ABSTRACT

CHARACTERIZATION OF ROOT PHOTOTROPISM IN NICOTIANA SYLVESTRIS

by Sasha Yvonne Lucas

Phototropism has been extensively studied in the shoots of plants, however the roots have not been well characterized. Most research has focused on the white and blue light induced negative phototropic response; but recently, a red light induced positive phototropic response was identified in Arabidopsis. In order to determine if this red light induced positive response in roots is common to other species or is specific to Arabidopsis, I characterized root phototropism in Nicotiana sylvestris. The results indicate that Nicotiana does not have a robust phototropic response and is not a good model plant to study root phototropism. Also tested was the hypothesis that gravitropism impaired starchless mutants are a useful for studying root phototropism. However, the starchless mutant of Nicotiana (NS 458) does not appear to be a useful tool for measuring root phototropism as no difference in response between the wild type and the mutant was found.
CHARACTERIZATION OF ROOT PHOTOTROPISM IN
*NICOTIANA SYLVESTIS*

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Introduction

Tropisms

Plants have evolved specific mechanisms to respond to the various stimuli of their surrounding environment. The interaction of these stimuli contribute to the final growth form of a plant (Hangarter 1997). Although rooting in the ground limits plant movement, they still have the ability to direct their growth in response to stimuli. In particular, they respond to the environment through differential growth toward or away from environmental stimuli. These responses, known as tropisms, are most commonly initiated by gravity, light and touch. The directed growth in response to these stimuli are termed gravitropism, phototropism and thigmotropism, respectively. It is necessary for plants to interact with their surroundings in order to survive and adapt to different living conditions and, thus, tropisms play an important role in plant development. For gravitropism, the differential growth in response to gravity is typically in the direction opposite of the gravity vector in shoots (negative gravitropism), while in roots differential growth is usually in the same direction as the gravity vector (positive gravitropism). For phototropism, the differential growth in response to light, shoots grow towards the light source (positive phototropism) while roots grow away from the light
source (negative phototropism). Thigmotropism, the differential growth in response to a solid object, is a directed growth response to touch such as tendrils wrapping around an object.

Of these signals, gravity and light are most important in plant development along with other factors such as temperature and hydration (Tsutsumi et al. 2002, Correll and Kiss 2002). The responses to gravity and light are the strongest, often overpowering the other responses. Plants integrate each of these environmental signals to best adapt to their growing conditions. For roots, gravitropism is the strongest response, often masking other responses in plant roots (Vitha et al. 2000, Kiss et al. 2003). While in shoots, phototropism is the strongest response, often masking other responses (Galland 2002). When the balance of the responses is changed due to a change in stimulus, the responses change to match the stimulus in the optimal way for plant growth. Thus, it is the combination of many factors that result in the final plant form, not just the one with the strongest response (Hangarter 1997).

Phototropism

Phototropism is the tendency of plant organs to reorient or bend with respect to the direction of light. The bending, induced by light, is due to unequal cell growth on either side of the organ, causing the plant to curve towards or away from the stimulus. Most commonly, stems and stem like organs exhibit positive phototropism, meaning they bend towards the light source (Hubert and Funke 1937).
Typically, roots are most often negatively phototropic in blue and white light, meaning they bend away from the light source. Recently, it was found, however, that roots of Arabidopsis curved toward red light (Ruppel et al. 2001). This indicates that white and blue light induce a negative phototropic response, while red light induces a positive phototropic response.

Phototropism has been separated into three events: perception of the light signal, transduction of the signal, and differential growth (Correll and Kiss, 2002). Recent research has isolated many of the light receptors known to cause phototropic effects. Phototropism has been well studied in stems, but, root phototropism has not been studied as often (Ruppel et al. 2001, Mullen et al. 2002). Although roots are covered by soil much of the time, light can penetrate the soil so it is important to also study how roots respond to light (Tamura 1992).

The perception of the light signal is controlled by many different photoreceptors, often specific to the wavelength of the source light. The primary photoreceptors involved in phototropism are the blue light photoreceptors in the phototropin family (PHOT1, PHOT2; Briggs and Christie 2002). The receptors PHOT1 and PHOT2 appear to be exclusive to plants, and have overlapping functions by mediating phototropism, chloroplast migration and stomatal opening in Arabidopsis (Kagawa and Wada 2002, Kinoshita 2001). PHOT1 and PHOT2 both contain segments that are transformed by the presence of light, oxygen or voltage and are called LOV domains. In the presence of blue light, the LOV domains undergo a spectral
change, which results in an absorption peak measured with a UV-Vis spectrophotometer at 390 nm, whereas, if the protein is kept in the dark, absorption at 450 nm is measured. This indicates that the molecule undergoes a change in the presence of blue light, resulting in a response from the plant (Briggs and Christie, 2002). The response to blue light induced by PHOT1 and PHOT2 consists of rapid inhibition of stem growth, resulting in positive phototropism. Conversely, the roots respond to the excitation of PHOT1 and 2 by bending away from the light source, resulting in negative phototropism. Blue light also causes chloroplast movement; in low light to maximize light capture, and in high light to minimize light capture and reduce photo-damage from too much light exposure. The other blue light receptors, the cryptochromes, CRY 1 and 2 appear to play less of a role in phototropism, however it is believed CRY 1 acts with one of the red light receptors, phytochrome B, to induce the amplification of the blue light response when seedlings are pretreated with red or far-red light (Batschauer 1999). CRY 1 and 2 are localized in the nucleus. Concentrations of CRY 1 appear not to be affected by light stimulation while concentrations of CRY 2 decrease when the plant is treated with blue light (Lin 2000).

