

# Green light signaling and adaptive response

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To a plant, the sun's light is not exclusively energy for photosynthesis, it also provides a package of data about time and prevailing conditions. The plant's surroundings may dampen or filter solar energies, altering spectral profiles of their light environment. Plants use this information to adjust form and physiology, tailoring gene expression to best match ambient conditions. Extensive literature exists on how blue, red and far-red light contribute to plant adaptive responses. A growing body of work identifies discrete effects of green light (500–565 nm) that also shape plant biology. Green light responses are known to be either mediated through, or independent of, the cryptochrome blue light receptors. Responses to green light share a general tendency to oppose blue- or red-light-induced responses, including stem growth rate inhibition, anthocyanin accumulation or chloroplast gene expression. Recent evidence demonstrates a role for green light in sensing a shaded environment, independent from far-red shade responses.

Plants frequently experience changes in their surrounding light environment. In fact, changes in light quantity, quality and duration are more the rule than the exception in nature. Alterations as transient as the shade of cloud cover, or as long-term as adjacent plant encroachment or changing seasons, require plants to adopt adaptive strategies to optimize light capture. Acclimation to the light environment requires continual adjustment of gene expression, physiology and form to best dovetail with natural conditions. It is therefore not surprising that plants utilize information from discrete sections of the light spectrum to guide these adaptive responses.

A set of sensitive light receptors monitors incident energies from various parts of the light spectrum. Detailed reviews have been recently published.<sup>1–3</sup> The phytochromes(phy) are red and far-red light-sensing pigments that exist in two interconvertible forms. In general, red light activation of phytochromes may be reversed by far-red light. An equilibrium is established between red and far-red absorbing forms that dictates downstream gene expression patterns and ultimately physiology. Phytochromes play a central role in adaptation to the light environment, sensing of shade, flowering and many other plant processes. The cryptochrome(cry) blue light receptors mediate responses including control of plant stature, anthocyanin accumulation and flowering.<sup>3</sup>

Recent studies have shown that certain cry-mediated responses are reversible by green light.<sup>4,5</sup> The phototropin receptors contribute to blue light responses such as leaf expansion, phototropism and chloroplast accumulation and avoidance. Three other LOV domain receptors mediate responses to blue and UV-A light, with specific contributions to conditioning circadian oscillator rate,<sup>6</sup> and photoperiodic flowering.<sup>7,8</sup> Together, the combined inputs from the light sensor collection permit a plant cell to interpret characteristics of the light environment, guiding precise acclimation to a particular set of conditions.

Several studies over the past 50 years have described specific effects on plant form, function or content that appear to be related to illumination with green (520–550 nm) wavebands. Comprehensive review of the work before 2000 is presented elsewhere.<sup>9,10</sup> Recent studies further demonstrate the role of green light in plant growth and development. Green-light exposure reversibly decreases stomatal conductance in lettuce,<sup>11</sup> while the combination of green light with blue and red light improves plant biomass and chlorophyll content in lettuce seedlings.<sup>12</sup> Studies in *Vicia faba* and *Arabidopsis thaliana* have identified a role for green wavebands as a modulator of stomatal aperture, reversing the blue light response.<sup>13</sup> Stomatal opening stimulated by blue light is reversed by green light in both pulse and continuous illumination experiments. The opposition is fluence-rate dependent and full reverse requires the 2:1 ratio of green/blue. In the *npq1* mutant, blue-specific stomatal opening was not observed in high-fluence conditions and the opening under low-fluence light is reversed by far-red light instead of green light. The results suggest that zeaxanthin plays a role in modulating the blue-induced stomata aperture.<sup>13,14</sup> It was also observed that monochromatic green light induces changes in *Arabidopsis* leaf position that are independent of phytochromes and cryptochromes.<sup>15</sup> In sunflower, both green monochromatic light and light transmitted through its own canopy induce the opening of abaxial stomata, while adaxial stomata remain unresponsive.<sup>16</sup> The time to heading in wheat is accelerated by green light in a fluence-rate-dependent manner,<sup>17</sup> and an action spectrum shows a peak at 540–550 nm.<sup>18</sup> This peak is consistent with that described as maximum for stomatal opening reversal,<sup>13</sup> green-induced dormancy maintenance in ryegrass<sup>19</sup> and green-driven inhibition of elongation in cress roots during gravitropic bending.<sup>20</sup> These findings represent some of the physiological alterations brought on by green light illumination, alone, or in concert with other wavelengths. The alignment of action spectra maxima provides independent evidence that all of these responses are guided by a similar receptor.

