a combination of wavelength, frequency, timing and length of exposure) can cause differential responses in multiple aspects of plant development including de-etiolation, growth rate, branching and the circadian rhythm (Quail, 2002). After the light signal has been perceived, there are conformational changes in the photoreceptor that activates a kinase domain which leads to the downstream signal transduction pathway thought to be regulated by calcium (Correll and Kiss, 2002). The mechanism by which signaling and transfer occur is not completely understood, but includes a series of intermediate signaling factors which alter gene expression and thus direct various growth and developmental responses (Quail, 2002). In terms of the response phase, differential auxin flow leads to differential growth in a manner similar to gravitropism (Koeplpi et al., 1938).

Almost all of the photoreceptors discussed in the next section have multiple overlapping pathways (Briggs and Olney, 2001; Quail, 2002). It has also been hypothesized that the gravitropic and phototropic mechanisms have overlapping pathways (Poppe et al., 1996, Berkovich et al., 2005). In addition, responses to various stimuli are prioritized differently. For example, it has been noted that *Arabidopsis* roots grow away from blue light, negative phototropism, and toward gravity, positive gravitropism. In studies done with starchless mutants of *Arabidopsis*, which are impaired in gravitropism, the phototropic response is more readily observed (Kiss, 2000). Also, in studies done by Berkovich et al., (2005) it was shown that when a light source was placed below Chinese cabbage plants and wheat seedlings, the morphology of the shoot did not change significantly, again suggesting that gravity affects the morphology more than the

direction of the light vector. Similar studies have also been done with *Arabidopsis* seedlings. For instance, a similar experiment was done by Okada and Shimura (1992) with both WT and starchless mutants exposing seedling roots to lateral light sources. They found that mutant roots had a greater orientation away from the light source (negative phototropism) than did the WT. Also, when the light source was positioned under the root of vertical seedlings, the absolute angle curvature away from the light source was only 37° in WT strains, suggesting that gravitropism is a stronger response because the negative phototropism response was limited due to the positive gravitropism response. (Vitha et al., 2000).

Finally, numerous studies with *Arabidopsis* have correlated all five genes of phytochrome (*PHYA*, *PHYB*, *PHYC*, *PHYD*, and *PHYE*) that encode a red-light photoreceptor discussed in Section 1.4, with gravitropism functions. For example, a study done by Kumar et al., (2008) showed that *phyD* mutants, lacking one of five forms of the phytochrome photoreceptor, showed a significant reduction of hypocotyl curvature in gravitropism experiments. *PHYA* has been shown to be involved in the inhibition of gravity response of hypocotyls and roots (Correll et al., 2003; Lariguet and Fankhauser 2004), while *PHYA*, *PHYB*, *PHYD* and *PHYE* also may play a role in the response of roots to a gravity vector (Correll and Kiss, 2005).

1.3. Photoreceptors and Function

As mentioned briefly in Section 1.2, there are three main photoreceptors which have been identified; cryptochromes, phototropins and phytochromes. Cryptochromes and phototropins are flavoproteins which are responsible for the response to blue and ultraviolet-A light (350-500 nm) while phytochromes, linear tetrapyrrole chromophores attached to apoproteins, are associated with red and far-red wavelengths (600-800 nm) (Christie, 2007). Cryptochromes and phytochromes are involved in photomorphogenesis (light-induced growth changes, e.g. de-etiolation) while phototropins play a role in the light dependent processes of phototropism (Christie, 2007).

In addition to their role in photosynthesis, phototropins are also involved in the responses of roots and stems to blue-light, in which the roots grow away from the light source, while shoots grow towards it (Galen et al., 2004). In *Arabidopsis* two different phototropins control two signaling pathways (Galen et al, 2004). Phototropin 1 (PHOT1) controls the root and shoot bending response toward or away from the light source of $< 1\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$ while phototropin 2 (PHOT2) is involved in the response to brighter blue light wavelengths of $> 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Galen et al, 2002).

There are three main forms of cryptochromes, cryptochrome 1 (CRY1) and cryptochrome 2 (CRY2) and cryptochrome 3 (CRY3) (DeBlasio et al., 2003). These photoreceptors are mainly responsible for responding to blue light, causing the inhibition of seedling hypocotyl growth (Henning, 1999). It has also been shown that cryptochromes are involved in the entrainment of the circadian clock and are involved in transmitting blue-light fluences to the clock (Somers, 1998). As seen with the phototropins, the CRY1 gene is correlated with response to light of high irradiance while CRY2 is involved in responding to lower irradiances with overlap in between (Henning, 1999).

1.4. Phytochromes

Phytochrome is a single linear tetrapyrrole chromophore (bilin) (Fig. 3) attached to an apoprotein localized in plastids (Montgomery, 2008). These bilin photoreceptors are homodimers consisting of two major domains operating via photointerconversion in which the molecules have an active and inactive form (Hennig and Schäfer, 2001). They are inactivated by red-light in the Pr form and are activated by far-red-light, Pfr (Franklin et al, 2003). They are synthesized as the inactive Pf form and then convert to the active Pfr form once they absorb far-red light (Hennig and Schäfer, 2001). Depending on the type of light available, phytochromes can switch back and forth between their active and inactive forms. These molecular “light switches” can control many aspects of plant development including seed germination, stem and leaf growth, and flowering (Franklin et al., 2003).

Phytochromes are the photoreversible biliprotein photoreceptors mainly responsible for the absorption and response to red and far-red light (Montgomery, 2008). While roots have a negative phototropic response to blue-light, they have been shown to exhibit positive phototropism when exposed to red light (Ruppel et al., 2001). However this response is weaker than other root tropisms and is mainly observable in mutants impaired in gravitropism or by using special instrumentation (Kiss et al., 2003).

In *Arabidopsis*, there are five known genes (*PHYA*, *PHYB*, *PHYC*, *PHYD* and *PHYE*) that encode the phytochrome apoprotein, PHYA-PHYE, which can be divided into two subfamilies. *PHYA* makes up the first subfamily, being a light-labile phytochrome mainly responsible for the far-red light sensing (Montgomery, 2008). The second subfamily includes *PHYB* through *PHYE*, light-stable phytochromes (Montgomery, 2008). Studies on mutant strains of one or multiple photoreceptors have given insight into the roles each play, and there many redundancies among them (DeBlasio et al., 2003). *PHYA* and *PHYB* have been identified as involved in the phototropic response of roots to red light (Kiss et al., 2003). More specifically, *PHYB* controls the de-etiolation (stem growth inhibition) and delay in flowering when exposed to red-light (Tepperman et al., 2004). Studies using the *phyC* mutant have revealed that *PHYC* plays a part along with *PHYA* in the de-etiolation of the hypocotyl and leaves under blue-light conditions (Franklin et al., 2003), while *phyD* mutants have demonstrated *PHYD* involvement in shade avoidance and also possible involvement in the perception of blue-light, exhibiting overlapping functions with *CRY1* (Hennig, 1999). Finally, *PHYE* also appears to be involved in shade avoidance like *PHYD*, along with germination and maintenance of rosettes (Hennig et al, 2002).

1.5. Transgenic Lines

Phytochromes are linear tetrapyrrole chromophores (bilin) attached to an apoprotein. This bilin group is necessary for phytochrome photosensory activity and phytochromes are less able to respond to red and far-red-light when the synthesis of this bilin is inhibited (Lagarias et al., 1997). Biliverdin is a precursor of phytochromobilin and is

reduced by BVR (Fig. 2). In addition, it can metabolize phytochromobilin to rubinoid, making it unable to join with apophytochrome (Lagarias et al., 1997). BVR does not recognize chlorophylls; therefore the expression of BVR only affects the synthesis of the phytochrome chromophore (Lagarias et al., 1997). These expression-specific lines provided the opportunity to study the spatial-specific roles phytochromes play on the photoregulatory system (Warnasooriya and Montgomery, 2009), including phototropism.

1.6. Research Questions

The overarching goal of this research is to determine the role of phytochromes in tropistic responses. The experiments will answer the following research questions: Does red-light sensing via phytochromes occur directly in the root itself? Alternatively, if sensing does not occur directly in the roots, then is the signal transmitted from the shoot to the root? If sensing occurs in the root itself, I would expect to see an attenuated response in root curvature toward red-light in the root-specific transgenic line (M0062/UASBVR) compared to its wild-type. Otherwise, if sensing is occurring primarily in the shoot and being transmitted to the root, I would expect to see an attenuated response in root curvature toward red-light in the shoot-specific line (CAB3::pBVR) compared to the wild-type. This research will answer some basic questions in plant biology by adding additional knowledge of phytochrome involvement in light perception as well as to providing new insight into the mechanism by which plant roots respond to light, whether it is via shoots or roots or both.

2. Methods and Materials

2.1. Plant Materials

The research of this thesis aimed to study the role of phytochromes in roots compared to their role in shoots. Since PHYA-PHYE have overlapping functions, the transgenic lines in these experiments lack all five forms of phytochrome. I used *Arabidopsis thaliana* seedlings from the C24 and Nossen (No-O) strains as the wild-type and two transgenic lines, M0062/UASBVR and CAB3::pBVR, from the C24 and No-O wild-type strains, respectively. The transgenic line M0062/UASBVR is deficient of phytochromes in the roots only (Costigan et al., in press), while the CAB3::pBVR line is cotyledon specific, lacking phytochromes in the cotyledons only (Warnasooriya and Montgomery, 2009). CAB (Chlorophyll a/b binding) proteins are a major component of the light-harvesting complex and are induced by light in photosynthetic cells (Mitra et al., 1989).

The two transgenic lines utilized in these experiments were prepared with cell- and tissue-specific expression of biliverdin IXa reductase (BVR), a mammalian enzyme and precursor of phytochrome, as seen in Fig. 2, which induces a phytochrome deficiency in transgenic plants and can locally inactivate all or nearly all phytochromes (Warnasooriya and Montgomery, 2009). pUAS1380-BVR (hereafter termed UASBVR) was constructed by cloning the full-length *BVR* coding region using primers UASBVR_S (CGTCTAGAATGGATGCCGAGCCAAAG) and UASBVR_AS (CGAGATCTTTACTTCTTCTGGTGGCAAAG) using the *Xba*I and *Bgl*II restriction sites, respectively (Costigan et al., in press). Next a template was created by using PCR-amplification of the BVR coding region using pASK-FLBVR (BL Montgomery and JC Lagarias, unpublished data). The resulting PCR product was restricted with *Xba*I and *Bgl*II enzymes (New England Biolabs). Finally, the processed PCR product was ligated to the pUAS1380 plant transformation vector (digested in a similar manner) using a

TaKaRa DNA Ligation Kit Ver. 2.1 (Takara Bio U.S.A., Inc.) (Costigan et al., in press). Using this UASBVR construct, the wild-type (WT) *Arabidopsis* ecotype C24 plants were transformed via standard methods for *Agrobacterium*-mediated floral dip (described in Clough and Bent, 1998; Costigan et al., in press). Antibiotic selection of putative UASBVR transformants was performed in Petri dishes on media containing 1X Murashige and Skoog salts (MS salts; Caisson Laboratories), 0.8% (w/v) Phytoblend (Caisson Laboratories), 1% (w/v) sucrose, and 50 g/mL kanamycin, adjusted to pH 5.7 with KOH (Costigan et al., in press). Next, to isolate a homozygous UASBVR transformant (UASBVR1), PCR-based screening was performed and T3 plants of UASBVR1 were crossed with the M0062 enhancer trap line, exhibiting root-specific GFP accumulation (Haseloff, 1999; Costigan et al., in press). Genotyping of the F1 seedlings was performed with *BVR*- (forward, 5'-ggctgagggactgaaggatccac-3', reverse, 5'-cacttctctggtggcaaagcttc-3') and *GAL4*-specific primers (forward, 5'-agtgtctgaagaacaactgggag-3', reverse, 5'-cgagtttgagcagatgtttacc-3') (Costigan et al., in press). F1 seedlings positive with both primer sets were relocated to soil and propagated obtaining F3 seeds, a cross between M0062 and UASBVR, giving M0062/UASBVR (Costigan et al., in press).

The CAB3::pBVR line was transformed in a similar manner, but with the plasmid vector pBIB/CAB3-TPBVR (Warnasooriya and Montgomery, 2009). The promoter -400-9bp region of CAB3::pBVR directed the expression of BVR to the mesophyll tissue (Warnasooriya and Montgomery, 2009). Both transgenic lines were kindly provided by Dr. Beronda Montgomery (Michigan University, East Lansing, MI).

Plants were grown in Sun Gro Sunshine LC1 mix soil at room temperature (23°C) under continuous white light florescent lamps (70-80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and watered with tap water and nutrient solution as needed. Harvested seeds were then stored at 4°C.

2.2. Growth Conditions for ROTATO experiments

ROTATO is a high resolution feedback system for monitoring root curvature in response to a tropistic stimulus such as light or gravity (Mullen et al., 2000; Kiss et al., 2003).