The phytochrome gene family PHY A through PHY E absorb light in the red and far-red wavelengths, which in turn affects the blue light receptors as well. Pretreatment with red or far-red light causes an increase in the blue light response, indicating that the phytochromes mediate phototropic responses in light grown plants (Liscum and Stowe-Evans 2000). Studies have shown that the PHYA
PHYB mutants of *Arabidopsis* have reduced curvature when exposed to low fluence rates of blue light, however, when high fluences are used, there is no difference between mutant and wild type (Liscum and Stowe-Evans 2000). Therefore the role phytocromes A and B play in modulating phototropism may be fluence dependent. Interestingly, phytocromes C-E appear to have no effect on phototropism and show no difference when exposed to either low or high fluence rates of blue light, therefore, none of them are thought to be the second receptor working with phyA and/or phyB (Sakai et al. 2000). Ruppel et al. (2001) described a red light induced positive phototropic response in *Arabidopsis* roots, *which* was thought to be mediated by the phytochrome red light receptors. PhyA and phyB have recently been identified as the primary photoreceptors in this red light response in roots (Kiss et al. 2003). While it has been proposed that *PHYA* and *PHYB* alone are responsible for the red-light-induced positive response, *phy B* along with *phy D* and *E* all act to control other light induced responses such as flowering time, leaf elongation and leaf development (Franklin et al. 2003).

Phototropic curvature can also be induced or enhanced by red/far-red light. This has been seen in lower plants such as the protonemata of the moss *Ceratodon*, in which red light results in a strong positive phototropic response and the fern *Adiantum*, which has both red and blue light induced responses (Lamparter et al. 1998, Kawai 2003). As stated above, a red-light-induced positive phototropic response has also been identified in *Arabidopsis* roots, suggesting that this response may also be present in some higher
plants (Kiss et al. 2001, Ruppel et al. 2001). Pre-treating plants with red/far-red light can also enhance the phototropic curvature in a blue light response (Liu et al. 1996). It is thought that there is an interaction between \textit{PHYB} and \textit{CRY1} to create this enhanced response (Gil et al. 2002). It, therefore, appears that wavelength and intensity are both important factors in whether or not plants exhibit a phototropic response (Sakai et al. 2000, Ruppel et al. 2001, Kiss et al. 2003)

\textit{Root Phototropism}
Root phototropism has been difficult to study as the roots of plants are usually surrounded by soil and because of the relatively strong gravitropic response in roots. In a survey of root phototropism in 152 plants, a negative phototropic response was found in 50% of the species (Hubert and Funke 1937). Many of the other species showed no response, although a few appeared to have a positive phototropic response. In this early study, the two species of tobacco that were surveyed, *Nicotiana affinis* and *Nicotiana tabacum*, demonstrated no response to light in their roots. With genetic and molecular advances and the use of mutants, new ways of studying the root phototropism system are emerging. Recently, blue-light-based phototropism has been characterized in *maize* roots; the sensing occurs in the root cap, while the response occurs in the zone of elongation (Mullen et al. 2002).

Mutants of Arabidopsis have been isolated which display a reduced gravitropic or phototropic response. These mutant strains have defects in all stages of the signal cascade of phototropism from perception to response (Correll and Kiss 2002). Use of mutants reduced in phototropism or gravitropism may help to separate the influence of one stimulus from another. In *Arabidopsis*, for example, there are mutants lacking the blue (*phot1*) and red/far-red (*PHYA-E*) photoreceptors. There are mutants in the signal transduction of auxin (*axr1*) and auxin response factor (*nph4*; Correll and Kiss 2002). If a mutant has a reduced response to light, the main influential factor is likely to be gravity, whereas if there is a reduced response to gravity the main influential factor is likely to be light. Another method to
separate influences between gravity and light is to perform experiments in microgravity, however, if this is not possible, gravity perceiving mutants have been shown to be good tools for measuring the light response (Ruppel et al. 2001). There appears to be a combination in roots of negative phototropism and positive gravitropism, orienting the root downwards most of the time (Mullen et al. 2002).

**Starchless Mutants**

Starchless mutants, which have a reduced gravitropic response, are useful in studying the response of phototropism (Ruppel et al. 2001, Kiss et al. 2002, Correll and Kiss 2002). The reduced gravity perception isolates the reaction of the roots to light, thus making it easier to study root phototropism. Starchless mutants of both *Arabidopsis* and *Nicotiana* have been shown to reduce gravitropic responses (Kiss et al. 1989, Kiss and Sack 1989, Kiss et al. 1996). The *pgm* (phosphoglucomutase) mutant of *Arabidopsis* has been shown to exhibit a novel red- light- dependent positive phototropic response (Ruppel et al. 2001). The phototropic response is very subtle and was easier to measure in the starchless mutant compared to the wild type plants. Because there is only one report of this positive gravitropism in roots, it is very important to retest and verify the response in a number of plant species.

