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Note

Responses to intermittent exposure to green light during the dark period in *Arabidopsis thaliana* and possible involvement of cryptochrome 2

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Abstract The effects of green light treatment during the dark period were examined in *Arabidopsis thaliana* as a first step to understanding the mechanism of artificial green light effects in plants. Plants were grown under long-day conditions (an 18-h light and a 6-h dark cycle) and were intermittently exposed to green light during the dark period for 2 h 3 times a week. This green light treatment suppressed the elongation of roots and hypocotyls in wild-type plants. However, the green light-induced changes were not significant in the *cry2* mutant that is deficient in the blue light receptor cryptochrome 2. The green light treatment elevated both jasmonic acid and salicylic acid levels in the wild-type plants but the elevation of the jasmonic acid level was impaired in the *cry2* mutant plants. These results suggest that intermittent exposure to green light triggers artificial responses in *Arabidopsis* plants that do not occur in the natural environment, and that cryptochrome 2-dependent and jasmonic acid-mediated responses may be partly involved in the effect of green light on plants.

Key words: cryptochrome, green light effect, jasmonic acid, salicylic acid.

Plants utilize light as an energy source for photosynthesis and percept it as a signal to control their growth and development. The effects of red and far-red light on plant development have been intensively studied because phytochromes have been identified. In addition, blue light responses are well elucidated because blue light receptors, e.g. cryptochromes and phototropins, have been identified (Taiz and Zeiger 2010). Green light is believed to be less effective for photosynthesis because chlorophylls weakly absorb this type of light. Studies on green light responses in plants are limited partly because a specific photoreceptor for green light remains to be identified. However, green light can be transmitted through plant tissues and there is a green light enriched environment in canopy shade (Folta and Maruhnich 2007; Franklin and Whitelam 2005; Taiz and Zeiger 2010). It is proposed that green light is effective for photosynthesis in certain environments that are green light enriched (Terashima et al. 2009). In addition, green light induces shade avoidance symptoms that result in rapid growth (Wang and Folta 2013; Zhang et al. 2011).

In plant factories where vegetables and fruits are cultivated in a closed system for year-round production, artificial light conditions, especially the use of monochromatic light, have been tested to improve the growth yield, appearance of products and content of functional molecules to conform to market needs (Hata et al. 2013; Hidaka et al. 2013; Johkan et al. 2012). A continuous exposure to green light at a high intensity enhanced the growth of leaf lettuce (Johkan et al. 2012). Moreover, exposure to green light for a short period during the night induced resistance against strawberry anthracnose and showed a repellent action on herbivorous spiders from strawberry plants (Kudo et al. 2011). If the green light exposure is effective for pest control, it is attractive in plant factories as a safe and economically feasible method to reduce the usage of agricultural chemicals. Hence, we tested the effects of the exposure to green light during the night on strawberry plants (Sato, unpublished results). The exposure to green light was effective in reducing the damage caused by herbivorous spiders. The fruit yield of strawberry increased slightly but the difference was not statistically significant (Sato, unpublished results).

However, little is known about the mechanisms of the action of green light in plants, and studies on the physiological responses to green light are limited thus far (Wang and Folta 2013). In this study, as a first step of a comprehensive study of green light effects on plants, the effects of intermittent green light treatment during the dark period, which was used in our strawberry cultivation, were examined in the model plant

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Arabidopsis thaliana. We tested a possible involvement of known photoreceptors in the intermittent green light responses by using the *cry1* and *cry2* mutants. The green light exposure may affect on plant growth and defense responses and jasmonates are known to play important roles in the regulation of growth and defense response in plants. Thus we examined the green light responses in the *jar1* mutant. In addition, the contents of jasmonic acid (JA) and salycilic acid (SA) were examined because the antagonism between JA and SA is well known.