Under a laminar flow hood, seeds were surface sterilized in a 70% (v/v) ethanol and 0.002% (v/v) Triton X-100 solution for 5 min, rinsed for 1 min twice in 95% (v/v) ethanol, and finally rinsed 1 min four times in sterilized H₂O. Seeds were then cold treated at 4°C for 1 day in sterilized H₂O. Under a laminar flow hood, two to three sterilized seeds were then sown onto sterile, round (60x15mm) plates containing 1.2% (w/v) AGM bacto-agar (described in Kiss et al., 1996) with one-half-strength Murashige and Skoog salts medium and 1% (w/v) sucrose at a pH 5.5. Plates were then sealed with two layers of parafilm while still in the laminar flow hood, and then transferred for a second 1 day cold treatment. Seeds were placed on their edge so agar surface was vertical and grown under continuous white light florescent lamps (70-80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 96 hours (4d) at 23°C.

The four-day-old seedlings were next set up on the feedback system ROTATO stage (Mullen et al., 2000). In order to fully study the effects of blue and red light on the roots of the phytochrome deficient transgenic lines, the plates were set up so the roots were vertical in dark conditions for approximately one hour to adjust. During the dark adjustment period, the growth rate of the root tip was collected on half of the plates using the ADAPT software program one hour prior to experiment initiation (Mullen et al., 1998). For phototropism studies (Fig. 4A), the seedlings were subjected to a unilateral light source of the appropriate wavelength using LED's (red, 660nm at 10-20 $\mu\text{mol m}^{-2} \text{s}^{-1}$; blue, 450nm at 5-10 $\mu\text{mol m}^{-2} \text{s}^{-1}$). For gravitropism studies, the plates were first placed so the roots were vertical for approximately one hour to adjust to dark conditions. Next the plates were rotated 90° so the root tips were then horizontal, as depicted in Fig. 4B. Replicates were screened based on the run time and were accepted in the final data set if the plants successfully ran on the ROTATO system for a minimum of ten hours giving at least ten replicates per experiment set.

2.3. Growth Conditions for Tropism Experiments on Petri Dishes

In this study, *Arabidopsis* seeds were surface sterilized and cold treated, as described in Section 2.2. Six seeds per row, 12 per plate, were sown on (100 x 100 x 15 mm), 1.2 % agar (w/v) plates with one-half-strength MS nutrients, 1% (w/v) sucrose, and a top nitrocellulose film (Promega Corp., Cat. # V7131). Plates were poured and the film laid on the solidified agar on the same day and then cold treated at 4°C for 1 day to allow the film to adhere to the agar (Yamamoto and Kiss, 2002). Sown plates were wrapped in two layers of parafilm and cold treated at 4°C for 1 day, then placed under one of two differing light treatments. The first treatment (light grown) was the same as described above for ROTATO (grown in fluorescent light for 96 hours), while the second treatment (dark grown) included placing the plates under fluorescent light for 24 hours then transferring them to dark conditions, at 23°C, for the remaining 72 hours. These etiolated shoots then have been shown to have a greater response to light sources than do shorter, light grown seedling shoots (Quail et al., 1995).

Four-day-old seedlings, of both light and dark growth treatments, were then transferred to a dark room and exposed to a unilateral blue light source for phototropic experiments. The light was delivered through a blue Plexiglas filter (Rohm and Haas No. 2424; transmission maximum 490 nm, Kumar et al., 2008) at a fluence rate of 15-20 $\mu\text{mol m}^{-2}\text{s}^{-1}$ Plexiglas filter. For gravitropic studies plates were kept in complete darkness and rotated 90° from the vertical. Images were then taken using a digital camera using green safe light (fluence rate $< 0.8 \mu\text{mol m}^{-2}\text{s}^{-1}$) for gravitropic studies and using the ambient blue light of the experiment for phototropic studies at the following time points; 0h, 0.5h, 1h, 2h, 4h, 8h, and 24h. Each experiment consisted of three replicates, with three plates per repetition equaling a total of nine plates with 90-100 plants.

2.4. Data Analyses

From the plate study images, shoot and root lengths and curvature measurements were acquired using Image Pro Plus 6.0 software; an image analysis software. From the length measurements the growth rate (G; mm/h) was determined by calculating:

$$G = \frac{L_{24} - L_0}{24h}$$

Here L_{24} is the length in millimeters at 24h and L_0 is the length in millimeters at 0h. Next, to determine any significant differences between the transgenic lines and their respective WT, their growth rates were compared by means of t-tests performed in Sigma Plot 11.0. A Shapiro-Wilk Normality Test was performed to determine if the data were normally distributed. Data conforming to normality were analyzed with a t-test, symbolized by (t) in the thesis, otherwise the non-parametric Mann-Whitney Rank Sum Test (T) was performed.

For the tropistic curvature measurements, growth toward the light source or gravity vector was measured as a positive angle, while growth away from the stimuli was measured as a negative angle. Curvature measurements were taken at select time points (0, 0.5, 1, 2, 4, 8, 24h) for plate studies and every 45 sec for ROTATO experiments. Data obtained from plate studies were filtered prior to statistical analysis by eliminating measurements of plants which had late or no germination, were highly undulate, upside down, had fallen off of the agar or were either touching the side of the dish or another seedling. In addition, plants with a shoot or root angle of $\pm 30^\circ$ from the gravity vector at $t=0$ were not included in the final data set for angle measurements (Kiss et al., 1996). Sample size of plate studies ranged from 78-98 plants depending on filtering process, and resulted in a range of observations from 1092-1371. For ROTATO studies, plants exhibiting a 10h experiment duration were selected for statistical analysis. Data obtained from ROTATO studies were further processed by selecting hourly time points to reduce the size of the data set since the unfiltered data set exceeded SAS 9.2 limits (SAS

Institute, 2004). Data points from each genotype were selected for each hour. Sample size of ROTATO plates ranged from 9-13, giving a range of observation from 126-312.

Statistical analysis was conducted using SAS 9.2 to determine linear trend through time and then to compare the slopes of the transgenic lines to their respective WT. A linear regression analysis was conducted using PROC REG procedure in SAS 9.2 (SAS Institute, 2004) in order to determine trend in curvature response through time. Next, PROC GLM was used to determine any significant difference ($p < 0.05$) in the curvature response between the two genotypes by comparing their regression coefficients (slopes) through time. A contrast statement was used to test if slopes were equal.

3. Results

3.1. Blue-light Phototropism in Roots

In order to study the effects of phytochrome deficiency (on phototropism and gravitropism) in roots and shoots, I performed time-course of curvature analyses and compared the responses of transgenic lines altered in phytochrome production to their respective WT. One transgenic line, M0062/UASBVR, was deficient in phytochromes specifically in the root (Costigan et al., in press), while the other line, CAB3::pBVR, was deficient in phytochromes specifically in the mesophyll of cotyledons and leaves (Warnasooriya and Montgomery, 2009). In phototropism and gravitropism plate studies, the curvature responses and growth rates of the roots and shoots of light- and dark-grown seedlings were analyzed. In addition, root-tip curvature was analyzed in the feedback system ROTATO (Mullen et al., 2000; Kiss et al., 2003) in response to either unidirectional red or blue light. Significant difference between the WT and the respective transgenic line were tested by comparing the percent differences in curvature response to the percent differences in growth rate.

In blue-light phototropism plate experiments of light-grown seedlings, time-course studies showed no significant differences ($p > 0.05$) in root curvature in the root-specific transgenic line, i.e., M0062/UASBVR (Fig. 5A) nor where any significant differences observed in the cotyledon-specific transgenic line, i.e., CAB3::pBVR, (Fig. 5A).

In the studies of dark-grown seedlings, there was a significant decrease ($p < 0.05$) in curvature response in the M0062/UASBVR line, (Fig. 5B), while phytochrome absence in the cotyledons of dark-grown seedlings showed no significant difference in the curvature response of CAB3::pBVR to blue light (Fig. 5B).

The phototropic differences were then compared to the growth rates of the transgenic and WT lines in order to determine whether any changes in tropism are largely due to differences in growth. In the blue light plate studies of light-grown seedlings,

there was a significant increase in growth rate of the root-specific line ($p < 0.05$; Fig. 6A), however, this increase did not cause a significant difference in the phototropic response of this line (Fig. 5A). There was a significant decrease in the growth rate of the cotyledon-specific line compared to its WT ($p < 0.05$; Fig. 6A); however, this did not cause a significant difference in root response of CAB3::pBVR to blue light.

In phototropism studies of dark-grown seedlings, a significant decrease in the growth rate was noted in both the M0062/UASBVR line and the CAB3::pBVR line ($p < 0.05$; Fig. 6B). The decrease in the growth rate of M0062/UASBVR was only 17.3% while the decrease in curvature was much greater. Therefore, phytochrome absence in roots of dark-grown seedlings inhibits blue-light phototropism in roots. In contrast, while there also was a significant decrease in the growth rate of the CAB3::pBVR line (Fig. 6B), this decreased growth rate did not cause a significant decrease in the root response to blue light.

3.2. Feedback Studies - Phototropism in Roots

In order to study the detailed kinetics of the tropistic curvature, we used a high resolution feedback system (Mullen et al., 2000). In the feedback study, there were no significant differences ($p > 0.05$) in root phototropic curvature in response to blue light in either the M0062/UASBVR or CAB3::pBVR line relative to their respective WTs (Fig. 7A).

However, in red-light feedback studies of root phototropism, the time-course study showed a significant decrease ($p < 0.05$) in the M0062/UASBVR transgenic line response to red light (Fig. 7B). These results suggest that phytochrome absence in roots inhibits red-light-based phototropism in roots. Representative images from individual experiments confirm that there is a significant decrease in red-light-based root phototropism in the M0062/UASBVR line (Fig. 8A).

In addition, there was a significant attenuation ($p < 0.05$) of the CAB3::pBVR to the phototropic response of the transgenic lines to red light compared to its WT (Fig. 7B), which suggests that phytochrome deficiency in shoots also plays some role in the

inhibition of red-light phototropism in roots. Representative images from individual experiments confirm that there is a significant decrease in red-light-based root phototropism in the CAB3::pBVR line relative to the WT (Fig. 8B). However, there was a greater inhibition of curvature response to red-light root phototropism when phytochromes were absent in roots (i.e., the M0062/UASBVR line).

In the blue-light phototropism feedback studies, there was a significant increase in the growth rate of the root-specific transgenic line ($p < 0.05$; Fig. 9A). However, this increase in growth rate did not cause a significant increase in the curvature response to blue light. No significant difference ($p > 0.05$) in root curvature in response to blue light in the CAB3::pBVR line (Fig. 9A) was noted.

In feedback studies of red-light phototropism, no significant difference ($p > 0.05$) was seen in the growth rate of the M0062/UASBVR line compared to the WT. In the root-specific line, the phototropic response at 24 h was impaired by 87.4% (Fig. 7B), while growth was promoted by 40.7 % relative to the WT (Fig. 9B). In the cotyledon transgenic line (i.e. CAB3::pBVR), the phototropic response was inhibited by 45.5% (Fig. 7B), while there also was a significant impairment ($p < 0.05$) in the growth rate of 34.4% (Fig. 9B). Thus, there was a far greater impairment in red-light phototropism in the M0062/UASBVR line which is lacking in phytochrome in the roots.

3.3. Gravitropism in Roots

In gravitropism plate studies of light-grown seedlings, there was a significant increase ($p < 0.05$) in curvature noted between the M0062/UASBVR strain and its WT (Fig. 10A), and there was a significant decrease ($p < 0.05$) in response noted between CAB3::pBVR and its WT (Fig. 10A). In the plate studies of dark-grown seedlings, there was a significant decrease in curvature response found between both the M0062/UASBVR line and CAB3::pBVR line ($p < 0.05$) and their respective WTs (Fig. 10B).

In light-grown seedling studies, a significant increase in growth rate was noted in the M0062/UASBVR line compared to its WT ($p < 0.05$; Fig.11A). However, when the

increase in growth rate was compared to the increased curvature, there was an increased curvature of 21.7% (Fig. 10A) and a promoted growth rate of only 12.3% (Fig. 11A).

There also was a significant decrease ($p < 0.05$) found between the growth rate of CAB3::pBVR and its WT in the gravitropism studies of light-grown seedlings (Fig. 11A). However, the significant decrease in curvature was only 36.3%, while the decrease in growth rate was 59.6%, suggesting that the decrease in growth rate could be responsible for the decrease in root gravitropism.

In the studies with dark-grown seedlings, there was a significant decrease ($p < 0.05$) in the growth rate of the root-specific line compared to its WT (Fig. 11B). When we compare this decreased growth rate to the decrease gravitropic response, there was a decreased growth rate of 19.1% and a decreased curvature of 8.5%. Therefore, the decreased growth rate is greater than the decrease in curvature and is likely the cause of the inhibition in gravitropism in the M0062/UASBVR line.

In the CAB3::pBVR dark-grown seedlings, there was no significant difference ($p > 0.05$) in the growth rate compared to its WT. Therefore, phytochrome absence in shoots of dark-grown seedlings inhibits root gravitropism since the 49.5% inhibition of gravitropism was not caused by an inhibition of growth.

In feedback studies of gravitropism of light-grown seedlings, there was no significant difference noted in curvature response between M0062/UASBVR and its WT (Fig. 12). Representative images from individual experiments confirm that there is no significant decrease in root gravitropism in the M0062/UASBVR line relative to the WT (Fig. 13A).