In order to confirm that the red light induced positive response in *Arabidopsis* is not a species-specific phenomenon a starchless
mutant in *Nicotiana*, NS458 was used in this study to test for phototropic responses in roots to red, blue and white illumination. *Nicotiana sylvestris*, commonly known as tobacco, has often been used as a model plant for other physiology studies. The mutant NS458 has a point mutation in the phosphoglucomutase gene (Kiss and Sack 1989), which results in a starchless mutant comparable to the *pgm* mutant of *Arabidopsis* (Kiss et al. 1989). It has been shown to have reduced gravitropic activity in the shoots and roots while still being a viable mutant (Kiss and Sack 1989). Preliminary studies identified a positive induced red light response in NS458, thus it is a good model plant to use for these studies (Ruppel et al. 2001).

*The Present Studies on Root Phototropism in Nicotiana*

It has been demonstrated that phototropism in *Arabidopsis* is mediated by two sensory systems, one for blue light and one for red light. The blue light sensory system is controlled by the blue light receptors, the phototropins, and commonly results in a negative phototropic response in both blue and white light. This response has been well documented, and it was believed that most plants would respond to blue and white light in a similar manner. The red light sensory system is controlled by the red/far-red receptors, the phytochromes, and results in a positive phototropic response in red light (Ruppel et al. 2001, Kiss et al. 2003).

Roots of *Nicotiana*, while still exhibiting a phototropic response, have a different photo-response compared to the roots of
*Arabidopsis*. When roots are illuminated with blue light, rather than the expected negative response often seen in roots, a positive blue-light-induced response was observed. The red light response is similar in both *Arabidopsis* and *Nicotiana* roots, which results in a positive growth towards the red light source. White light results in a positive root growth towards the light source in *Nicotiana* while in *Arabidopsis* white light results in a negative phototropic in the response in the roots.

**Materials and Methods**

*Plant Material and Culture Conditions*

Experiments were performed using wild type (WT) and a starchless mutant of *Nicotiana sylvestris* (NS 458). NS 458 has a recessive mutation in a single nuclear gene, which makes it deficient in the activity of the enzyme phosphoglucomutase (Hanson and McHale 1988, Kiss and Sack 1989).

Seeds were surface sterilized in 30% (v/v) commercial bleach with 0.002% (v/v) Triton X-100 for 20 minutes, and then rinsed five times in sterile distilled water. Seeds were sown into onto a sheet of pre-sterilized cellophane that was placed on top of *Arabidopsis* growth medium (Kiss et al. 1996) with 1% (w/v) sucrose in 1.2% (w/v) agar in square (100 mm x 100 mm x 15 mm) petri dishes. Dishes were sealed with Parafilm and placed on edge so that the surface of the agar was vertical. Plates were incubated at 23 - 25°C in the light, with the wavelength and location of the light specific to each
experiment. Seedlings used in experiments were approximately 1 cm in length approximately 7 days after the seeds were sown and grown.

Light Sources

Fluence rates were measured with a Li-Cor LI-189 Quantum Radiometer Photometer equipped with a LI-190SA Quantum sensor. Experiments with white light had a fluence rate of 70 - 90 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) obtained from 34 W “cool-white” florescent lamps. Blue and red illumination were obtained by passing light from fluorescent bulbs through Plexiglas filters. The fluence rate through the blue filter (Rohm and Haas No. 2424 Dayton Plastics, Columbus, OH) was 12 - 14 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) with a transmission maximum of 490 nm, while the fluence rate through the red filter (Rohm and Haas No. 2423) was 12-14 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) with a transmission maximum of 630 nm.

Experiments were performed in continuous light applied to the vertically oriented Petri dishes. In orientation experiments, light sources were placed either directly above or directly below the Petri dishes for up to 9 days. Continuous white and red light was applied to seedlings in the orientation experiments, while in blue light experiments a 24 hour pre-treatment immediately after sowing with white light was applied to initiate germination. For time course and side illumination experiments, seedlings were grown for 7 days in continuous white light. In time course experiments, after seven days seedlings were reoriented 90° and the light wavelength was changed depending on the experiment; while the side illumination experiments were set up by rotating the light source 90° after seven days and
changing the wavelength depending on the wavelength being tested. The long-term unidirectional experiments were set up as in the orientation experiments, however photographs were taken over a series of days rather than for just one day. Dark controls were tested by reorienting seedlings that had been grown in white light from above for seven days $90^\circ$ and placing them in the dark to take measurements.

Measurement of Orientation and Curvature

In the orientation experiments with light from above or below, root angles were measured relative to the gravity vector, and the gravity vector was defined as $0^\circ$. In time course experiments, roots from vertically grown seedlings were reoriented $90^\circ$ after seven days, while the light source remained in the same place. Root curvature in the time course and dark control experiments was measured as the change from the starting point (i.e. $90^\circ$ from the gravity vector) with positive angles measured as curvature towards the gravity vector and negative angles measured as curvature in the opposite direction of the gravity vector. Seedlings for the side illumination experiments were grown vertically in white light for 7 days, after which the light source was moved $90^\circ$ to illuminate seedlings from the side. Orientation was measured with roots growing toward the light source defined as positive angles and those growing away from the light source defined as negative angles. It is possible that some of the conclusions of this study could be affected if I had used a relative scale for angle measurement.
Seedlings were photographed with a 35 mm camera equipped with a macro lens, using Kodak Technical Pan and Tmax-100 film (No. 2415) at ASA 50. Images were digitally captured using a Nikon supercool scanner 4000 equipped with the long strip adapter while angle measurements were made using Image-Pro Plus (version 4.0; Media Cybernetics, Silver Spring, MD, USA) on a PC computer. Seedlings were excluded from the study if their roots contacted neighboring plants. Each experiment was repeated at least three times with values reported as the mean ± standard error (SE).