Genetic studies have demonstrated that some of the responses to green light are attributable to cryptochromes. Green light has

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been demonstrated to reverse blue-mediated inhibition of hypocotyl elongation and anthocyanin accumulation in *Arabidopsis* seedlings.<sup>4,5,21</sup> Green wavebands also inhibit blue-induced flowering induction, *FT* expression and *cry2* degradation.<sup>4</sup> All of these blue-green reversible responses are mediated by cryptochrome receptors, as they fail to persist in *cry* mutants. The mechanism of blue-green cryptochrome reversibility has been proposed to be based on switching between states of a semiquinone active form and reduced inactive form of the chromophore,<sup>4,5</sup> or autophosphorylation of cryptochromes caused by a photolyase-like cyclic electron shuttle.<sup>22</sup> In these cases green light reverses the course of plant physiology by interrupting the signaling status of the cryptochrome blue light receptors, demonstrating that some green responses are cryptochrome dependent.

In other cases the blue light driven cryptochrome response cannot be reversed by green light. Dormancy maintenance in imbibed annual ryegrass (*Lolium rigidum*) seeds also is likely cryptochrome mediated, yet green light does not reverse the blue response.<sup>23</sup> Instead, green illumination effects are comparable to the effects of blue light. The authors conclude that either the green response is cryptochrome mediated in seeds (not reversing cryptochrome, but working in the same direction), or is mediated by an independent, non-phytochrome receptor. The action spectrum for the response suggests the latter, as mentioned above.

The effects of specific wavelengths and genetic factors can be precisely described by monitoring the growth of the hypocotyl during photomorphogenesis. The dark-grown seedling's hypocotyl growth rate is extremely sensitive to transition to the light environment. Red, blue and far-red light suppress hypocotyl elongation.<sup>24</sup> However, when a short, single pulse of green light is given to a dark-grown seedling, it transiently elongates at a rate that eclipses the dark rate. This increase in growth rate persists in *cry*, *phy* and *phot* receptor mutant backgrounds. This finding, in conjunction with the fact that the response is the opposite of normal light responses mediated by characterized receptors, suggests that the green response is driven by a novel photosensor. Using the green-induced growth kinetics as a guide, microarray experiments (performed precisely at the peak of green-light response) presented changes in the transcriptome that accompanied the green-light-induced increase in growth rate.<sup>25</sup> Two classes of transcripts were significantly affected. The first class is similar to those induced by *phyA*, reinforcing the dictum that *phyA* is a sensitive receptor for all visible wavelengths. These included *Elip*, *Hy5* and *PKS1* transcripts.

The second class of transcripts decreased following green light treatment. Surprisingly these were plastid resident transcripts, especially those encoding proteins destined to support the photosynthetic apparatus such as *psaA*, *rbcL* and *psbD*. These transcripts have long been known to be induced by light, namely red or blue light. Here the green light system drives their abundance down in contrast to the action of other light qualities.

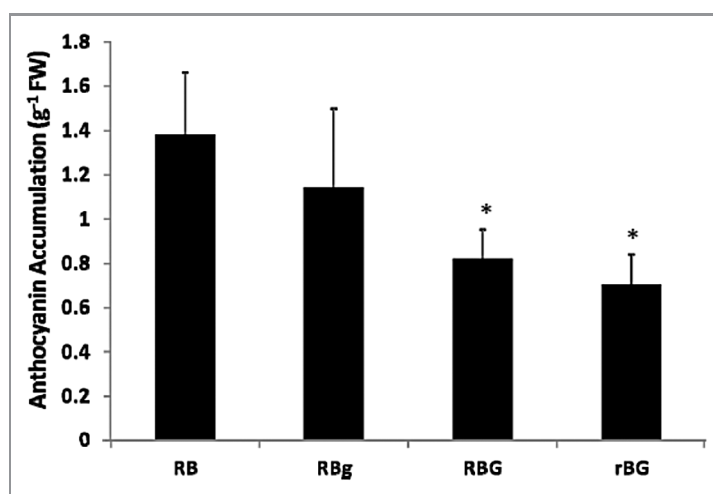
Together the cryptochrome-dependent and the cryptochrome-independent green light responses share a common theme of opposing light-driven physiological or developmental

responses. However, new evidence shows roles for both systems in adjusting plant architecture in a green enriched environment.