A significant decrease in curvature was observed between the CAB3::pBVR line and its WT ($p < 0.05$; Fig. 12). Representative images from individual experiments are illustrated in Figure 13B.

No significant difference ($p > 0.05$) in the growth rate of M0062/UASBVR compared to its WT was noted (Fig. 14). There also was no significant decrease ($p > 0.05$) noted in the CAB3::pBVR line, and when we compare the percent differences, we note that the 22.6% decrease in growth rate is likely responsible for the 23.7% decrease in root gravitropism.

3.4. Phototropism in Hypocotyls

There was a significant inhibition in hypocotyl phototropic response ($p < 0.05$) to blue light when phytochromes were absent in both roots and cotyledons of the light-grown seedlings (Fig. 15A). There also was a significant inhibition in hypocotyl phototropic response of dark-grown M0062/UASBVR seedlings ($p < 0.05$) to blue light and no significant difference between CAB3::pBVR and its WT (Fig. 15B).

However, when the curvature differences in the studies of light grown seedlings were compared to their growth rates, there was a significant decrease ($p < 0.05$) of 52.5% in the growth rate of M0062/UASBVR (Fig. 16A) which could be responsible for the 40.1% decrease in curvature (Fig 15A). In addition, CAB3::pBVR had an inhibited phototropic response of 31.1% and a significant decrease in growth rate ($p < 0.05$) of 33.7%, also suggesting that the decreased growth rate likely caused the decreased phototropic response.

In studies of dark-grown seedlings, there was a significant decrease ($p < 0.05$) in the M0062/UASBVR growth rate (Fig. 16B) of 34.5%, accounting for the inhibited hypocotyl response to gravity of 17.9%. There was no significant difference ($p > 0.05$) in the growth rates of the dark-grown CAB3::pBVR seedlings and their WT. Therefore, the phytochrome absence in roots or cotyledons of both light- and dark-grown seedlings did not affect hypocotyl phototropism.

3.5. Gravitropism in Hypocotyls

In gravitropism studies of light-grown seedlings, there was a significant decrease ($p < 0.05$) found in hypocotyl phototropism between M0062/UASBVR and its WT (Fig. 17A). In addition, the absence of phytochrome in cotyledons significantly increased ($p < 0.05$) the hypocotyl curvature response in the transgenic line (CAB3::pBVR) compared to the WT (Fig. 17A).

In the gravitropism plate studies of dark-grown seedlings, it was shown that the absence of phytochromes in both roots and cotyledons significantly promotes ($p < 0.05$) hypocotyl gravitropism (Fig. 17B).

When these gravitropic responses were compared to their respective growth rates, there was a significant decrease in growth ($p < 0.05$) in both of the light-grown M0062/UASBVR and CAB3::pBVR lines. Overall, phytochrome absence in roots of light-grown seedlings showed a decreased growth rate in the M0062/UASBVR line of 45.3% (Fig. 18A), while the decrease in curvature was only 33.5% (Fig. 17A). These results suggest that the greater decrease in growth rate is largely responsible for the decreased curvature response. In contrast, phytochrome absence in cotyledons of light-grown seedlings, promoted hypocotyl gravitropism with an increased phototropic curvature of 26.9% (Fig. 17A) and a decreased growth rate of 22.2% (Fig. 18A).

In dark-grown seedlings, when the M0062/UASBVR curvature response was compared to its growth rate, there was a significant decrease in growth ($p < 0.05$) of 36.4% (Fig. 18B), with an increase of 33.2% in gravitropic curvature (Fig. 17B). Therefore, these results suggest that phytochrome absence in roots of dark-grown seedlings promotes hypocotyl gravitropism.

In addition, a significant difference ($p < 0.05$) was noted between the cotyledon transgenic line and its WT. Phytochrome absence in cotyledons of dark-grown seedlings promotes hypocotyl gravitropism with a decreased growth rate at 24h of 11.7% (Fig. 18B), while an increase of 23.9% in hypocotyl phototropism (Fig. 17B). As can be observed in Fig. 17B, as well as with the percent differences listed above, there was a greater promotion of hypocotyl gravitropism when phytochromes were absent in the root than when they were absent in the cotyledon.

Table 1 promotes a summary of all experiments in this study and illustrates the significant effects on tropistic curvature of phytochrome deficiency in roots (M0062/UASBVR) and cotyledons (CAB3::pBVR).

4. Discussion

4.1. Does red-light sensing occur in the root?

The expression-specific lines used in this research (M0062/UASBVR, root-specific line, and CAB3::pBVR, cotyledon-specific line) provide a tool to study the spatial-specific roles phytochromes play on the photoregulatory system (Warnasooriya and Montgomery, 2009), including phototropism. In addition, use of these lines allow for the study of phytochrome involvement in light perception, providing new insights into the mechanisms by which plant roots respond to light, whether it is via shoots or roots or both.

In red-light-based root phototropism, phytochrome deficiency attenuated both the root-specific (M0062/UASBVR) transgenic line and the shoot specific (CAB3::pBVR) transgenic line. However, a greater magnitude of inhibition was noted when phytochrome was absent in the roots (i.e. in the M0062/UASBVR line). These results suggest that root-localized phytochrome plays a significant role in modulating the seedling curvature response in terms of red-light root phototropism. In addition, the attenuation in the phototropic response of roots of the CAB3::pBVR line suggests that cells in the shoot may also be perceiving the red-light signal and transducing signal to the roots to some degree. However, since this attenuation was less than that noted between the root-specific line and the WT, these results suggest that the root primarily obtains its signaling from phytochrome present in the root.

In blue-light-based root phototropism, phytochrome deficiency did not significantly affect light-grown seedlings of either the root- or shoot-specific line. However, a significant attenuation in blue-light root phototropism was noted when phytochromes were absent in the roots of dark-grown seedlings (i.e. in the M0062/UASBVR line). Therefore, the absence of phytochrome in the roots of dark-

grown seedlings inhibits blue-light phototropism in roots, which supports the idea of sensing of light in the root itself there by moderating tropistic curvature.

One of the main goals of this research was to more precisely determine the role of phytochromes in tropistic responses. Specifically, we aimed to discover whether red-light sensing via phytochromes occurs directly in the root itself or if a signal is transmitted from the shoot to the root resulting in root phototropic curvature. In a study done in which the shoots of *Arabidopsis* seedlings were covered to block the light source thereby leaving the roots exposed (Kiss et al., 2003), roots still exhibited a positive phototropic response to red light. However, when the root was covered with foil allowing no light penetration, there was no phototropic response, strongly suggesting that the light perception is occurring in the root and not the shoot (Kiss et al., 2003). In addition, a study by Hall et al. (2001) also showed phyA localized in the root cap.

While phototropins and cryptochromes are the pigments primarily responsible for light sensing in blue-light phototropism (Christie, 2007), phytochromes modulate some aspects of blue-light phototropism in addition to their role in red-light phototropism (Montgomery, 2008). More specifically, PHYA and PHYB are involved in hypocotyl gravitropism (Parks et al., 1996; Janoudi et al., 1997) and red-light root phototropism (Correll et al., 2003; Kiss et al., 2003; Molas and Kiss, 2008), while PHYA-E modulate hypocotyl blue-light phototropism (Whippo and Hangarter, 2004; Kumar and Kiss, 2006; Kumar et al., 2008). Studies done using mutants altered in specific photoreceptors suggest interesting interactions among and between these sets of photoreceptor families (Mas et al., 2000; Whippo and Hangarter, 2003; Kumar and Kiss, 2006).

The specific mechanism by which phytochromes function in blue-light-based phototropism has not been fully elucidated. However, there are three main hypothesis regarding hypocotyl phototropism. The first model proposes that phytochromes modulate phototropism via signaling by phototropins (Whippo and Hangarter, 2004), and a second hypothesis suggests that phytochromes modulate phototropism via cryptochrome interaction (Mas et al., 2000; Correll et al., 2003; Whippo and Hangarter, 2003). Alternatively, the third hypothesis proposes that phytochromes attenuate the gravitropic response, thus allowing for a promoted phototropic response (Parks et al., 1996).

There have only been relatively few studies on root phototropism; however, it has been shown that the perception of blue light occurs in the root cap of maize (Mullen et al., 2002). It is further suggested that the perception of blue light is primarily localized in the root cap due to the coaction of the blue-light photoreceptor with phytochromes, which are also localized in the root cap (Johnson et al., 1991). While even less investigation has been done on phytochrome mediated red-light phototropism at the root level, it has been revealed by studies in our lab that red light induces positive phototropism in roots (Kiss et al., 2003). More specifically, we found that PKS1 (Phytochrome Kinase Substrate 1), a negative regulator of the phytochrome-based response (Fankhauser et al., 1999) gene transcription is up-regulated in seedling roots after exposure to red light (Molas et al., 2006). Therefore, PKS1 modulates the phototropic response of red light in roots while PHYA and PHYB act together to control red-light phototropism in roots (Kiss et al., 2003; Molas et al., 2006). Since PHYA and PHYB are also known to regulate hypocotyl phototropism and PKS1 negatively regulates phytochrome-based responses, it has now been suggested that PKS1 is necessary for hypocotyl phototropism (Lariguet et al., 2006). Thus, it is proposed that this protein is one of the key links between the red- and blue-light photoreceptor families (Molas and Kiss, 2008).

4.2. Gravitropic signaling in roots via phytochromes may involve a transfer of signals from the shoot

Numerous studies have suggested that gravitropism and phototropism have overlapping pathways (e.g., Poppe et al., 1996; Berkovich et al., 2005). However, since gravitropism has a more robust expression in roots (Vitha et al., 2000), the phototropic response is more readily observed in studies done with starchless mutants of *Arabidopsis*, which are impaired in gravitropism compared to the WT (Kiss, 2000). Our studies also included gravitropism experiments because of the role of phytochrome in gravitropic signaling mechanisms (Poppe et al., 1996). Furthermore, a study done by Wolverton et al. (2002) used decapped roots to find that the perception of gravity is not confined only to the root tip, but that the elongation zone is also gravistimulated. These results further

support the idea that signals can be perceived in differing parts of the plant and then be transferred to other regions.

In addition, numerous other studies have shown photoreceptor involvement in gravitropism pathways in shoots. For instance, Lariguet and Fankhauser (2004) found that while blue light triggers phototropism, it also represses hypocotyl gravitropism. With *phyAphot1* double mutants, they found that PHYA is necessary for the inhibition of hypocotyl gravitropism (Lariguet and Fankhauser, 2004). More specifically, a study by Kim et al. (2011) demonstrated that phytochrome inhibits hypocotyl gravitropism by converting the starch-filled amyloplasts, which play a role in gravity sensing, into plastids with chloroplastic or etioplastic characteristics. Other studies have shown that PHYA positively regulates phototropism by inhibiting gravitropism (Robson and Smith, 1996). Also, it was previously shown by Liscum and Hangarter (1993) that PHYB is involved in hypocotyl gravitropism in *Arabidopsis*.

Other work has demonstrated that phytochromes also play an integral role in gravitropic root response. For example, Feldman and Briggs (1987) found that in *Zea mays*, red light exposure promoted root gravitropism. Their studies showed that the effect of blue light on root gravitropism was 50-100 times less than that of red light, therefore making phytochrome, not phototropin, the photoreceptor more directly associated with root gravitropism (Feldman and Briggs, 1987). Studies with our group showed that root gravitropism is impaired in both light- and dark-grown *phyAB* and *phyB* mutants (Correll and Kiss, 2005) and further studies on *phyA-phyE* mutants showed that PHYA and PHYB are involved in the regulation of root gravitropism (Hennig et al., 2002; Correll and Kiss, 2005).

In our present studies of root gravitropism, we found a significant decrease in root curvature response found in the shoot-specific CAB3::pBVR line compared to its WT (Table 1). While there have been many studies of shoot signals transferred to the root, these results suggest there may be transfer of signals from the shoot to the root via phytochromes during gravitropism.

4.3. Signaling also may occur from root to shoot in hypocotyl gravitropism

Phytochrome A is necessary for the inhibition of hypocotyl gravitropism, and this inhibition of gravitropic response positively regulates hypocotyl phototropism (Lariguet and Fankhauser, 2004). In our gravitropism studies of light-grown seedlings, the absence of phytochrome in cotyledons significantly increased the hypocotyl curvature response in the cotyledon-specific transgenic line (CAB3::pBVR) compared to the WT (Table 1). In addition, in the gravitropism studies of dark-grown seedlings, it was shown that the absence of phytochromes in both roots and cotyledons significantly promotes hypocotyl gravitropism.

Thus, these results support the hypothesis of Lariguet and Fankhauser (2004) that phytochrome is involved in the inhibition of gravitropic response because we observed that the lack of phytochrome in root- and cotyledon-specific lines promote hypocotyl gravitropism. In addition, there was a greater promotion of hypocotyl gravitropism when phytochrome was absent in the root compared to when absent in the cotyledon of dark-grown seedlings (Table 1). These results suggest that there may be some signaling from root to shoot in hypocotyl gravitropism since the lack of phytochrome in the root promoted hypocotyl gravitropism to a great degree. These results also are supported by a recent study by Martin-Vertedor and Dodd (2011) on soil moisture and ABA concentrations. They found that roots that are in a drying soil produce several chemical signals including abscisic acid (ABA) and that these signals can then be transported from the root to the shoot, changing many aspects of their physiology.

