

ROOT PHOTOTROPISM: HOW LIGHT AND GRAVITY INTERACT IN SHAPING PLANT FORM

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ABSTRACT

The interactions among tropisms can be critical in determining the final growth form of plants and plant organs. We have studied tropistic responses in roots as an example of these type of interactions. While gravitropism is the predominant tropistic response in roots, phototropism also plays a role in the oriented growth in this organ in flowering plants. In blue or white light, roots exhibit negative phototropism, but red light induces positive phototropism. In the flowering plant *Arabidopsis*, the photosensitive pigments phytochrome A (phyA) and phytochrome B (phyB) mediate this positive red-light-based photoresponse in roots since single mutants (and the double *phyAB* mutant) were severely impaired in this response. While blue-light-based negative phototropism is primarily mediated by the phototropin family of photoreceptors, the *phyA* and *phyAB* mutants (but not *phyB*) were inhibited in this response relative to the WT. The differences observed in phototropic responses were not due to growth limitations since the growth rates among all the mutants tested were not significantly different from that of the WT. Thus, our study shows that the blue-light and red-light systems interact in plants and that phytochrome plays a key role in integrating multiple environmental stimuli.

INTRODUCTION

Plant responses to environmental stimuli often involve some type of movement. Plants generally can exhibit two broad categories of movements: tropisms and nastic movements (Srivastava, 2002). A tropism is a directed growth response to a stimulus (e.g., phototropism in response to light, gravitropism in response to gravity) while nastic movements are in response to a more diffuse stimulus (e.g., circumnutation, an oscillatory movement).

Interactions among tropisms (and nastic responses) can be important in determining the final growth form of a plant. In roots, gravitropism has been well-characterized since gravity is the most critical signal for growth and development in this organ (Kiss, 2000; Boonsirichai *et al.*, 2002). However, recent research has shown that gravitropism interacts with other tropistic responses, including phototropism, thigmotropism and hydrotropism, in determining the final form of the entire root system (Hangarter, 1997; Correll and Kiss, 2002). Studies of phototropism in roots have been reviewed by Hubert and Funke (1937), but this topic was more recently explored by Okada and Shimura (1992), who isolated mutants in root phototropism which were later shown to be deficient in the blue-light receptor phototropin 1 (phot1; Briggs and

Christie, 2002). Roots are typically negatively phototropic in response to white and blue light and use the same photoreceptors that are involved in positive phototropism in stem-like organs (Sakai *et al.*, 2000).

In addition to blue-light-based negative phototropism in roots, there is a red-light-induced positive phototropism in primary roots of *Arabidopsis* (Ruppel *et al.*, 2001; Kiss *et al.*, 2003). The photoresponse to red light appears to be relatively weak compared to other root tropisms but is readily apparent in mutants that are impaired in gravisensing (e.g., starchless mutants; see Kiss, 2000). This red-light-induced positive phototropism also occurs in the lateral roots of *Arabidopsis* (Kiss *et al.*, 2002).

In this paper, we compare positive, red-light-induced phototropism and negative, blue-light-induced phototropism in *Arabidopsis* roots. Our results show that the red-light-absorbing phytochrome (phy) family of photoreceptors (Quail, 2002), specifically phyA and phyB, play a role in both types of phototropic responses in *Arabidopsis* roots.

MATERIALS AND METHODS

Plant material and culture conditions

In these experiments, we used wild-type *Arabidopsis thaliana* of the Landsberg erecta (Ler) or Wassilewskija (Ws) ecotype. The phytochrome mutants utilized were *phyA-201*, *phyB-1*, and *phyAB-1*; the characteristics of these strains (which are in the Ler background) are summarized in a recent paper by Hennig *et al.* (2002). The starchless mutant (in the Ws background) used in these studies is deficient in phosphoglucomutase (pgm) and is described in Ruppel *et al.* (2001).