Most lights used for artificial growth environments spend their energy on producing red and blue light to best support photosynthesis. Scientists at NASA were concerned about long-term effects of growing plants under such conditions, not because the plants would suffer, but because they would appear unusually dark-purple or black to the attending crew.<sup>26</sup> Small amounts of added green light would make the plants appear normal and at the same time aid in surveillance of nutritional deficiencies or pathogen problems in growing plants that may not be conspicuous under red/blue illumination. These studies are currently continued in candidate space crop plants like lettuce, radish, tomatoes and herbs.

When similar studies were performed using *Arabidopsis*, it became immediately clear that the addition of green light to a background of red and blue had conspicuous, yet counterintuitive effects. A recent study from our laboratory details how *Arabidopsis* plants generate shaded symptoms after the addition of green light.<sup>27</sup> This work is exciting because plants paradoxically take on a shaded, low-light morphological reconfiguration when visible light is increased. Plants grown in a dense canopy exhibit extensive remodeling of architecture, including petiole elongation, upward leaf reorientation, and leaf area reduction.<sup>28-30</sup> Together these symptoms are described as "shade avoidance syndrome." These alterations are typically induced by a low red to far-red ratio in foliar canopy.<sup>31</sup>

Green light, like far-red light, also passes through plant tissue with greater efficiency than red or blue light, leading to



**Figure 1. Supplemental green light decreases anthocyanin accumulation in wild-type *Arabidopsis* (Col-0).** Wild-type (Col-0) *Arabidopsis* plants were grown under white light for approximately three weeks and then transferred to one of four light treatments: 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  red and 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  blue LED light (RB); 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  red, 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  blue and 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  green light (RBg); 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  red, 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  blue and 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  green light (RBG); 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  red, 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  blue and 40  $\mu\text{mol m}^{-2} \text{s}^{-1}$  green light (rBG) for 3–5 d. Anthocyanin was extracted and measured. Error bars represent standard error of the mean of three independent biological replicates. Asterisks represents statistically significant difference comparing to the treatment RB ( $p < 0.05$ ).

enrichment of green wavebands within the understory.<sup>3,9,10,31</sup> To test whether this green light portion also contributed to shade avoidance responses, wild-type *Arabidopsis* plants were treated with different amounts of green light added on a constant red and blue background utilizing a narrow-bandwidth LED-based lighting system. The plants unexpectedly exhibited a shade growth habit in non-shade conditions due to the supplemental green light. As the light became brighter, the plants behaved as though they were growing in shade. To determine whether this green response was mediated by a known class of light sensors (especially the known reversal of blue-light cry responses) multiple *cry* and *phy* mutants were tested in these same conditions. The results showed that these mutants, including double mutants, also exhibited green-induced shade symptoms. The addition of light causing a shaded effect, coupled to persistent effects in photomorphogenic mutants suggested that the green-induced shade avoidance response was possibly mediated by a novel signaling pathway.