4.4. Differences in tropisms among *Arabidopsis* ecotypes

Two transgenic lines were used in this study, M0062/UASBVR which is deficient of phytochrome in the root and CAB3::pBVR which is deficient of phytochrome in the mesophyll of the cotyledons and leaves. The two lines were derived

from two different genotypes, with the M0062/UASBVR line being derived from the C24 ecotype, while the CAB3::pBVR line was from the Nossen (No-O) ecotype.

Several researchers have noted physiological differences among different ecotypes of *Arabidopsis thaliana*. For instance, in previous studies using light- and dark-grown seedlings, a stronger red-light phototropic response in light-grown seedlings (using the Landsberg-Ler ecotype) was observed compared to dark-grown seedlings (Sakai et al., 2000; Kiss et al., 2003). In contrast, another study (using the RLD ecotype) found there to be a stronger red-light-based phototropic response in the roots of dark-grown seedlings (McCoshum and Kiss, in press). Differences in light- versus dark-grown seedling root expression to red-light phototropism could be due to varying ecotypes used in different studies, and the variation in responses can fluctuate in red-light root expression as much as 10°-15° in the Columbia ecotype to 30°-35° in the Ler strain, while the Wassilewskija (WS) ecotype showed a minimal response to red light (Kumar et al., 2008). These results suggest that the degree of curvature response between the root and shoot-specific lines could be due to the different ecotypes from which they were derived.

There has also been an extensive study done by Johanson et al. (2000) which depicted the variations in flowering times of over thirty different *Arabidopsis thaliana* ecotypes. A study by Li et al. (1998) also demonstrated the differences in the growth of forty different *Arabidopsis* ecotypes. They found that ecotypes from higher latitudes tended to have smaller relative growth rates (including entire plant size, seed size and rosette size) compared to those from lower latitudes. Their results demonstrated that there is significant variation among *Arabidopsis* ecotypes with relative growth rates which are negatively correlated with latitude.

4.5. Conclusions

The mechanisms by which plants respond to light and gravity are not only complex, but have been shown to be interconnected and overlapping. The focus of this research was to determine whether roots directly perceive light signals in their

environment. An alternative hypothesis is that the signal may be perceived in the shoot and transmitted to the root.

In this investigation, we found that when phytochrome (PHYA-E) was lacking in roots that there was a drastic decrease in the root phototropic response to red light, suggesting that roots are involved in the perception of red light. In addition, there was an attenuated response in blue-light-based root phototropism when phytochrome was lacking in cotyledons. However, this attenuation was less than that noted between the root-specific line and the WT. Therefore, not only is the perception of red light spatially localized in the root, but the response in the root is greater than the light signal being perceived in the shoot and transmitted to the root. In addition, the absence of phytochrome in the roots of dark-grown seedlings also inhibited blue-light phototropism in roots, further supporting the idea of sensing of light in the root itself modulating tropistic curvature.

While little work on root to shoot signaling has been done, these studies on gravitropism show that sensing of light in roots may have an effect on shoot gravitropism since phytochrome absence in roots of dark-grown seedlings promoted hypocotyl gravitropism. In these studies, we also found that the lack of phytochrome in hypocotyls promoted hypocotyl gravitropism of light- and dark-grown seedlings, further supporting that there are interactions between the phototropism and gravitropism pathways. Further research will focus on elucidating signaling pathways in root and shoot tropistic responses.

Figures

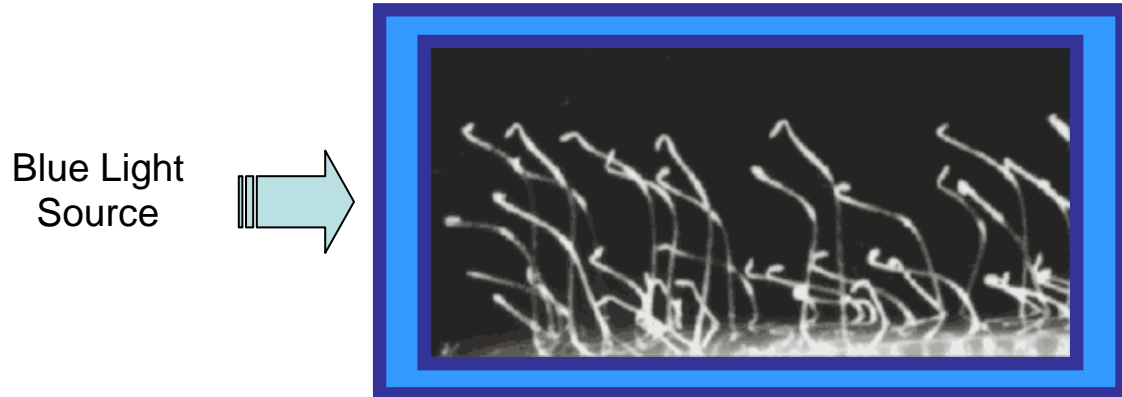


Figure 1: *Arabidopsis* hypocotyls bending toward blue light, thereby exhibiting a positive phototropism.

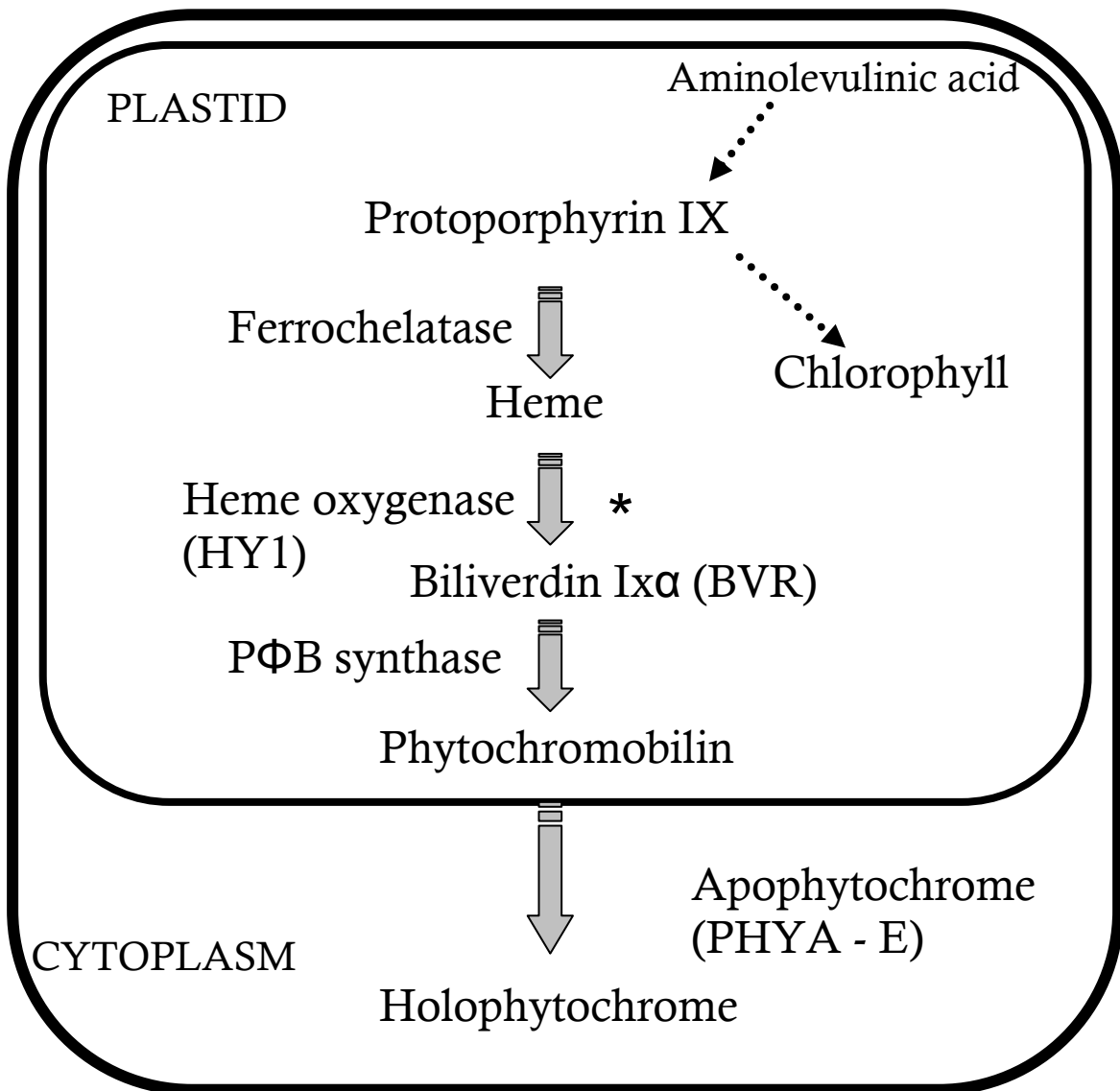


Figure 2: Phytochrome biosynthesis pathway adapted from Kohchi et al., 2001. The * indicates the step in the synthetic pathway which is repressed by the addition of biliverdin IX α reductase (BVR), inhibiting the production of bilin, a precursor of phytochrome.

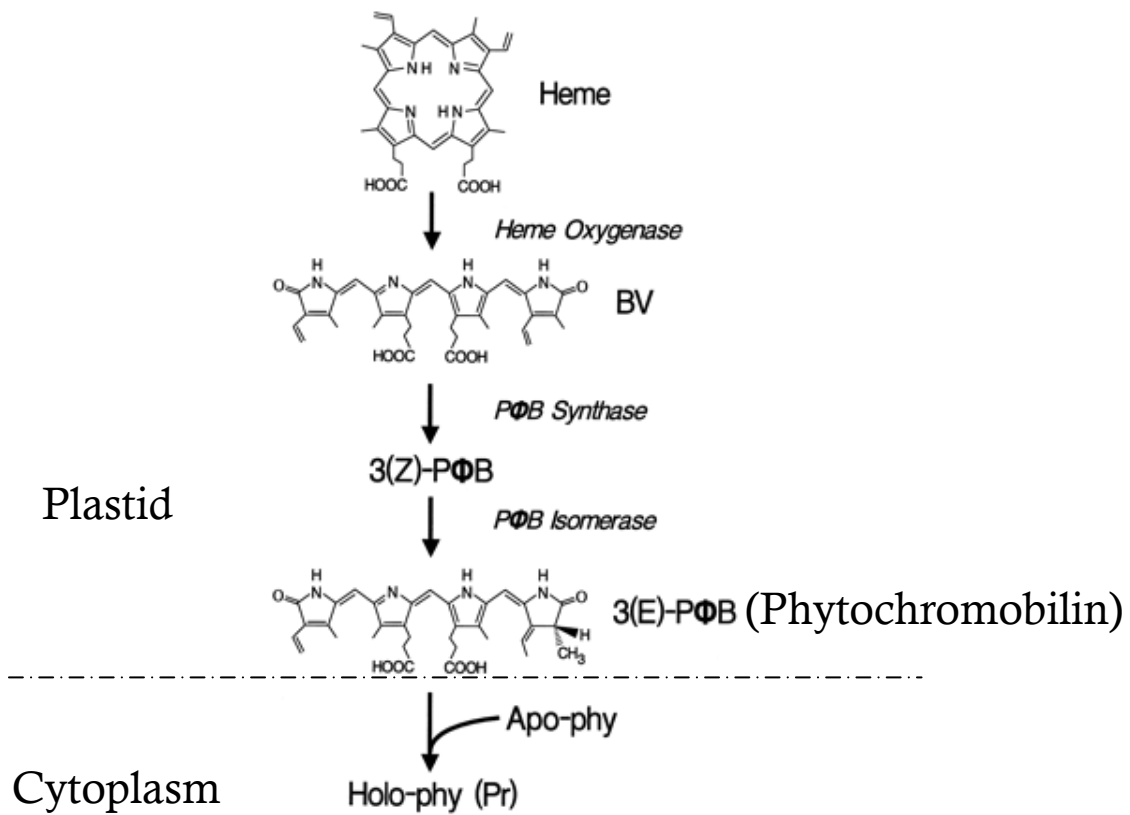


Figure 3: Phytochrome synthetic pathway adapted from Davis et al., 1999.