Arabidopsis thaliana mutants were derived from the wild-type Columbia ecotype (Col-0). The *cry1* mutant that is deficient in the blue light receptor cryptochrome 1, the *cry2* mutant that is deficient in another blue light receptor, i.e., cryptochrome 2, and the *jar1* mutant that is deficient in JA-Ile were obtained from the Arabidopsis Biological Resource Center (ABRC). Seeds were sown on Murashige and Skoog medium containing 2% (w/v) sucrose and 0.8% (w/v) plant agar (Gelrite; Wako, Osaka, Japan) after 2 days of treatment at 4°C. The plants were grown in a plant growth chamber with a temperature and humidity control system (Koitotron KG-50HLA) under a long-day cycle of 18 h light at 23°C (5:00–21:00) and 6 h dark at 17°C (21:00–5:00). White light was provided from fluorescence lamps at an intensity of 60–85 μmol m^{-2} s^{-1} during the light period. The humidity was maintained at 70% relative humidity. The plants were exposed to green or blue light illumination during the dark period for 2 h three times per week on Sunday, Tuesday and Thursday nights (1:00–3:00) (Figure 1). Green light was provided from color fluorescence lamps (FL20SG, Toshiba, Yokosuka, Japan) with an emission range of 500 to 600 nm (λ_{max} at 530 nm) at an intensity of 29 μmol m^{-2} s^{-1}. Blue light was provided from color fluorescence lamps (FL20SB, Toshiba, Yokosuka, Japan) with an emission range of 400 to 600 nm (λ_{max} at 430 nm) at an intensity of 26 μmol m^{-2} s^{-1}. The light treatment conditions were set according to our cultivation conditions to test the effects of green light on strawberry (Sato, unpublished results) and corresponded to those used in the report by Kudo et al. (2011). We independently established our own plant growth system using a controlled illumination device. After 2 weeks, the root length, hypocotyl length, and number of leaves of the seedlings were measured. Plants were grown for 2 weeks and JA and SA in whole plants, including shoots and roots, were measured in the House Food Analytical Laboratory Inc. (Yotsukaido, Japan) using LC-MS/MS according to Segarra et al. (2006).

Wild type (Col-0) *Arabidopsis* plants, the *cry1* and *cry2* cryptochrome mutants, and the *jar1* jasmonic acid mutant were grown under the light conditions shown

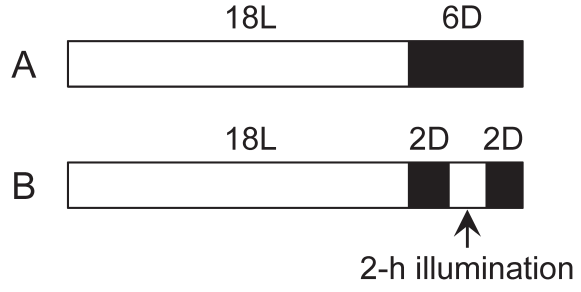

Figure 1. Schematic representation of the light treatment conditions. Plants were grown under a long-day cycle of 18 h light and 6 h dark (A) and exposed to green or blue illumination for 2 h during the dark period three times per week (B).

Table 1. Effect of the intermittent light treatment during the dark period on the root and hypocotyl length of WT and mutant *Arabidopsis* plants.

	Control[a]	Green[b]	Blue[c]
Root length (mm)			
WT			
Col-0	31.5±1.2 (*N*=8)	25.3±1.5* (*N*=7)	31.9±1.7 (*N*=6)
Mutant			
cry1	22.9±1.4 (*N*=5)	14.9±1.5* (*N*=4)	24.4±2.4 (*N*=6)
cry2	42.8±5.4 (*N*=6)	41.2±2.4 (*N*=6)	48.9±3.4 (*N*=6)
jar1	30.2±4.1 (*N*=5)	50.0±2.7** (*N*=6)	Not measured[d]
Hypocotyl length (mm)			
WT			
Col-0	4.6±0.6 (*N*=8)	2.3±0.3** (*N*=7)	2.9±0.2** (*N*=6)
Mutant			
cry1	8.5±0.9 (*N*=5)	4.0±0.6** (*N*=4)	5.1±0.5** (*N*=6)
cry2	3.3±0.3 (*N*=6)	2.7±0.1 (*N*=6)	4.8±0.1** (*N*=6)
jar1	3.9±0.3 (*N*=5)	3.7±0.2 (*N*=6)	Not measured[d]
Number of leaves (Number)			
WT			
Col-0	8.0±0.2 (*N*=8)	7.7±0.5 (*N*=7)	7.0±0.4 (*N*=6)
Mutant			
cry1	7.4±0.3 (*N*=5)	6.8±0.9 (*N*=4)	6.2±0.3 (*N*=6)
cry2	7.5±0.3 (*N*=6)	7.7±0.2 (*N*=6)	7.8±0.3 (*N*=6)
jar1	8.4±0.3 (*N*=5)	8.0±0.3 (*N*=6)	Not measured[d]