Results

White, blue and red light quality

To test the overall response of roots to different wavelengths, continuous illumination with white, blue and red light was placed either directly above or below the Petri dishes. Seedlings were photographed after 7 days of growth under the light treatment, with the exception of blue light experiments in which an initial 24 hour period with white light was used to stimulate germination. All wavelengths tested produced a positive phototropic response, or growth towards the light source (Fig. 1). This resulted in larger root angles when light was positioned above the petri dishes and smaller root angles when light was positioned below the petri dishes (Fig. 1).

When white light was used, a positive phototropic response was observed in both the WT and NS 458 mutant (Fig 1A). This
positive response resulted in a higher average root angle in WT than in NS 458 when light was from above. However when light was below there was little difference between root angles of the WT and NS 458 seedlings (Fig 1A). The response in blue illumination was less than the response in white light (Fig 1B). By qualitative standards, there was little difference in root angle between light from above and light from below samples. Although there was a slight positive phototropism present in the WT, as the angles are higher when the light source was from above, there was no difference between NS 458 seedlings. The red light response was also positive, however this was much stronger than both the white and blue light responses. The response was slightly stronger in the starchless mutant NS 458 than in the WT plant (Fig 1C).

White, blue and red light time course of phototropic curvature

To measure the response of root phototropism combined with the gravitropic response, a time course analysis of curvature was done in white, blue and red light. Roots were grown vertically for seven days and then reoriented 90°, allowing for measurements to be taken combining the response to gravity and light. Simultaneously, a positive phototropic response will slow the gravitropic curvature when light is above and increase the curvature when light is below, while a negative phototropic response will increase the gravitropic curvature when light is above and slow the gravitropic curvature when light is below. As a control, seedlings were grown for seven days on vertical agar with light above to orient them and then reoriented 90° and
placed in the dark. This allowed the gravitropic curvature to be measured without the interfering effects of phototropism.

White light slowed gravitropic curvature when light was applied from above, while it increased curvature when light was from below (Fig 2A). This indicates a positive phototropic response; however, the dark control had increased curvature compared to both white light from above and below in the WT seedlings, which indicates that the phototropic response is weak, if present at all. While, in NS 458, the dark control was similar to the light below response, indicating that white light from above had slowed the curvature response (Fig 2B). Blue light affected gravitropic curvature similarly, by slowing the curvature when light was from above and increasing the curvature when light was below (Fig 3 A, B). The dark control, when compared to both the WT and NS 458, seedlings in blue light showed greater curvature than when light was below or above (Fig 3 A, B). Red light also caused the curvature to slow when light was above and increased when light was below in both the WT and NS 458 seedlings. Again, the dark control had more curvature than both the WT and NS 458 when light was below (Fig 4 A, B). The blue and red light responses, when compared to the dark control, indicate that there is little if any response in the roots to the light stimulation.

**Side Illumination Experiments**

In order to check strength of the phototropic curvature relative to gravitropic curvature, we measured root curvature in
response to unilateral illumination while seedlings were in vertically oriented Petri dishes. Seedlings were grown for seven days in continuous unidirectional white light from above, after which the light source was moved to the side, keeping the petri dish orientation the same. If there is a positive phototropic response, the average root angle will increase, while if there is a negative phototropic response the average root angle will decrease.

When white light was unidirectionally applied (from the side) to the seedlings, the average root angle increased over 48 hours in both the WT and NS 458 seedlings (Fig. 5A). There appeared to be no difference between the response in WT and NS 458 when plants were exposed to unidirectional illumination (Fig. 5A). When blue light was unidirectionally applied to the seedlings the average root angle increased over 48 hours in both WT and NS 458 seedlings (Fig. 5B). Again, there appeared to be no difference between the response of the WT and mutant seedlings (Fig. 5B). Red light also caused a positive response in both WT and NS 458 seedlings (Fig. 5C). In red light, maximum curvature occurred at 12 hours and then decreased slightly. In white and blue light, the root angles for the WT are noisy until 12 hours, at which point they increase at 24 hours and plateau at 48 hours. NS 458 in comparison has a smooth increase over the full 48 hours in white light and for 24 hours in blue light, after which the angle plateau.

*Long-term effects of unidirectional illumination*
To see if the curvature of the roots changed over a longer period of time, the orientation experiments were repeated with photographs being taken over 4-5 days rather than at just one time point (see Fig. 1). Plants were grown in white, blue or red light with unidirectional illumination from either above or below. Photographs were taken for extended time periods at the same time every day after germination, which usually occurred when the seedlings are 3-4 days old. Experiments performed in blue light were illuminated in white light for one day to promote seed germination.

When white light was applied without change over eight days, there was a change in the growth direction of the roots (Fig 6). When light was from either above or below, the WT roots exhibited positive phototropism until day five, after which they were oriented away from the light (Fig 6A). When light was positioned above the WT roots the growth began as positively phototropic, then switched to negatively phototropic. The roots illuminated from below had an increasingly negative phototropic response over the whole eight days (Fig. 6A). When NS 458 seedlings were used in these experiments (Fig 6B), the seedlings with light positioned above had an increase in root angle for the first five days, after which the angles switched to a decrease in root angle from days six to eight. NS 458 roots lit from below began with a high angle, which slowly decreased over the whole eight days.