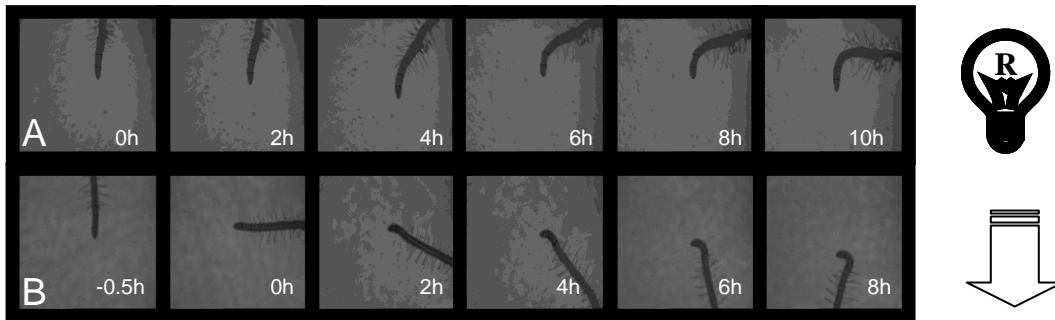


Figure 4: The images in A illustrate how the feedback system ROTATO (Mullen et al., 2000; Kiss et al., 2003) operates during a red phototropism experiment (unilateral red light from the right) by rotating the plate in order to keep the tip of the root tip at a constant 0° angle during phototropism experiments. The images in B represent this same operation during gravitropism experiments in which the root tip was maintained at a 90° angle.

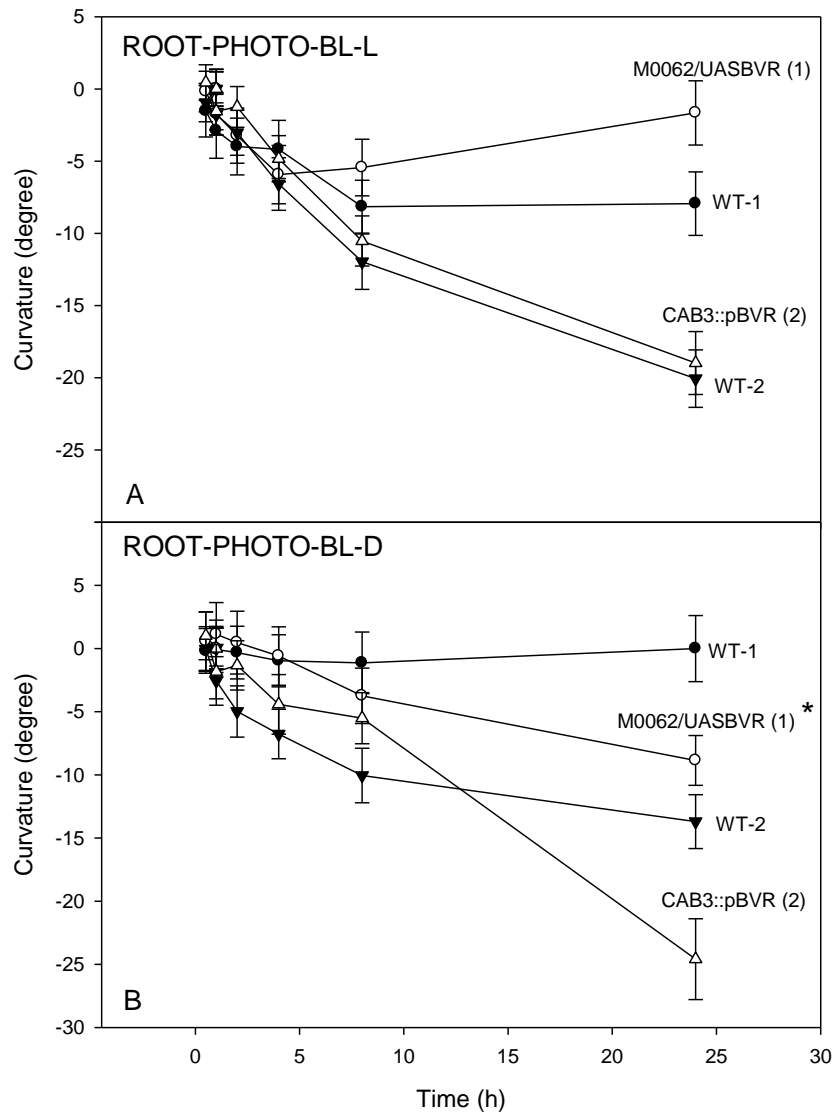


Figure 5: Time-course of curvature of blue-light phototropism in light-grown (A) and dark-grown (B) roots of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). The mean curvature at each data point was calculated for 73-96 plants. Error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) through time in the M0062/UASBVR line compared to the WT are indicated by an (*).

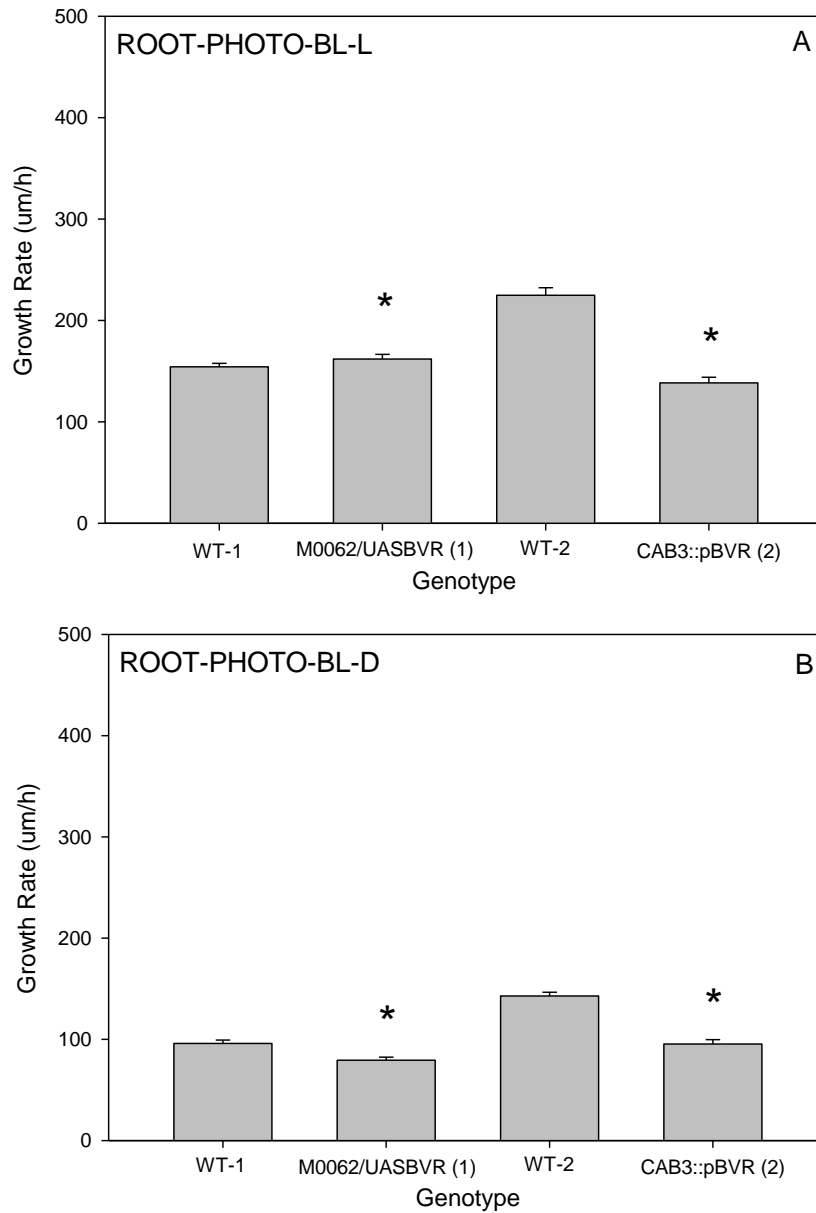


Figure 6: Growth rate of blue-light phototropism in light-grown (A) and dark-grown (B) roots of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). N= 82-98 plants; and error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) in the transgenic line compared to its WT are indicated by an (*).

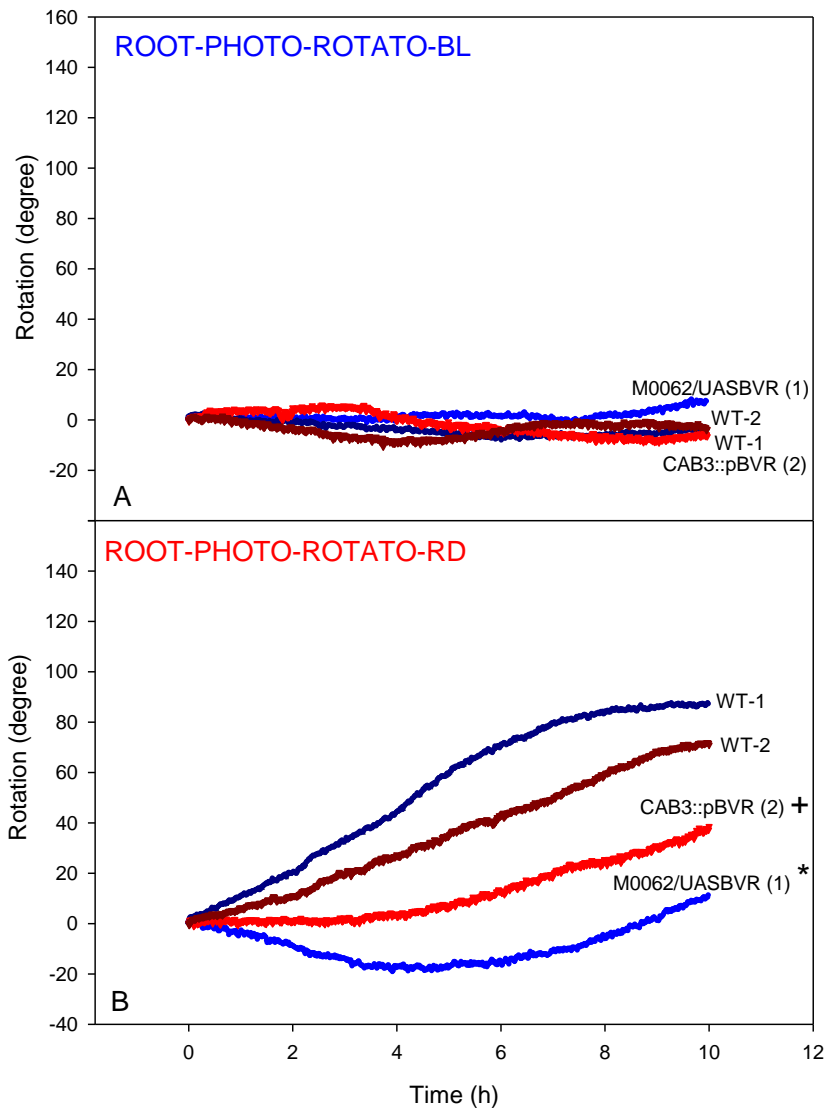


Figure 7: Feedback system studies of blue-light (A) and red-light (B) phototropism in roots of light-grown 4-day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). Curvature measurements were taken every 45 seconds to determine the change in curvature over a 10h time period. The mean curvature was calculated for 10-15 plants. Statistically significant differences ($p < 0.05$) through time in the M0062/UASBVR line compared to its WT are indicated by an (*) while significant differences in the CAB3::pBVR line are indicated by a (+).

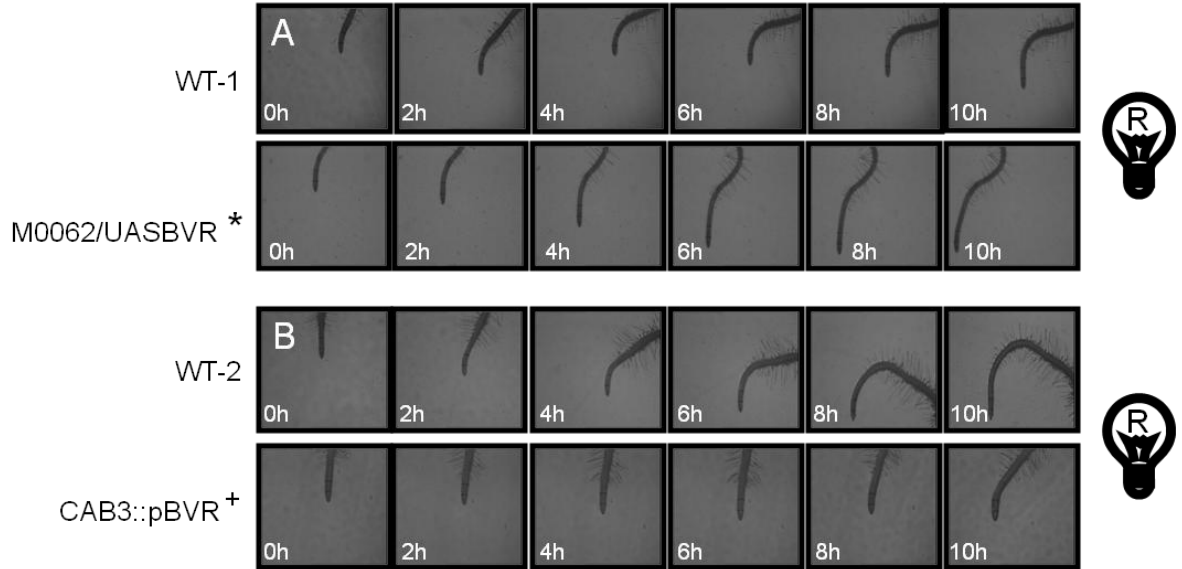


Figure 8: Representative images from the time-course of phototropism studies conducted using the feedback system. Seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). Petri dishes of both treatments were exposed to a unilateral red-light source ($10\text{-}20 \mu\text{mol m}^{-2}\text{s}^{-1}$) throughout the experiment. In this experiment, red light was provided from the right side (indicated by the light bulbs) with the root tip constrained at 0° (vertical). Images were taken every 30 minutes over a 10h time period and are shown here at 2h intervals. Note the obvious attenuation in curvature in the roots of both the M0062/UASBVR and CAB3::pBVR transgenic lines compared to their WTs. Statistically significant difference ($p < 0.05$) through time in the M0062/UASBVR line compared to its WT is indicated by an (*) while significant difference in the CAB3::pBVR line is indicated by a (+).