Seeds were surface sterilized and grown in a medium of one-half-strength Murashige-Skoog salts with 1% (w/v) sucrose and 1 mM MES (pH 5.5) in 1.2% (w/v) agar according to Kiss *et al.* (2003). Seedlings were grown in white light of 70–90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and were used in experiments when the roots were approximately 1 cm in length.

Light sources and the computer-based feedback system used in phototropism experiments

In some phototropism experiments, blue and red light were obtained by passing light from fluorescent bulbs through plexiglass filters. The transmission maxima through the blue filter (Rohm and Haas No. 2424; Dayton Plastics, Columbus, OH) was 490 nm, and it was 630 nm for the red filter (Rohm and Haas No. 2423). Both filters provided a fluence rate of 12–14 $\mu\text{mol m}^{-2} \text{sec}^{-1}$. In phototropism experiments which involved the feedback system, a red light-emitting diode (LED) of 660 nm or a blue LED of 468 nm were used at 10–20 $\mu\text{mol m}^{-2} \text{sec}^{-1}$.

The seedling to be observed was first repositioned so that its root tip was at the center of the Petri dish.

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Following a 12–15 hr equilibration period, the dish containing the seedling was attached to a vertical stage in the dark, and growth was analyzed with a digital imaging system described by Mullen *et al.* (1998). Briefly, roots were imaged using infrared illumination (940 nm LED) and a CCD camera interfaced to a PC computer. In addition, a computer feedback system (as described by Mullen *et al.*, 2000 with modifications by Kiss *et al.*, 2003) was used to constrain the root tip angle to the vertical during either unilateral red or blue light stimulation.

Measurement of curvature and statistical analyses

In phototropism experiments, roots of light-grown seedlings that grew toward the light source were assigned positive angles, and roots that grew away from the light were assigned negative angles. Root curvature was defined as the change in angle from the starting point. Statistical significance was determined by using a one-way ANOVA test ($P < 0.05$), and if necessary, this was followed by Dunnett’s post-test ($P < 0.05$). Where the criteria of an ANOVA test were not met, an ANOVA on ranks ($P < 0.05$) followed by a Dunn’s method ($P < 0.05$) was used for multiple comparisons. Additional details on image capture and processing are provided in Kiss *et al.* (2003).

RESULTS

Experiments with *Arabidopsis* seedlings grown in constant unilateral illumination demonstrated that red and blue light had opposite effects on root orientation, as shown in Figure 1. Roots of starchless *pgm* mutant seedlings (which are impaired in gravitropism, see Kiss, 2000) grown in unilateral light clearly showed differences in orientation with roots growing towards red light, and in blue light, the roots grew away from the light source. Hypocotyls grew toward blue and were disoriented in red illumination as described by Hangarter (1997). Thus,

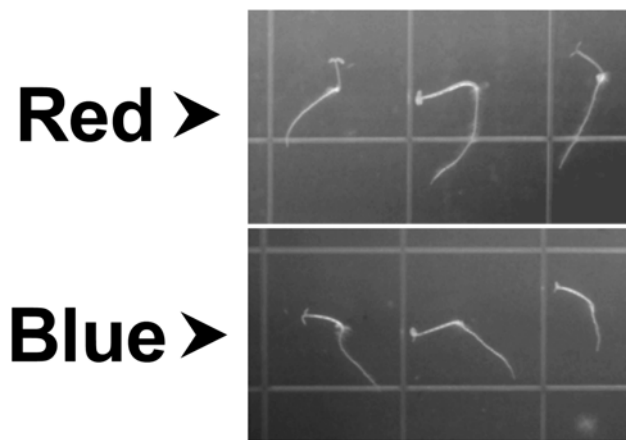


Figure 1. Photographs of starchless *pgm* seedlings grown with unilateral illumination. In red light, the roots grew toward the light source, and the hypocotyls were disoriented. In blue light, the roots grew away from the light source, and the hypocotyls grew toward the light. Seedlings were grown in vertically-held Petri dishes. The distance between the grid lines is 13 mm.

these observations suggest that roots exhibit negative phototropism in blue (as had been previously established; Okada and Shimura, 1992) and positive phototropism in red light.