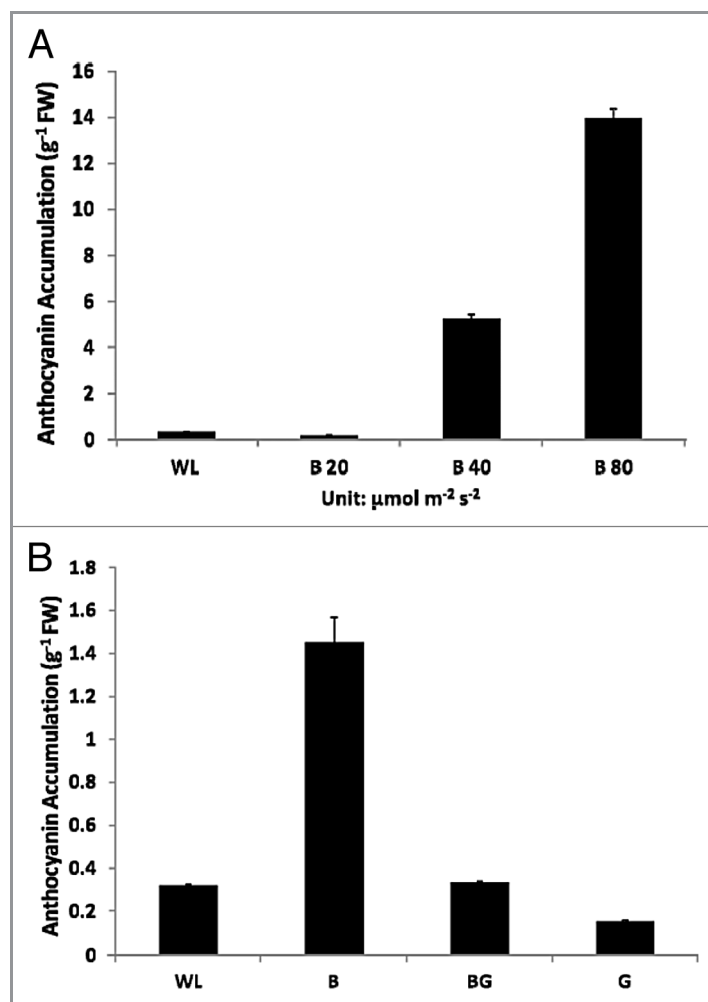
The gene expression patterns coincident with shade avoidance responses have been well described.<sup>32</sup> Some transcripts are strongly induced by a low red/far-red ratio. For example, the *HAT4* and *PIL1* transcripts rapidly accumulate in far-red-induced shade. These same transcripts would serve as excellent indicators to test if the far-red shade response could be uncoupled from the new, green-sensitive response.

In wild-type plants, the *HAT4* and *PIL1* transcripts that spike in response to far-red treatment did not increase in response to a green-enriched environment indicating that the far-red and green responses do not excite entirely parallel responses. This finding suggests separate signaling mechanisms. Examination of photosensory mutants shows that *HAT4* and *PIL1* transcripts do increase in the cryptochrome mutants when green light is added. The interpretation of these data is that cryptochromes are being activated by green light to block the induction of these classical shade-related transcripts in relative green-enriched environment. The result is an example of how the green absorbing form of cryptochromes actively represses a light-driven response.

As noted earlier, green light negates blue-light-induced anthocyanin accumulation.<sup>5</sup> In the recently cited study by Zhang et al.<sup>27</sup>, it was observed that mature *Arabidopsis* plants grown in red and blue conditions with supplemental green light contained visibly less anthocyanin than those in red and blue conditions alone. Anthocyanins were quantified and the results are presented in **Figure 1**. As the green light component increases, the amount of anthocyanin decreases, approaching half the level present in red + blue conditions alone. The same principles were tested in ‘Red Sails’ lettuce, a lettuce variety that has a wide linear dose-response accumulation of pigments in response to blue light (K. Folta and K. Price, unpublished). In ‘Red Sails’ lettuce, anthocyanin accumulates dramatically along with the increasing fluence rates of blue light (**Fig. 2A**). As in *Arabidopsis*, green light also reverses this blue-induced response. Compared with lettuce grown in blue light alone, the levels of anthocyanin were sharply lower than when grown in blue + green or

green alone (**Fig. 2B**). These results are consistent with the anthocyanin accumulation data presented by Bouly<sup>5</sup> and add an additional example of how green light antagonizes other light-induced responses in mature plants across species.

With this knowledge, our thoughts can turn to application. Can “white” artificial lighting be optimized to better control plant stature, pigment production or gene expression? Can removal of green wavelengths change the value of pigmented specialty crops like ‘micro mixes’, where deep purple pigments are highly desirable? These are important questions that can be addressed as our understanding of fundamental light signaling pathways gains additional relevance in plant husbandry and food production.



**Figure 2.** Green light reverses blue-induced anthocyanin accumulation in lettuce. Lettuce were grown under white light for approximately one month and then transferred to different light treatments. Anthocyanin was extracted and measured. Panel A represents anthocyanin levels in lettuce treated with  $90 \mu\text{mol m}^{-2} \text{s}^{-1}$  white fluorescent light (WL) and blue LED light (B) at the amount of 20, 40 and  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$  for 7 d, respectively. Panel B represents the anthocyanin accumulation in lettuce treated with  $90 \mu\text{mol m}^{-2} \text{s}^{-1}$  white fluorescent light (WL),  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  blue (B),  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  blue LED light plus  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  green (BG) and  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  green (G) LED light, respectively. Error bars represent standard error of the mean of three independent biological replicates.

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