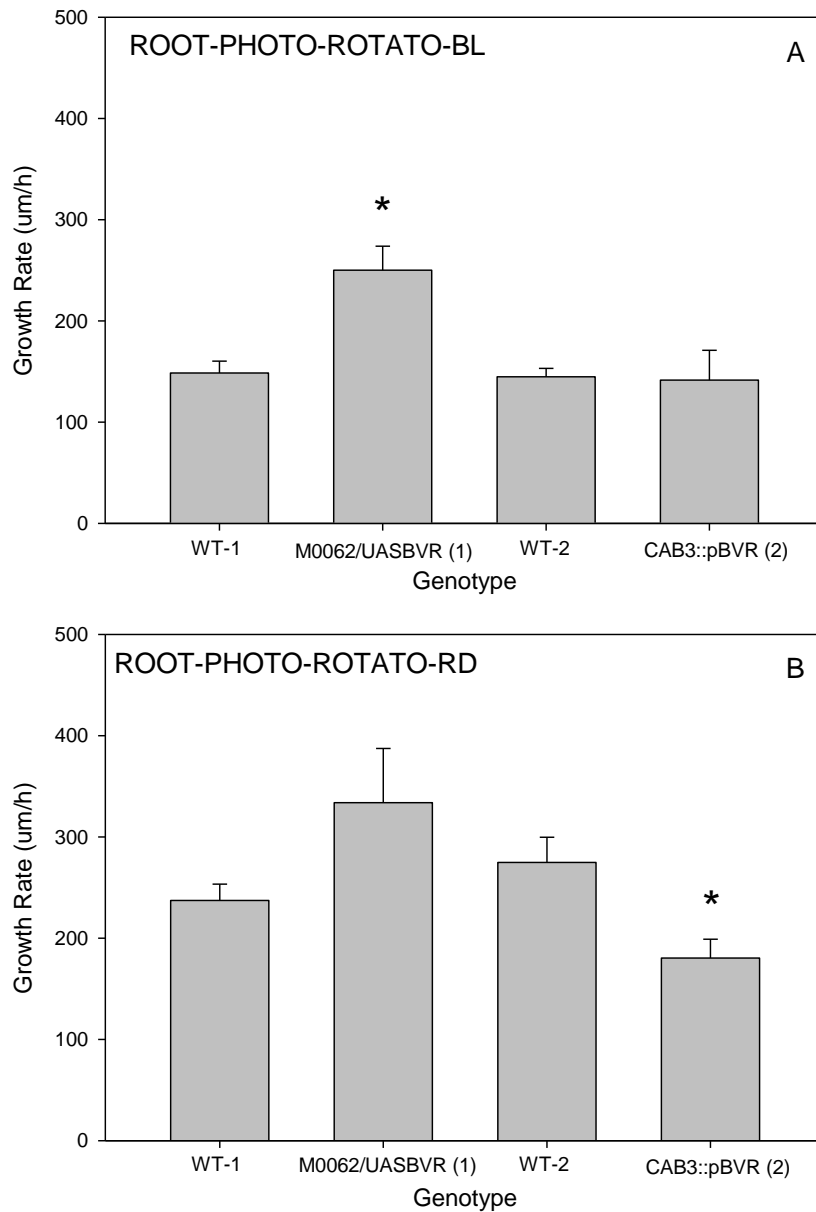


Figure 9: Growth rate of feedback system studies of blue-light (A) and red-light (B) phototropism in light-grown roots of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). N=5-14 plants; and error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) in the transgenic line compared to its WT are indicated by an (*).

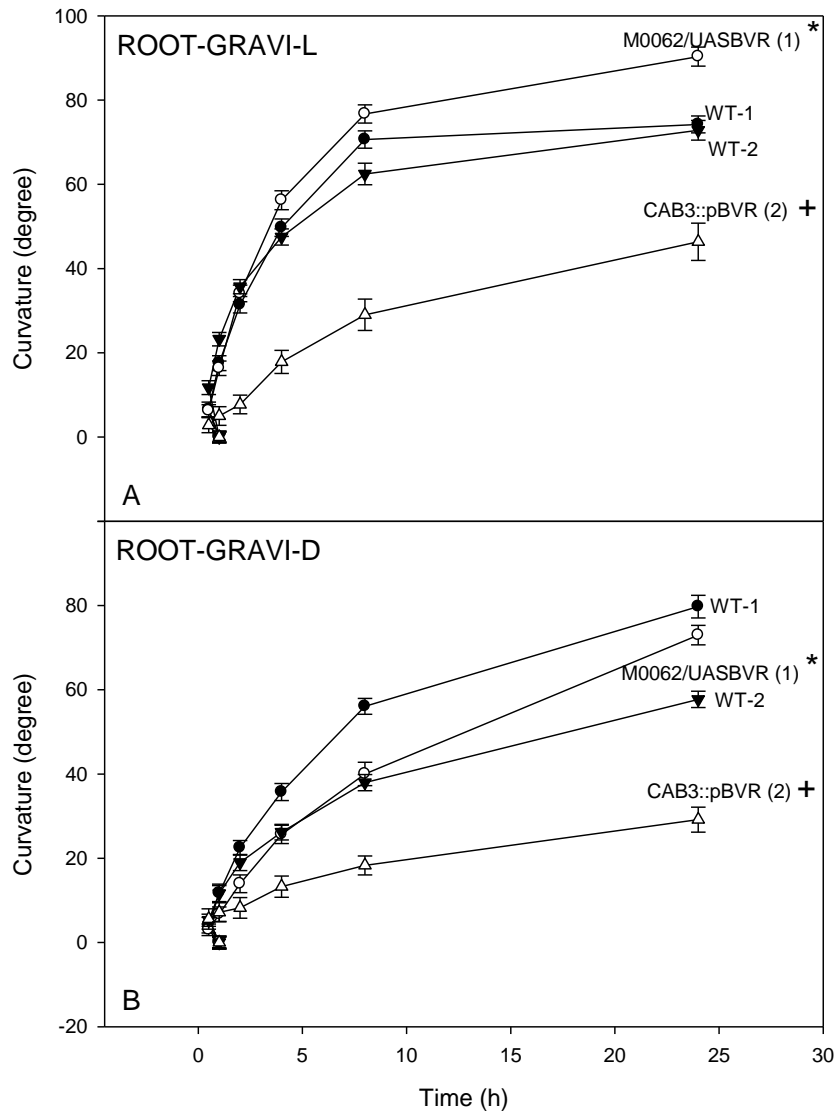


Figure 10: Time-course of curvature of gravitropism in light-grown (A) and dark-grown (B) roots of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). The mean curvature at each data point was calculated for 63-94 plants. Error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) through time in the M0062/UASBVR line compared to its WT are indicated by an (*) while significant differences in the CAB3::pBVR line are indicated by a (+).

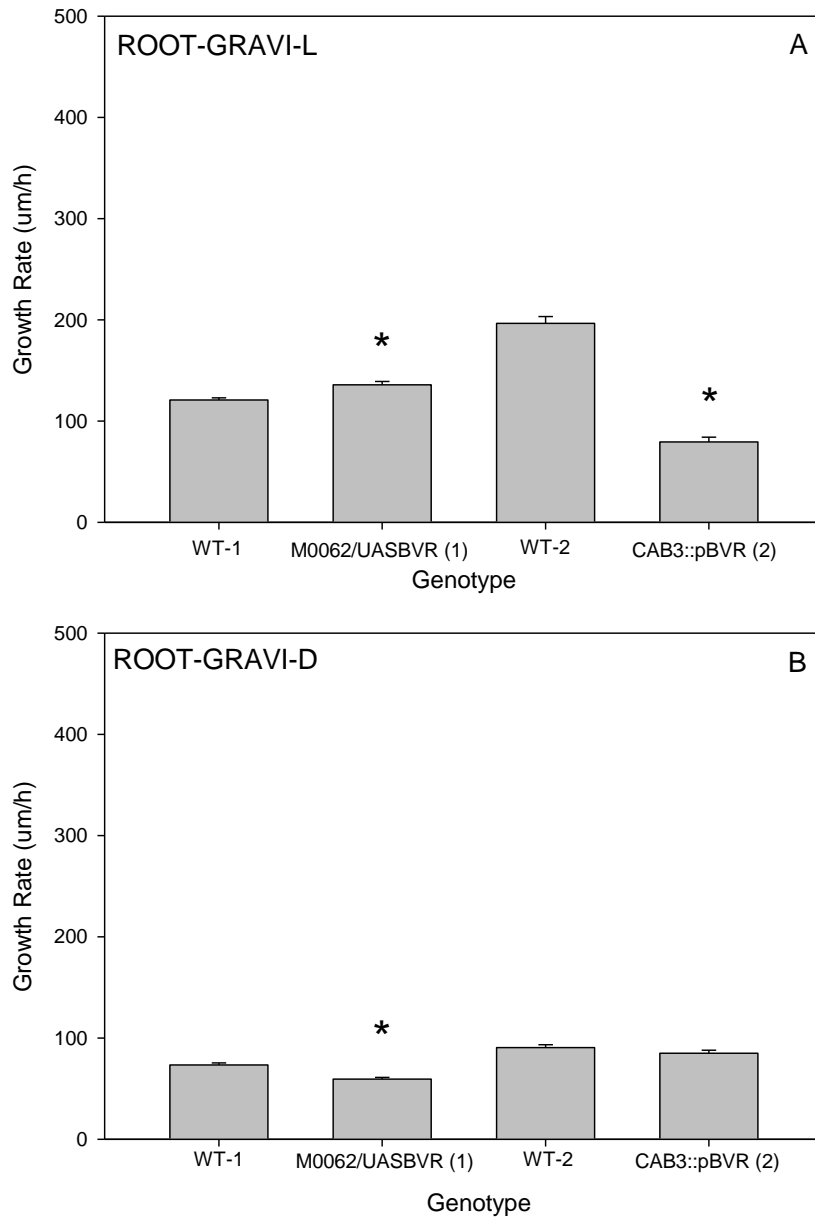


Figure 11: Growth rate of gravitropism in light-grown (A) and dark-grown (B) roots of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). N=84-101 plants; and error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) in the transgenic line compared to its WT are indicated by an (*).

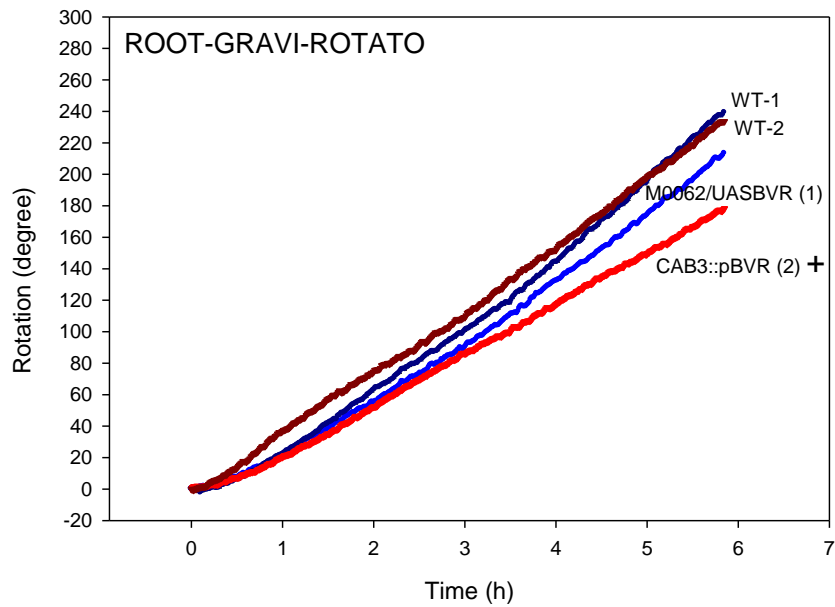


Figure 12: Feedback systems studies of gravitropism in light-grown roots of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). Curvature measurements were taken every 45 seconds to determine the change in curvature over a 6h time period. The mean curvature was calculated for 9-11 plants. Statistically significant difference ($p < 0.05$) through time in the CAB3::pBVR line compared to its WT is indicated by a (+).