Fluence rate response curves for both WT and *pgm* show that the phototropic response is readily apparent at $0.1 \mu\text{mol m}^{-2}\text{sec}^{-1}$ and saturates by $10 \mu\text{mol m}^{-2}\text{sec}^{-1}$, as illustrated in Figure 2. Positive phototropism is stronger in the *pgm* mutant compared to the WT, as previously reported (Ruppel *et al.*, 2001). The increased phototropic response in the *pgm* mutant was especially evident at higher fluence rates.

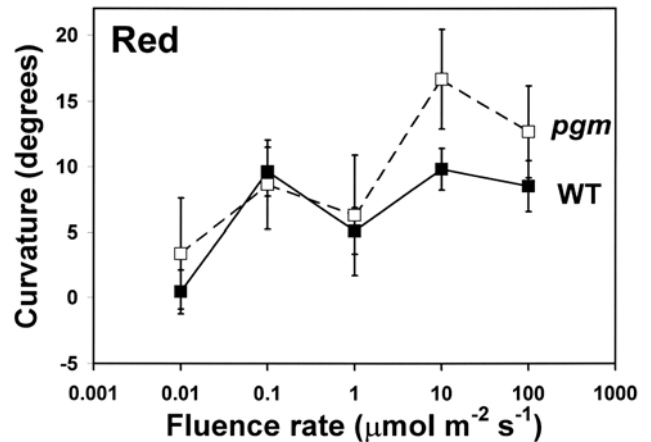


Figure 2. Fluence rate response of roots of WT and *pgm* seedlings to continuous unilateral red light. The measured curvature was the change in the orientation of the root tip 48 hr after the unilateral light was applied to the seedlings. (Results for a similar experiment with blue light is given in Sakai *et al.*, 2000.) Bars represent SE, and $n = 84$ to 172.

It is clear that the photoresponse in roots is weak relative to the graviresponse. Thus, in an attempt to separate root phototropism from other responses, we performed studies with a relatively new technique to assay tropistic responses. This involved using a feedback system to rotate a seedling so that its root tip is constrained to a particular angle. In our studies, we chose to constrain the root apex to 0° (vertical). This allowed us to study phototropism without the complications of a constantly changing gravitational stimulus (Kiss *et al.*, 2003). Under these conditions, roots exhibited a vigorous red-induced positive phototropism that is illustrated in Figure 3. Furthermore, the response obtained with the feedback system (Figure 3) was greater in magnitude compared to results obtained with unconstrained roots (Figure 2). After a latent period of 1–2 hr, the roots responded to the red light stimulus and achieved a constant rate of curvature for several hours, which was followed by a plateau phase (approx. 30°) in the response curve (Figure 3).

Most red light responses in plants are mediated by the photoreceptor pigment phytochrome (Quail, 2002). Thus, we wanted to determine if phytochrome is involved in this photoresponse, and if so, which of the five-member

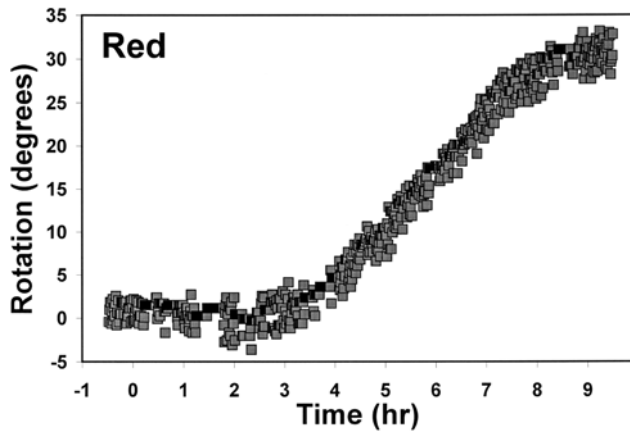


Figure 3. Kinetics of the positive phototropic response of a typical WT root in response to unilateral red illumination as measured with the feedback system. The plot shows the rotation of the stage necessary to keep the root tip constrained at 0° (vertical). Seedlings were kept in the dark, and unilateral illumination began at time 0. This experiment was repeated 25 times with similar results.