Blue light caused a positive phototropic effect when applied from above the plants. The positive effect was the greatest magnitude at five days and slowly decreased for the rest of the experiment in the WT seedlings (Fig 7A). The same pattern appeared in NS 458
however the positive response was not as strong and the negative response at the end was greater (Fig. 7B). Both the WT and NS 458 responded the same when light was below, both seedling strains had a positive phototropic response that stayed did not change for the entire experiment.

Red light resulted in an initial positive phototropic response for both WT and NS 458 when light was above (Fig. 8). The WT seedlings had a large root angle for the first five days of growth which then decreased until the last day in which the light from above and below were the same (Fig. 8A). When the wild type seedlings were illuminated from below, the root angle stayed relatively constant, decreasing from the fifth to sixth day and then again slightly from day seven to eight (Fig. 8A). The NS 458 seedlings also have a positive phototropic effect in red light (Fig 8B). The average root angle increased between days four and five and stayed relatively level for the rest of the experiment. There was also little change in the seedlings illuminated from below, the root angle changed slightly however the average root angle was the same on day four as it was on day eight of growth (Fig. 8).
Figure 1: Orientation of *Nicotiana* roots grown in continuous white (A), blue (B) or red (C) light respectively positioned either directly above or below the plants. Positive phototropism was seen in all light colors. In white and blue light, there is a weak positive phototropic response, however it is stronger in WT than in NS 458. The positive phototropic response in red light is the strongest in the starchless mutant NS 458, although there is a strong response in the WT as well. In all graphs, 0° represents the gravity vector, or down while 180° represents up. Bar = SE (n = 111-230), WT = Wild Type NS 458 = gravitropic/starchless mutant.
Figure 2 (A-B): Time course of phototropic curvature of NS WT and NS 458 in white light. Seedlings are grown for 5-7 days on vertical agar with light positioned either above or below the petri dishes. The roots are then reoriented 90° and curvature is measured for 48 hours. When the light is above the roots, the curvature is decreased in both WT and NS 458 while illumination from below results in less curvature. The angle of curvature was higher with NS 458 (B), however there was a stronger difference between the light above and light below response in the WT (A). Bars = SE (n=43-118)
Figure 3 (A-B): Time course of phototropic curvature in blue light. Seedlings were grown for 5-7 days in petri dished oriented vertically. Plants were reoriented 90°, illuminated from either above or below and curvature was measured for 48 hours. The WT strain has increased curvature when light is below, and less curvature when light is above (A). The starchless mutant (NS 458, B) has increased curvature when light is below, and decreased curvature when light is above. The response is more apparent in the WT than in the starchless mutant NS 458. Bars = SE, (n=53-105)
**Figure 4 (A-B):** Time course of phototropic curvature in red light. Seedlings were grown for 5-7 days in petri dishes oriented vertically. Plants were reoriented 90°, illuminated from either above or below and curvature was measured for 48 hours. When red light is placed above, both WT (A) and NS 458 (B) have decreased curvature than when red light is placed below the seedlings. For dark control, seedlings were grown in light but then rotated 90° and kept in the dark for 48 hours. Bars = SE (n= 65-101)
A. Red

Curvature (degrees) vs Time (hours)

WT Above 🟢 WT Below 🟡 Control 🔴

B. Red

Curvature (degrees) vs Time (hours)

458 Above 🟢 458 Below 🟡 Control 🔴
Figure 5: Side Illumination phototropic curvature of roots in white (A), blue (B) or white (C) light. Seedlings are grown for 5-7 days on petri dishes oriented vertically. The light source is then moved 90° so that the seedlings are illuminated from the side and curvature is measured over 48 hours. 0° is the vector of gravity, or down while towards the light source is a positive angle and away from the light source is a negative angle. In all wavelengths of light the curvature is positive for both WT and NS 458. WT and NS 458 responded similarly to all wavelengths of light. The amount in curvature was strongest in white and blue light respectively, while there was little difference in curvature in red light. Bars = SE (n = 138-176 in white light, 35-138 in blue light and 138-236 in red light)
Figure 6 (A-B): Developmental experiments in white light. Seedlings were grown on vertical petri dishes for up to 8 days with measurements taken daily beginning with 4 days. Light was oriented either above or below petri dishes. When light is above, WT root orientation increases and then decreases over four days. When light is below, the orientation increases continuously over four days. When NS 458 is illuminated from above, the response increases and then decreases over the four days. When illuminated from below, the curvature decreases continuously over the four days. Bars = SE (n = 50-116)
**Figure 7 (A-B):** Developmental Experiments with blue illumination, seedlings are grown on vertical petri dished lit from above or below in continuous blue light for up to eight days with measurements daily. When both WT and NS 458 are illuminated from above, the curvature increases and then decreases over the four days. When the light source is below the seedlings, there is little change in the orientation over time, however the WT curvature increases slightly while the NS 458 decreases slightly. Bars = SE (n= 32-115)
Figure 8 (A-B): Red developmental experiments phototropic curvature was measured over four days when the light source is either above or below. The seedlings are grown on petri dishes oriented vertically in continuous red light with measurements being taken daily. When the light source is above, there is an increase and then a decrease in the curvature of the roots of both WT and NS 458. When the light source is below the seedlings, there is relatively no change in curvature. Bars = SE (n= 48-188)
A. Red