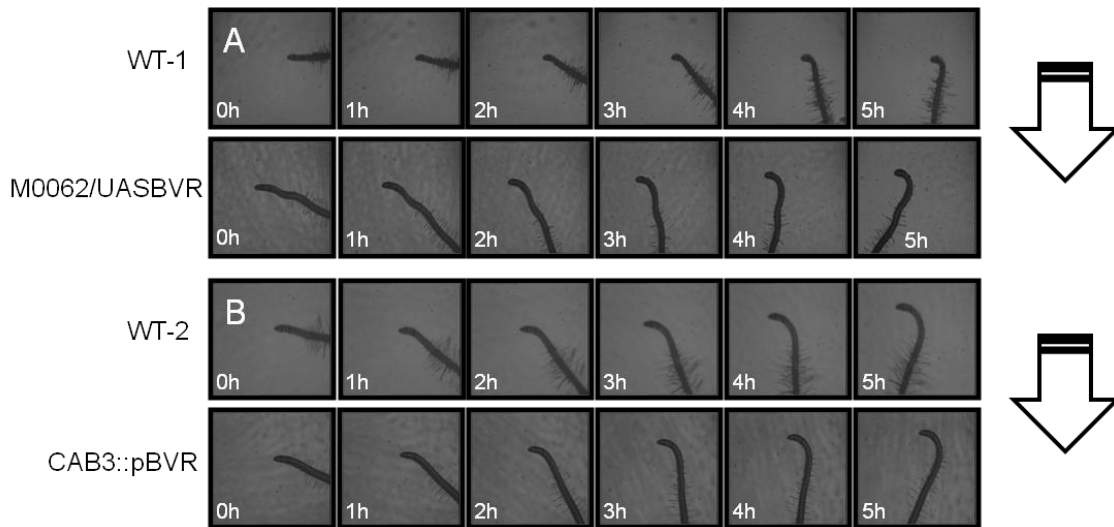


Figure 13: Representative images from the time-course of phototropism studies conducted using the feedback system. Seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). Petri dishes were rotated 90° from the vertical and kept in the dark throughout the experiment. Images were taken every 30 minutes over a 6h time period and are shown here at 1h intervals. Neither of the two transgenic lines had a significant difference in curvature when compared to their relative growth rates.

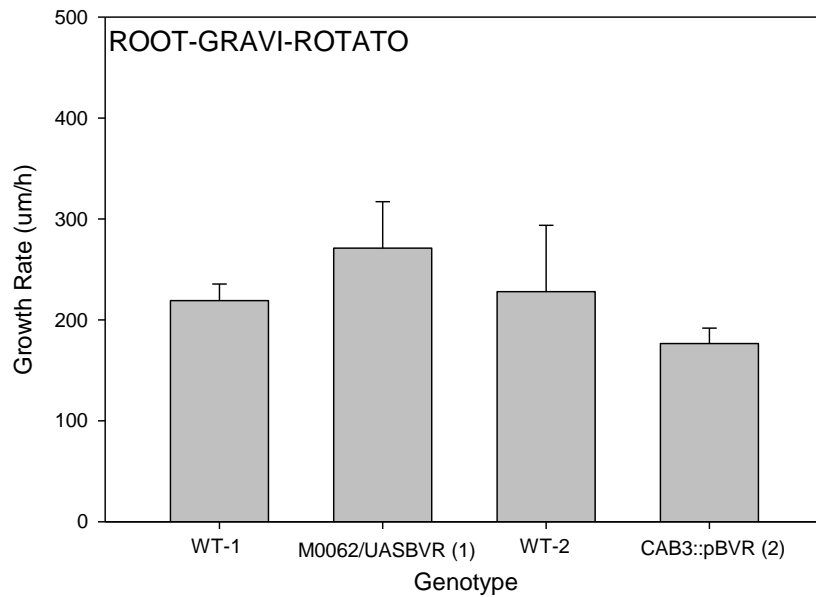


Figure 14: Growth rate of feedback system gravitropism in light-grown roots of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). Growth rate measurements were taken every 45 seconds to determine the change in growth over a 1h time period. N=5-12 plants; and error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) in the transgenic line compared to its WT are indicated by an (*).

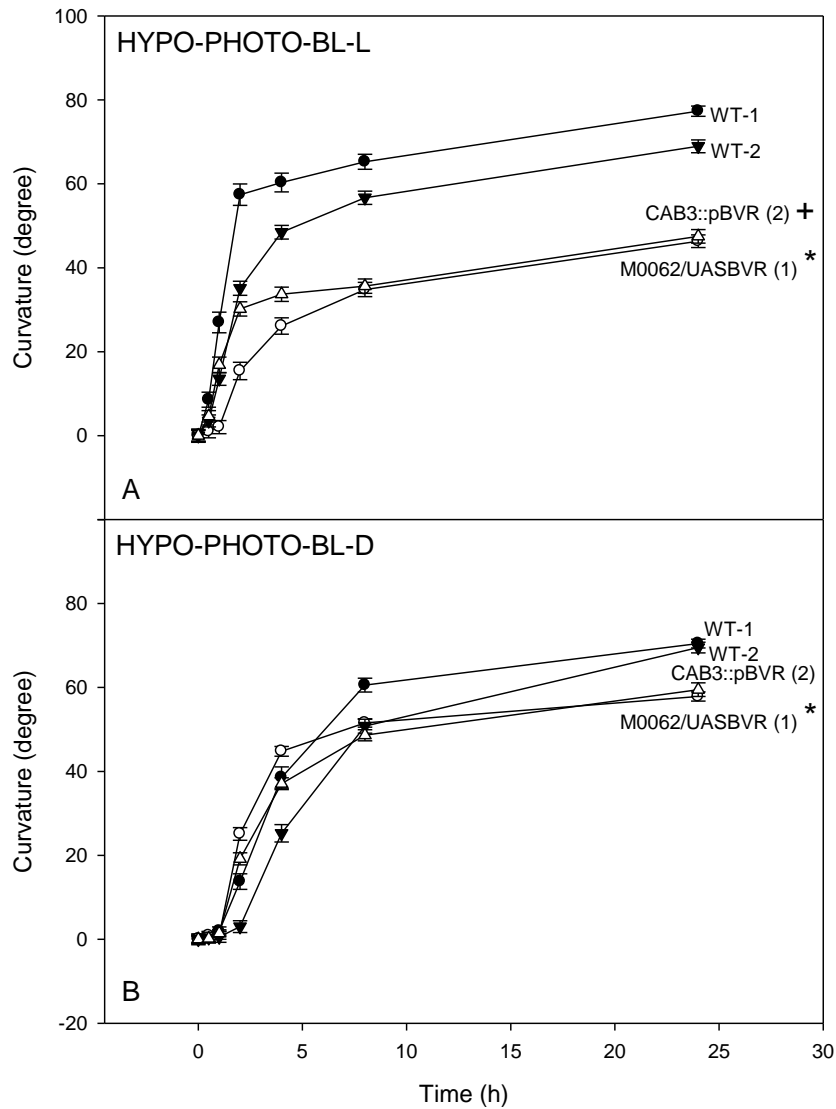


Figure 15: Time-course of curvature of blue-light phototropism in light-grown (A) and dark-grown (B) hypocotyls of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). The mean curvature at each data point was calculated for 74-94 plants. Error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) through time in the M0062/UASBVR line compared to its WT are indicated by an (*) while significant differences in the CAB3::pBVR line are indicated by a (+).

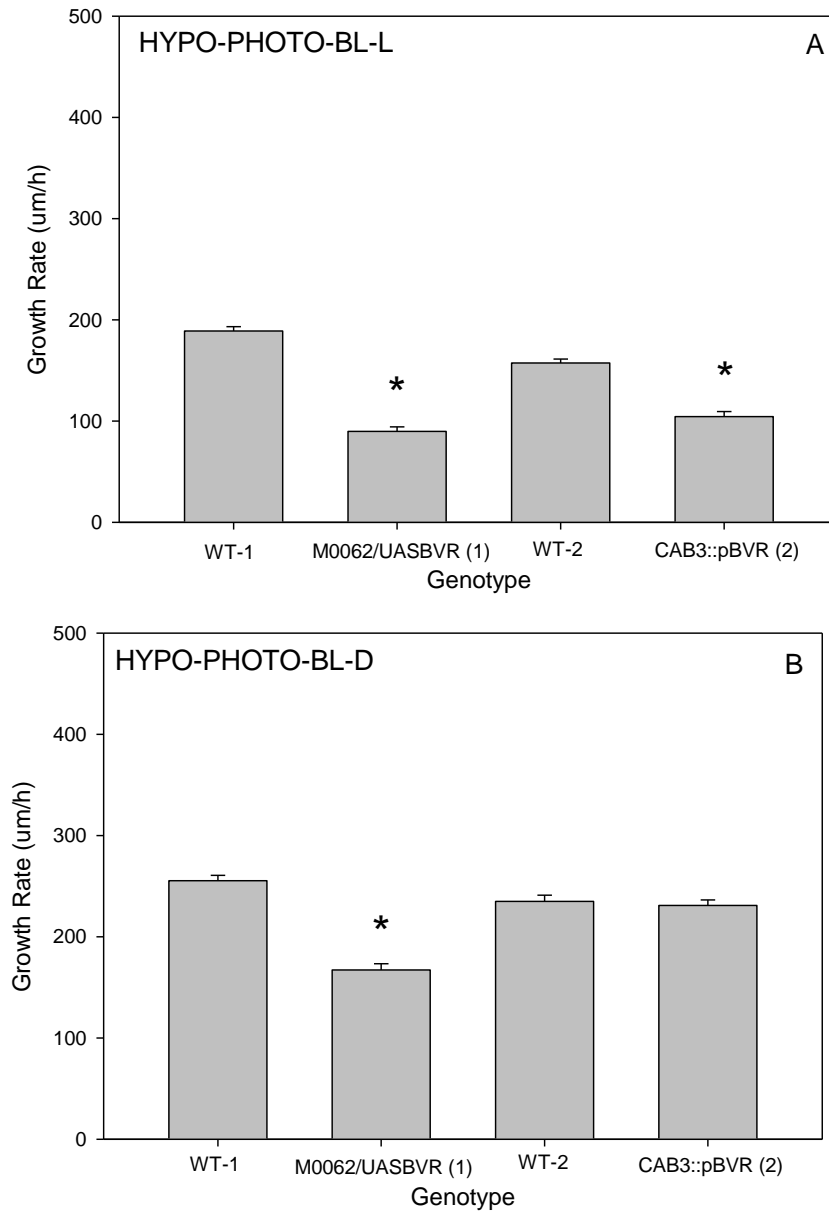


Figure 16: Growth rate of blue-light phototropism in light-grown (A) and dark-grown (B) hypocotyls of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). N= 82-98 plants; and error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) in the transgenic line compared to its WT are indicated by an (*).

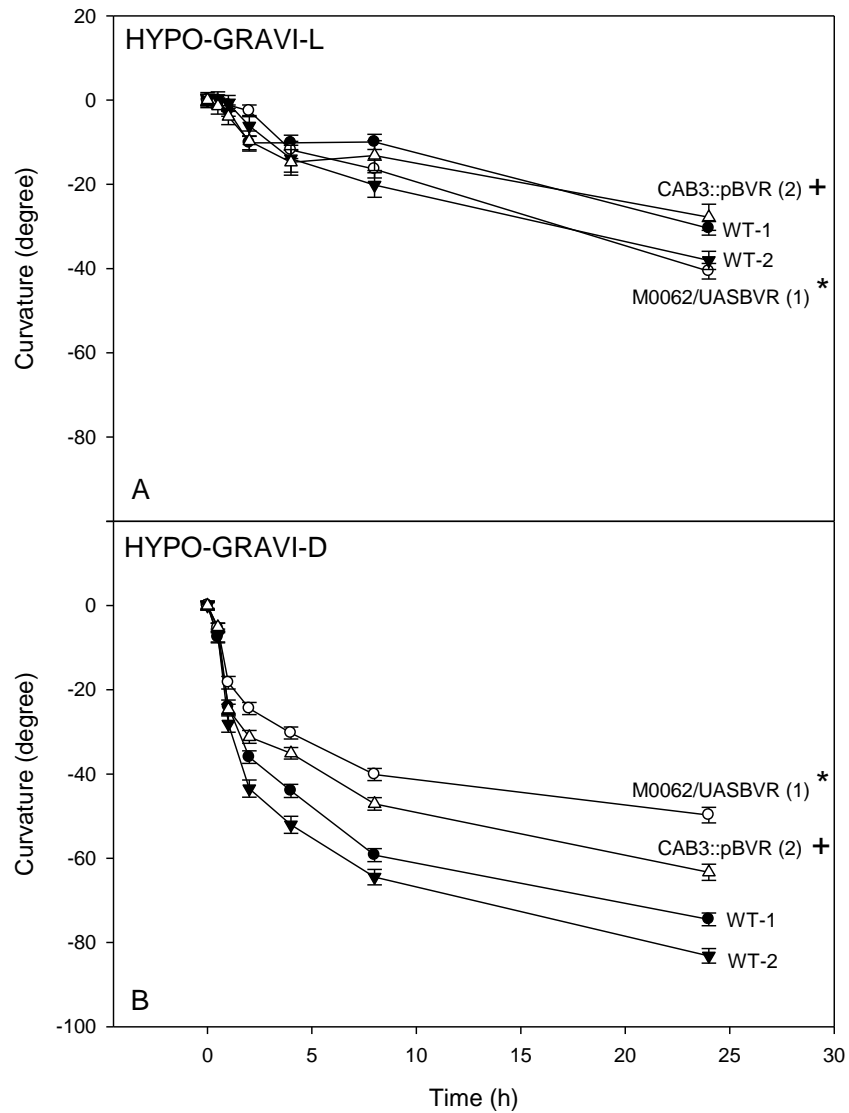


Figure 17: Time-course of curvature of gravitropism in light-grown (A) and dark-grown (B) hypocotyls of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). The mean curvature at each data point was calculated for 79-100 plants. Error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) through time in the M0062/UASBVR line compared to its WT are indicated by an (*) while significant differences in the CAB3::pBVR line are indicated by a (+).