phytochrome gene family (phyA – E) participates in light perception. Root phototropism was studied with the feedback system in *Arabidopsis* mutants deficient in various phytochromes (Figure 4). Roots of *phyA*, *phyB*, and *phyAB* were inhibited in the photoresponse compared to the WT roots. In terms of statistical significance as determined by an ANOVA ($P = 0.002$) and Dunnett's post-test ($P < 0.05$), *phyA*, *phyB* and the double mutant exhibited significantly less curvature relative to the WT. In addition, there were no significant differences among the growth rates so that growth did not contribute to differences in the phototropic response among these strains (as shown in Kiss *et al.*, 2003). In this previous report, we also had demonstrated that roots of the *phyD* and *phyE* mutants exhibited a normal response to red light.

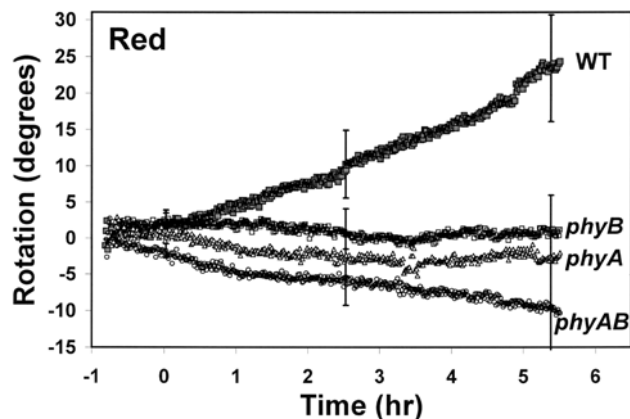


Figure 4. Mean response of WT and phy mutant roots ($n = 11 - 12$) to unilateral red illumination as measured with the feedback system. The responses of the *phyA*, *phyB*, and *phyAB* mutants were significantly less than that of the WT as determined by an ANOVA ($P = 0.002$) and Dunnett's post-test ($P < 0.05$). Bars represent SE.

Using the computer-based feedback apparatus, we studied the early kinetics of the red-light-induced positive phototropism and compared it to that of the blue-light-induced negative root phototropism, as illustrated in Figure 5. The latent period for the red-light response ranges from 1 – 2 hr while this value typically is 30 – 40 min for the blue-light response in *Arabidopsis* roots (see also Wolverton *et al.*, 2002; Kiss *et al.*, 2003). In addition, the magnitude of the response was greater for blue-light-based compared to red-light-based phototropism.

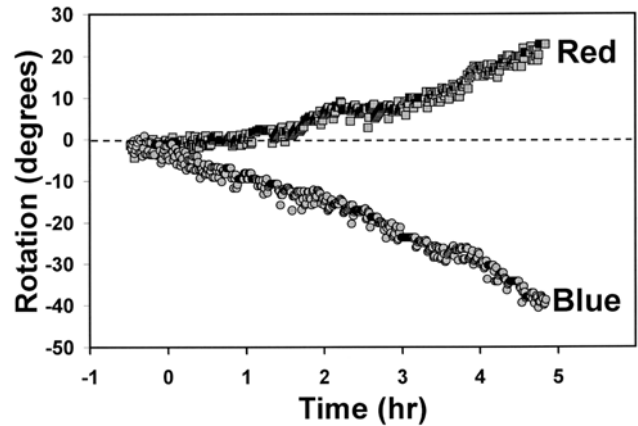


Figure 5. Early kinetics of the red-light-induced positive and blue-light-induced negative phototropic responses of typical WT roots (as measured with the feedback system). The plot shows the rotation of the stage necessary to keep the root tip constrained at 0° (vertical), which is indicated by the dashed line. This experiment was repeated at least 10 times with similar results.