![Graph showing orientation changes over time for WT Above and WT Below.](image)

B. Red

![Graph showing orientation changes over time for 458 Above and 458 Below.](image)
Discussion

Root Phototropism in Nicotiana

These studies tested two different hypotheses. First, is there a red light based positive phototropic response in Nicotiana roots? Second, is there a difference in response between the wild type Nicotiana and the starchless pgm mutant? A red-light-induced positive phototropic response in Arabidopsis roots was first described by Ruppel et al. (2001), and once found, other species were tested for this positive response in roots. In preliminary studies (Kiss et al. 2001), Nicotiana was one of the seedlings tested that also had a positive red light phototropic response. A positive phototropic red light response was seen in all of these experiments except for the side illumination experiment (Table 1). However, in the side illumination experiment roots did not exhibit a phototropic response for any quality of light studied.

A negative response to white and blue light, (Mullen et al. 2002), which has been previously studied was also expected in Nicotiana. There was no negative response seen in either white or blue light. However, there was a positive phototropic response in our time course experiments and our development experiments along with a slight positive response in the orientation experiments. In addition, there was no phototropism detected in blue and white light in the orientation and side illumination experiments. Combined, these results indicate that there is a red light induced positive phototropic response in Nicotiana, however, in blue and white light the responses
can vary depending on the assay. When compared to *Arabidopsis*,
the phototropic response of *Nicotiana* is not as robust, and is
measured as a positive response to all wavelengths of light rather
than the negative response to white and blue light and the positive
response to red light seen in *Arabidopsis* (Ruppel et al. 2001)

Ruppel et al. (2001) demonstrated that starchless
mutants, deficient in gravity perception, are helpful in measuring the
phototropic response. According to the starch statolith hypothesis,
amyloplasts in the cytoplasm of the plant cells fall to the bottom of a
specialized cell (termed stratocytes) with the pull of gravity, and
thereby play a role in sensing the gravity vector for the cell. Without
these amyloplasts, the cell cannot effectively determine the direction
of the pull of gravity and therefore have reduces gravity perception
(Caspar and Pickard 1989, Kiss and Sack 1991). In the study by
Ruppel et al. (2001), the starchless mutants of *Arabidopsis* (*pgm*)
demonstrated a stronger phototropic response in the roots than did
the wild type. In this present study, the starchless mutant seedlings
exhibit a greater phototropic response, but only in a few of the
experimental assays, specifically the blue time course and blue and
red developmental experiments (Table 2). In experiments performed
in white light, there was no difference between the starchless mutant
and the wild type seedlings of *Nicotiana*. In blue light, there was no
difference in the orientation and side illumination experiments, but in
the time course experiment, the mutant responds more relative to the
WT, and in the development experiment the mutant responds less
relative to the WT. In red light, there is no difference except in the
development experiment, where the mutant responds more. Overall,
these results demonstrate that starchless mutants are not as good
tool for studying root phototropism in *Nicotiana* as they are for
Arabidopsis (Ruppel et al. 2001).
Table 1. This table presents a summary of root phototropism in experiments in all light wavelengths tested. There was no phototropism detected for the orientation experiments in white and blue light, however red light resulted in positive phototropism. The time course experiments produced a positive response in all wavelengths used. Side illumination experiments on the other hand resulted in no phototropism detected in all color qualities used. Lastly, the development experiments resulted in a negative response, which changed to a positive response for white light, and a positive response for the whole experiment in blue and white light.

<table>
<thead>
<tr>
<th>Phototropism Assay</th>
<th>White</th>
<th>Blue</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orientation</td>
<td>O</td>
<td>O</td>
<td>+</td>
</tr>
<tr>
<td>Time Course</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Side Illumination</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Development</td>
<td>O / +</td>
<td>+ / +</td>
<td>+ / +</td>
</tr>
</tbody>
</table>

O = No Phototropism detected  
- = Negative Phototropism  
+ = Positive Phototropism
Table 2. This table summarizes the differences in root phototropism between the wild type strain and the gravitropism-impaired \textit{pgm} (phosphoglucomutase) mutant in various assays. There was no difference between the WT and the mutant for all experiments in white light. In blue light however, there was no difference in the orientation and side illumination experiments, but there was a difference in response in the time course experiment in which the mutant responds more and the development experiment in which the mutant responds less. In red light, there is no difference between the WT and the mutant in all experiments except the development experiment where the mutant responds more.

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>White</th>
<th>Blue</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phototropism Assay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orientation</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Time Course</td>
<td>No</td>
<td>Yes, Mutant responds more</td>
<td>No</td>
</tr>
<tr>
<td>Side Illumination</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Development</td>
<td>No</td>
<td>Yes, Mutant responds less</td>
<td>Yes/No, Mutant responds more when light is above</td>
</tr>
</tbody>
</table>
Negative root phototropism

According to an early study, root phototropism appeared to be negative in 50% of the plants studied (Hubert and Funke 1937). Upon further study, it appeared that there was a negative response well documented in plant studies, specifically to white and blue light. Okada and Shimura (1992) isolated mutants deficient in root phototropism (rpt-root phototropism). These mutants helped to isolate the effects of light and gravity on the plant root. The interaction of light and gravity on Arabidopsis roots was studied using starchless mutants (Vitha et al. 2000, Ruppel et al. 2001). It was shown that the balance between gravitropism and phototropism changes depending on the position of light and the length of treatment when measuring for gravitropism, but that starchless mutants with reduced gravitropism were a good tool for studying phototropism in these studies (Vitha et al. 2000).