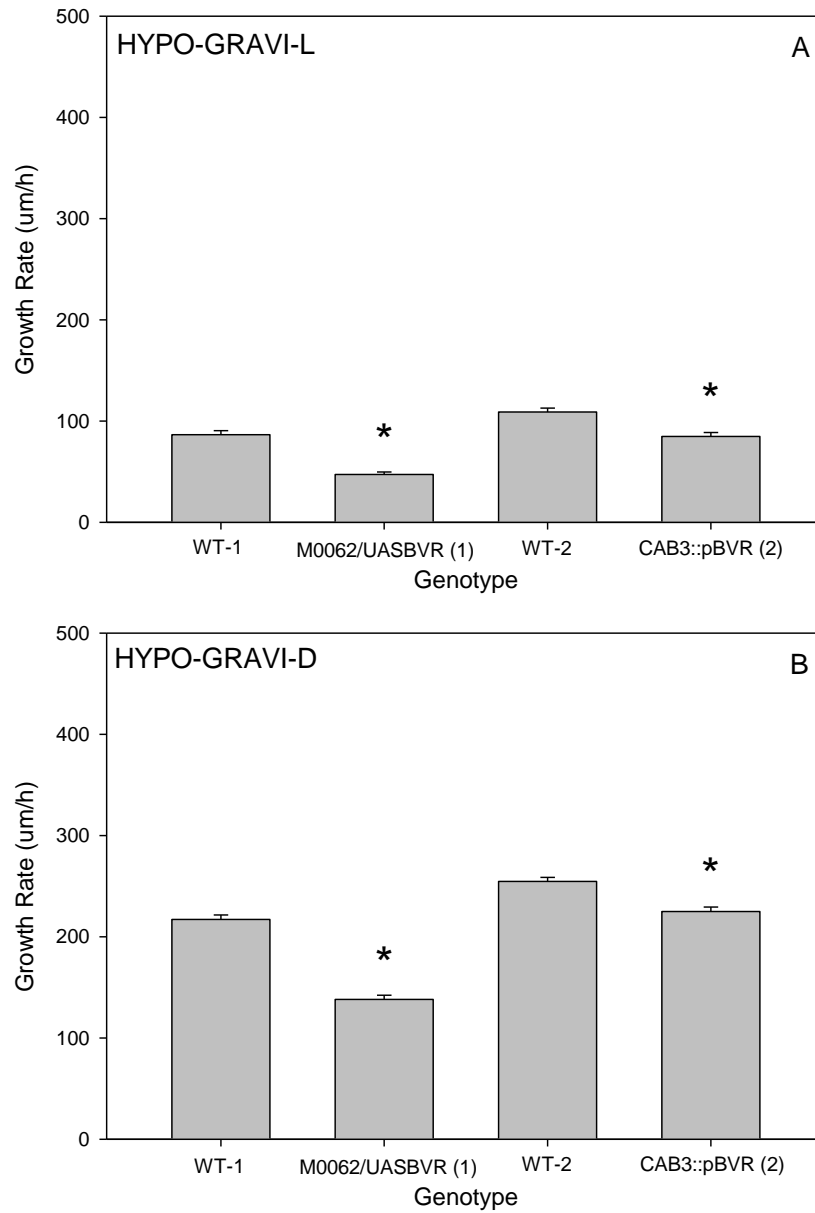


Figure 18: Growth rate of gravitropism in light-grown (A) and dark-grown (B) hypocotyls of 4 day-old seedlings of M0062/UASBVR, WT-1 (strain C24), CAB3::pBVR and WT-2 (strain No-O). N=84-101 plants; and error bars represent ± 1 S.E. Statistically significant differences ($p < 0.05$) in the transgenic line compared to its WT are indicated by an (*).

Table 1: Summary of the experiments of the effects on tropistic curvature of phytochrome deficiency in roots (M0062/UASBVR) and cotyledons (CAB3::pBVR) in light-grown and dark-grown seedlings. The lightly shaded region represents seedling root studies, while the darker shaded region represents hypocotyl studies. (+) indicates a promotion of curvature in the transgenic line compared to the WT; (++) indicates an even greater promotion; (-) indicates an inhibition; (--) indicates an even greater inhibition and (0) indicates no significant difference in curvature between the transgenic line and WT.

Treatment	M0062/UASBVR (Lacks PHY in roots)	CAB3::pBVR (Lacks PHY in cotyledons)
ROOT-PHOTO-BL-L	0	0
ROOT-PHOTO-BL-D	--	0
ROOT-PHOTO- ROTATO-BL	0	0
ROOT-PHOTO- ROTATO-RD	--	-
ROOT-GRAVI-L	0	0
ROOT-GRAVI-D	0	--
GRAV-ROOT- ROTATO	0	0
HYPO-PHOTO-BL-L	0	0
HYPO-PHOTO-BL-D	0	0
HYPO-GRAVI-L	0	++
HYPO-GRAVI-D	++	+

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Appendix

Stimuli	Ecotype	LG-HYPO	LG-ROOT	DG-HYPO	DG-ROOT
Gravitropism -Curvature	WT-1:M0062/UASBVR	F = 13.79 ; p = 0.0002	F = 21.53 ; p < .0001	F = 115.47 ; p < .0001	F = 11.40 ; p = 0.0008
	WT-2:CAB3::pBVR	F=7.32 ; p = 0.0069	F = 61.95 ; p < .0001	F = 74.49 ; p < .0001	F = 72.53 ; p < .0001
Blue Phototropism -Curvature	WT-1:M0062/UASBVR	F = 103.50; p < .0001	F = 1.84 ; p = 0.1750	F = 17.77 ; p < .0001	F = 4.20 ; p = 0.0408
	WT-2:CAB3::pBVR	F = 108.15 ; p < .0001	F = 0.16 ; p = 0.6918	F = 3.53 ; p = 0.0607	F = 2.23 ; p = 0.1354
Gravitropism -Growth Rate	WT-1:M0062/UASBVR	T = 10382.00; p < 0.001	T = 6345.000; p < 0.001	T = 5607.000; p < 0.001	t = 5.362; p < 0.001
	WT-2:CAB3::pBVR	T = 6622.000; p < 0.001	T = 4470.000; p < 0.001	T = 6989.000; p < 0.001	T = 8215.000; p = 0.057
Blue Phototropism -Growth Rate	WT-1:M0062/UASBVR	t = 16.121; p < 0.001	T = 6550.000; p = 0.047	t = 10.879; p < 0.001	t = 3.701; p < 0.001
	WT-2:CAB3::pBVR	T = 4949.000; p < 0.001	T = 4710.000; p < 0.001	T = 8433.000; p = 0.405	T = 5871.000; p < 0.001

Appendix 1: Statistical analysis of time-course of curvature and growth rate of blue-light phototropism and gravitropism in hypocotyls and roots of light-grown (LG) and dark-grown (DG) seedlings. The comparison is between the transgenic line and respective WT. For M0062/UASBVR, the WT-1 strain is C24. For CAB3::pBVR, the WT-2 strain is No-O. “T” represents a Mann-Whitney Rank Sum Test and “t” represents a Shapiro-Wilk Normality Test.

Stimuli	Ecotype	Regression Coefficient Comparison	Growth Rate – T-test
Gravitropism	WT-1:M0062/UASBVR	F = 0.61; p = 0.4375	t = -0.674; p = 0.509
	WT-2:CAB3::pBVR	F = 14.50; p = 0.0002	t = 0.834; p = 0.426
Red Phototropism	WT-1:M0062/UASBVR	F = 7.60; p = 0.0063	t = -1.825; p = 0.098
	WT-2:CAB3::pBVR	F = 22.69; p < .0001	T = 58.000; p = 0.022
Blue Phototropism	WT-1:M0062/UASBVR	F = 0.10; p = 0.7520	t = -3.669; p = 0.003
	WT-2:CAB3::pBVR	F = 0.00; p = 0.9997	T = 30.000; p = 0.351

Appendix 2: Statistical analysis of time-course of curvature and growth rate of studies with the feedback system of blue- and red-light phototropism and gravitropism in roots of light-grown seedlings. “T” represents a Mann-Whitney Rank Sum Test and “t” represents a Shapiro-Wilk Normality Test.

	BL-PHOTO-HYPO-L		BL-PHOTO-ROOT-L		GRAV-HYPO-L		GRAV-ROOT-L	
Ecotype	Growth	Tropism	Growth	Tropism	Growth	Tropism	Growth	Tropism
M0062 -----								
WT-1	52.46% v	40.09% v	0	0	45.34% v	33.5% v	12.33% ^	21.7% ^
CAB3 -----								
WT-2	33.71% v	31.10% v	38.45% v	5.39% ^	22.19% v	26.92% ^	59.63% v	36.34% v
	BL-PHOTO-HYPO-D		BL-PHOTO-ROOT-D		GRAV-HYPO-D		GRAV-ROOT-D	
Ecotype	Growth	Tropism	Growth	Tropism	Growth	Tropism	Growth	Tropism
M0062 -----								
WT-1	34.5% v	17.89% v	17.27% v	80427.3% v	36.39% v	33.22% ^	19.1% v	8.48% v
CAB3 -----								
WT-2	0	0	33.17% v	79.47% v	11.67% v	23.85% ^	6.11% v	49.46% v

Appendix 3: Percent comparisons of time-course of curvature and growth rate of blue-light phototropism and gravitropism in hypocotyls and roots of light-grown and dark-grown seedlings. (^) indicate an increase in curvature or growth, while (v) indicates a decrease in curvature or growth relative to the respective WT strain.

Stimuli	Ecotype	Growth	Tropism
GRAV-ROOT-ROTATO	M0062 -----		
	WT-1	23.70 % ^	10.83 % v
	CAB3 -----		
	WT-2	22.55 % v	23.68 % v
R-PHOTO-ROOT-ROTATO	M0062 -----		
	WT-1	40.69 % ^	87.38 %v
	CAB3 -----		
	WT-2	34.35 %v	45.53 %v
BL-PHOTO-ROOT-ROTATO	M0062 -----		
	WT-1	68.39 % ^	299.46 % ^
	CAB3 -----		
	WT-2	0	0

Appendix 4: Percent comparisons of time-course of curvature and growth rate of studies with the feedback system of blue- and red-light phototropism and gravitropism in roots of light-grown seedlings. (^) indicate an increase in curvature or growth, while (v) indicates a decrease in curvature or growth relative to the respective WT strain.

```

data blue;
input Time Plate Genotype Curv ;
datalines;

DATA

;
PROC REG Data=blue;
by genotype;
model curv=time;
run;

Proc glm data = blue;
class genotype;
model curv = time genotype time*genotype / solution;
contrast 'test equal slopes' time*genotype 1 -1,
time*genotype -1 1;
run;

proc means data=blue mean stderr skew kurt ; var curv; by
genotype ;
run;

```

Appendix 5: Procedure statement for SAS 9.2. Data sets were analyzed using SAS 9.2 to compare slopes of transgenic line to respective WT. A linear regression analysis was conducted using a PROC REG procedure to determine trends through time. Next, PROC GLM was used to determine any significant difference ($p < 0.05$) in the regression coefficients through time between the transgenic line and its WT.

Treatment	WT-1: M0062/UASBVR – Number of Plants per Strain	WT-1: M0062/UASBVR – Number of Total Observations	WT-2: CAB3::pBVR – Number of Plants per Strain	WT-2: CAB3::pBVR – Number of Total Observations
ROOT-PHOTO-BL-L	84	1176	88	1239
ROOT-PHOTO-BL-D	81	1134	78	1099
ROOT-PHOTO- ROTATO-BL	13	312	10	220
ROOT-PHOTO- ROTATO-RD	10	240	10	220
ROOT-GRAVI-L	82	1155	87	1218
ROOT-GRAVI-D	88	1239	78	1092
GRAV-ROOT- ROTATO	11	154	9	126
HYPO-PHOTO-BL-L	78	1099	85	1190
HYPO-PHOTO-BL-D	93	1309	90	1260
HYPO-GRAVI-L	84	1183	84	1183
HYPO-GRAVI-D	98	1371	92	1294

Appendix 6: Summary of the experiments of the number of plants and total observations used in the statistical analysis in comparing WT-1:M0062/UASBVR (deficient in roots) and WT-2:CAB3::pBVR (deficient in cotyledons) in light-grown and dark-grown seedlings. The lightly shaded region represents seedling root studies, while the darker shaded region represents hypocotyl studies.