Since root phototropism in red illumination was inhibited in phytochrome mutants, we also investigated the effects of phytochrome on blue-light-induced negative root phototropism. These studies were performed without the feedback system since the blue light response is more robust compared to the red light response. The experiments show that the blue-light negative phototropic response is reduced in *phyA*, and in a greater magnitude in *phyAB*, but this response is normal in the *phyB* mutant, as illustrated in Figure 6. In terms of statistical significance as determined by an ANOVA on Ranks ($P < 0.001$) and Dunn's post-test ($P < 0.05$), at the 12-hr point in the time course, *phyA* and *phyAB* (but not *phyB*) exhibited significantly less curvature relative to the WT. At the 24-hr point in the time course experiment, only the *phyAB* double mutant is still significantly different ($P < 0.05$) by the above criteria.

DISCUSSION

Characteristics of root phototropism

We have studied tropistic responses in roots as an example of how interactions among tropisms can be important in determining the final growth form of a plant organ. Phototropism as well as the overwhelming gravitropic reaction can play a role in the oriented growth

of roots in flowering plants. In blue or white light, roots exhibit negative phototropism (Okada and Shimura, 1992; Sakai *et al.*, 2000), but red light induces a positive phototropic response (Figure 1; see also Kiss *et al.*, 2003). In this paper, we demonstrate that the computer-based feedback system developed by Mullen *et al.* (2000) is useful to study root phototropism since the magnitude of the phototropic response is enhanced in both blue and red light (Figures 3, 5).

We found that the positive phototropic response could be observed at fluence rates from $0.1 \mu\text{mol m}^{-2}\text{sec}^{-1}$ to $100 \mu\text{mol m}^{-2}\text{sec}^{-1}$ (Figure 2). The response increased with increasing fluence rate up to about $10 \mu\text{mol m}^{-2}\text{sec}^{-1}$, with no reversal of curvature detected at any of the fluence rates tested. Except for the different direction, the fluence rate dependence for the positive phototropic response elicited by red light was similar to those reported for negative blue-light-induced phototropism in roots of *Arabidopsis* by Sakai *et al.* (2000), who found maximal curvature in the range of $10 - 100 \mu\text{mol m}^{-2}\text{sec}^{-1}$.

In terms of the relative strength of tropistic responses in roots, we propose that: gravitropism > negative phototropism (induced by blue light) > positive phototropism (induced by red light). In fact, the reason that root phototropism has been largely unstudied for decades is that the graviresponse in roots overwhelms the phototropic response (Hubert and Funke, 1937; Okada and Shimura, 1992). One indication of the relative strength of root tropisms can be determined by calculations of the latent period prior to response to the stimulus. These latent periods can be estimated by using the feedback system of Mullen *et al.* (2000). Thus, by these methods, the latent period for red-light phototropism is 1–2 hr (Figures 3, 5) while this value for blue-light phototropism is 30–40 min for *Arabidopsis* (Wolverton *et al.*, 2002). The value for the latent period for gravitropism in *Arabidopsis* roots is about 10 min (Mullen *et al.*, 2000). However, gravitropism clearly is the most robust tropism in roots as indicated by a number of parameters (Kiss, 2000). For example, gravitropic curvature as measured by the feedback system remains linear without reaching a distinct plateau phase (Mullen *et al.*, 2000) while both red- and blue-light based phototropism reach a plateau following a linear increase in response (Figure 3; see also Mullen *et al.*, 2002; Kiss *et al.*, 2003).

Role of phytochrome in root phototropism

Our results show that phytochrome is involved in both negative and positive phototropism in *Arabidopsis* roots. Firstly, *phyA* and *phyB* mediate the positive red-light-based photoresponse in roots since single mutants (and the double *phyAB* mutant) were severely impaired in this response (Figure 4). Secondly, in blue-light-based negative phototropism, *phyA* and *phyAB* (but not *phyB*) were inhibited in the response compared to the WT (Figure 6). In addition, in a recent study, we found that in root gravitropism, *phyB* and *phyAB* (but not *phyA*) were inhibited in the response compared to the WT (Correll

et al., 2003). Since both single and double mutants are impaired in red-light phototropism, these results suggest that red-light-based positive phototropism is directly regulated by phytochrome while phytochromes may have more of a modulation effect in gravitropism and blue-light-based phototropism. In all of these cases, the differences observed in tropistic responses were not due to growth limitations since the growth rates among all the mutants tested were not significantly different from that of the WT.