Negative root phototropism is controlled by two different blue light photoreceptors with separate signaling pathways downstream and which are specific to certain fluence rates (Sakai et al. 2000). These two photoreceptors are similar to those in shoots and are now called PHOT1 and PHOT2, and have been shown to be the mediators of the blue light response, as well as other redundant responses such as blue light induced stomatal opening and chloroplast migration (Briggs and Christie 2002). As no negative response was measured, this might indicate that the ratio of the gravitropic response and the phototropic response is greater in
Nicotiana than it is in Arabidopsis. Meaning, gravitropism plays more of a role in orienting the roots of Nicotiana than in Arabidopsis.

Positive root phototropism

A red-light-induced positive root phototropic response was recently discovered in Arabidopsis (Ruppel et al. 2001, Kiss et al. 2003). The response was weak and only apparent in the pgm (starchless) mutant, not the wild type. The red light positive response is also weak compared to the more robust blue light negative response. It has been proposed that the positive induced red light response might be more functional in orienting lateral roots rather than the primary roots (Ruppel et al. 2001, Kiss et al. 2002). In the research presented here, a strong positive red light response was measured in both the WT and mutant roots of Nicotiana. This response was strongest when there was a long treatment of continuous light (orientation and development experiments), which indicates that the response to red light may be slower in Nicotiana than in Arabidopsis.

An alternative interpretation for the orientation of the roots from seedlings grown in continuous red illumination as seen in Figure 1C (suggesting a positive phototropic response) is that continuous red light elicits some developmental response (related to photomorphogenesis) that is of the “high irradiance” type (Furuya and Shäfer 1996). This could possibly be a high irradiance effect on differential elongation rates or some other phytocrome-regulated developmental factor. Although we do not know the exact nature of
such a high irradiance response, continuous red illumination seems
to have a profound affect on the orientation and development of
seedlings of *Nicotiana sylvestris*, as has been reported for other
species.

In *Arabidopsis*, phytochromes (the red light receptors) are
thought to mediate the positive induced red light phototropic
response. The phytochrome family in *Arabidopsis* consists of five
members, *Phy* A-E. Recently, the red light induced positive response
was shown to be mediated by *phyA* and *phyB* alone (Kiss et al.
2003). *PhyD* and *E* appear to have no affect on the positive
response. In terms of other parameters, phytochromes B, D, and E
have all been shown to demonstrate redundancy in the functions of
flowering time, leaf development and regulation of specific genes,
indicating that they have separate functions in plant regulation and
growth (Franklin et al. 2003).

Since it is difficult to study root phototropism, researchers
have developed a feedback system in order to better characterize this
and other tropisms (Mullen et al. 2000). Using this feedback system,
which rotates a stage while keeping the root tip oriented at 0°, it was
possible to measure a response stronger than with unconstrained
*Arabidopsis* roots. When on the stage, single mutants for *phyD* and *E*
did not curve when illuminated with red light, while mutants for *phyA*
and *B* did. Using this feedback system, human error and the need to
move the seedlings over the time of the experiment is eliminated
allowing for a better isolation of the tropic response alone. Studies
using this feedback system would be beneficial to elucidate the red
light response in *Nicotiana*. 

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Mechanisms of phototropism in *Nicotiana* roots and stems

Shoot phototropism in *Nicotiana* has been studied and compared to *Arabidopsis* previously (Janoudi et al. 1992). The time threshold response to red light in *Nicotiana* and *Arabidopsis* was measured and found to be very different between the two species. The threshold for dark grown *Arabidopsis* seedlings was 15 minutes, while the threshold for *Nicotiana* seedlings was 60 minutes. Both of these time thresholds were decreased with a pretreatment of either 450 or 669 nanometer wavelengths of light. Therefore, the response in *Nicotiana* is slower than the response in *Arabidopsis*.

To further investigate mechanisms of red-light-based responses in plants, a number of investigations have measured phytochrome levels “in vivo” with GFP constructs. For instance, transport of phytochrome -A bound to GFP was compared using transgenic *Nicotiana* and Arabidopsis; the response was found to be different between the two (Kim et al. 2000). In *Arabidopsis*, VLFR (very low fluence response) light pulses and continuous HIR (high irradiance response) induced nuclear import of the phy-A:GFP was observed. However, in *Nicotiana* nuclear import of the phyA:GFP compound was induced only in continuous HIR light, but not VLFR light pulses. The authors suggest that because the plants responded differently to the cellular signals, the nuclear import of phyA maybe controlled by different cystolic factors in the two species (Kim et al. 2000).
Nuclear import of \textit{phyB} was also studied in \textit{Nicotiana}. Red, far-red and blue light was tested in pulses and continuously to determine what causes the import of a \textit{phyB}: GFP compound in \textit{Nicotiana} (Gil et al. 2000). The nuclear import is caused only by continuous light illumination, not pulses of red, far-red or blue light. Continuous far-red light did not cause nuclear import either, however continuous red light and to a lesser extent blue light does cause nuclear import of blue light. This indicates that the light induced partitioning of \textit{phyB} exhibits similar characteristics to the amplification of the blue light response with pretreatment of red or far-red light which is thought to be mediated by the photoreceptors \textit{phyB} and CRY 1.

\textit{Comparison of Phototropism in Nicotiana and Arabidopsis}

The phototropic response in the roots of \textit{Nicotiana} is much different than the response seen in the roots of \textit{Arabidopsis}. There is a vigorous phototropic response in \textit{Arabidopsis} while in \textit{Nicotiana} little response was seen. In \textit{Arabidopsis}, there is a stronger white and blue light induced negative response, however in \textit{Nicotiana}, the red-light-based response was the strongest (Fig. 1). The side illumination experiments resulted in no response from the roots regardless of the light quality used.

The blue-light-induced negative phototropic response in plant roots has been previously studied and documented in \textit{Arabidopsis} (Okada and Shimura 1992, Sakai et al. 2000). The data collected here does not support the presence of blue light induced
negative phototropism in *Nicotiana*. In the experiments when there was a response to blue light, the response was positive such as in the time course experiments and the development experiments (Fig. 1). There was no phototropic response to blue light in the light quality and side illumination experiments.

One of the main goals of this study was to see if there is a positive induced red light response in other species besides *Arabidopsis*. The response already identified in *Arabidopsis* could be a species-specific phenomenon and needed to be confirmed in another species (Ruppel et al. 2001). Some of the assays tested showed a response, however some did not. The light quality, time course and development experiments all demonstrated the presence of a red light induced positive red light response while the side illumination experiment did not (Fig. 1). Because the red light induced positive response was not seen in all of the assays tested, there could be a positive phototropic response in roots. However, further study is needed to resolve this issue.

*Studies with Starchless mutants*

Several studies have shown that starchless mutants impaired in gravitropism are useful in measuring root phototropism (Vitha et al. 2000, Ruppel et al. 2001, Kiss et al. 2003). These studies all use a gravitropism-impaired starchless mutant to measure the phototropic response. If the gravity response is reduced as in these mutants (reviewed in Kiss 2000), the phototropic response should be more apparent and easier to measure. The interactions of
gravitropism and phototropism and their effects on plant roots was found to be more complex than previously thought, however, starchless mutants impaired in gravity sensing can be good tools for studying phototropism (Vitha et al 2000). It was also shown that the positive induced red light response is only measurable in the starchless mutant, while the responses in white and blue light were also shown to be stronger in the starchless mutant (Ruppel et al. 2001). The starchless mutant was again used to study root phototropism in *Arabidopsis* to measure the red light response in both starchless mutants and phytochrome deficient mutants (Kiss et al. 2003).

It is well documented in *Arabidopsis* that gravity impaired starchless mutants are a good tool to isolate and measure the root phototropic response. The results from this study however, indicate that the starchless mutant of *Nicotiana* is not as useful in studies of root phototropism (Table 2). There was little difference between the starchless mutant and the wild type strains in most of the assays used. One major difference appeared in the blue light time course assay, in which case the mutant seedlings responded more than the wild type seedlings, however in the blue light development assay, the mutant seedlings responded less than the wild type seedlings. The only other major difference was that in the red light development assay, in which the mutant responds more when the light is above, but there is no difference when the light is below. Thus, the lack of differences between the starchless and wild type mutant indicates that starchless mutants are not a good tool for measuring phototropism in *Nicotiana* roots.
Future studies

In order to better determine root phototropism in *Nicotiana*, we plan to use a computer feedback system previously used to isolate the red light response in roots (Mullen et al. 2000, Kiss et al. 2003). Rather than eliminating the gravity response, the feedback system keeps the gravity vector constant in one direction so that the phototropic response can be more readily resolved. The feedback system was first used to measure the gravity response in *Arabidopsis* roots (Mullen et al. 2000). It has also been utilized to measure root phototropism in which the phytochrome that mediated the red light induced positive response was elucidated (Kiss et al. 2003). The feedback system also eliminates handling and manipulation of the seedlings after the experiment begins, which could remove a portion of the error in the experiment. Thus, it appears that the feedback system would be a good tool to measure root phototropism.

There could also be other tropic responses that are acting on the roots of *Nicotiana*. Plant growth is a balance of all the environmental factors that the plant processes and reacts to; there could be another environmental factor that is overpowering the phototropic response (Hangarter 1997, Correll and Kiss 2002). It would be useful to verify there is no hydrotropic or other tropic response acting to mask the phototropic response. For instance, a no hydrotropic response mutant of *Arabidopsis* has been isolated and
would be a useful comparison against the *Arabidopsis* phototropic response (Eapen et al. 2002).

Microscopy and molecular studies could be performed to compare the phototropic response on the cellular and molecular level. Do the amyloplasts act the same during phototropism in *Nicotiana* as in *Arabidopsis*? It has already been shown that *Nicotiana* and *Arabidopsis* regulate phyA differently, are the blue light receptors also regulated differently, and, if they are, what kind of environmental factors could attribute to these differences? Thus, in future studies, it is important to understand how species other than *Arabidopsis* regulate and control phototropism.
References


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