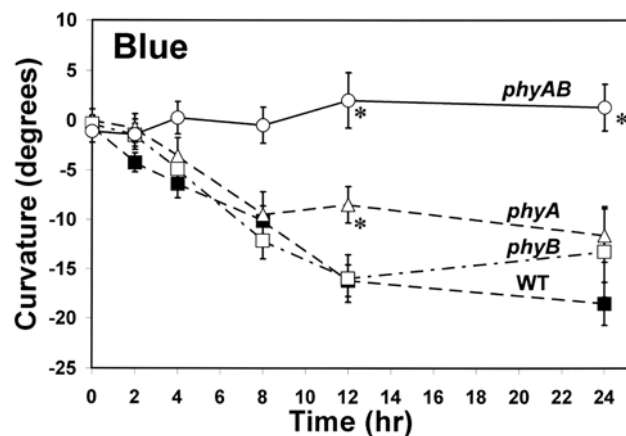


Figure 6. Time course of negative phototropic curvature (without the feedback system) of roots of WT and *phy* mutant seedlings illuminated with continuous unilateral blue light. Statistical differences ($P < 0.05$; relative to the WT) at the 12-hr and 24-hr time points are indicated by asterisks. Values represent the mean ($n = 57 - 107$), and bars represent SE.

Several studies have shown that phytochromes play a role in the modulation of the phototropic response in hypocotyls, and the results in this paper extend these results to root phototropism as well. As an example, Janoudi *et al.* (1997) demonstrated that *phyA* and *phyB* are required for the normal expression of hypocotyl phototropism and suggest a complex interaction between blue-light and red-light mediated processes in phototropism. Furthermore, Parks *et al.* (1996) have shown that *phyA* regulates red-light induction of phototropic enhancement in hypocotyls.

In addition to their direct role in the red-light-based positive phototropism and their role in modulating blue-light phototropism, *phyA* and *phyB* have been shown to have roles in gravitropism. For instance, light-induced reduction of negative gravitropism is controlled synergistically by these two members of the phytochrome family (Poppe *et al.*, 1996). Supporting these studies, other workers have proposed that the function of *phyA* and *phyB* in developing seedlings in the presence of light is to switch off negative gravitropism to allow for phototropic stimuli to determine the orientation of growth (Robson and Smith, 1996).

It is becoming increasingly clear that the blue-light and red-light signaling systems interact with each other, and also with the gravisensing system. In roots, phytochromes are involved in the regulation of all three systems.

However, a different combination of phytochromes modulates each of the responses. These differences could allow differential regulation of the signaling pathways and, thus, may be important in the integration of multiple environmental stimuli.

The use of microgravity to study root phototropism

In order to further study these complex interactions among tropistic responses, we plan to study phototropism, without the input of a gravistimulus, in microgravity conditions during a spaceflight experiment. We are in the development phase for an experiment on the International Space Station (ISS) which would use the European Modular Cultivation System (EMCS) in studies of root phototropism in *Arabidopsis*. The EMCS has an incubator, atmospheric control, a variety of LED's, and a high-resolution, movable video camera system that we need for this project (Brinckmann, 1999). The entire system is on a variable centrifuge palette so that an in-flight 1-g control can be performed and intermediate g-levels can be selected as well. A major advantage of EMCS is that this system is fairly sophisticated and automated (little crew time required) since there is limited availability of the crew during the current early utilization phase of the ISS. We presently are working with NASA to design unique experimental hardware to study root phototropism in EMCS. At the end of this project, these experiments should provide a more detailed characterization of root phototropism, provide insight into how red light enhances blue-light dependent phototropism, and provide a better understanding of how plants integrate sensory input from multiple light and gravity perception systems.

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