Values are mean±SE. Significant differences with respect to the control are indicated by * ($p<0.05$) and ** ($p<0.01$), according to Tukey's test. The growth experiments using the WT were repeated 4 times independently and the experiments using the mutants were repeated twice. [a]Control plants were grown under a long-day cycle of 18 h light and 6 h dark for 2 weeks. No illumination was provided during the dark period. [b]Green illumination was provided from color fluorescence lamps during the dark period for 2 h on Day 2, 4, 7, 9, 11 and 14 after germination. [c]Blue illumination was provided from color fluorescence lamps during the dark period for 2 h on Day 2, 4, 7, 9, 11 and 14 after germination. [d]Growth of plants was inhibited because of a fungal contamination. Blue light might influence on the growth of contaminated fungus (at least in our growth conditions) and the *jar1* mutant is defective in defense responses. Thus, we omitted the data of blue light response in the *jar1* mutant.

in Figure 1. The root lengths, hypocotyl lengths, and number of leaves of 2-week-old seedlings are shown in Table 1. In the wild type and *cry1* mutant plants, the intermittent exposure to green light during the dark period suppressed root elongation. In the *cry2* mutant plants, the green light treatment did not significantly affect the root lengths, although they were longer than those of the wild type plants (Table 1, Figure 2). The *jar1* mutant was used to test the involvement of JA-mediated responses in the green light-induced morphological changes. In the *jar1* mutant plants, root elongation was particularly enhanced by the green light treatment (Table 1). The green light treatment suppressed hypocotyl elongation in the wild type and *cry1* mutant plants, although the hypocotyl lengths of the *cry1* mutant plants were longer than those of the wild type plants under the control condition. There were no significant differences in the hypocotyl lengths in the *cry2* and *jar* mutant plants with respect to the green light treatment, although their hypocotyls were slightly shorter than those of the wild type plants under the control condition (Table 1). There was little or no effect of the green light treatment on the number of leaves of the seedlings (Table 1). These results suggest that the intermittent exposure to green light during the dark period triggers the suppression of root and hypocotyl elongation and these green light-induced changes are impaired in the *cry2* mutant plants.

To test the involvement of JA in the green light response in *Arabidopsis* plants, the wild type and the *cry2* mutant were grown under long-day conditions (18 L/6 D cycle) and were intermittently exposed to green or blue light during the dark period, and the JA and SA levels in whole plants were measured (Table 2). In the wild type plants, both JA and SA levels increased significantly in response to the green light treatment. In contrast, the JA level did not increase in the *cry2* mutant with respect to the green light treatment, although the control level was higher than that of the wild type plants. The SA level in the *cry2* mutant was increased by the green light treatment. The blue light treatment significantly elevated both JA and SA levels in the *cry2* mutant. In the wild-type plants, the JA level slightly increased and no significant change was observed in the SA level in response to the blue light treatment (Table 2). These results suggest that the increase of the JA level in response to green light is impaired in the *cry2* mutant plants and cryptochrome 2 is likely to be involved in the green-light triggered JA elevation.

Although a green light specific sensory system has not been elucidated in plants, monochromatic green light acts as a signal to control plant growth (Chory 1997; Folta 2004; Folta and Maruhnich 2007; von Arnim and Deng 1996; Wang and Folta 2013; Zhang et al. 2011). In addition, some, but not all green light responses are mediated via blue-light receptors, i.e., cryptochromes (Zhang et al. 2011). This study shows that the intermittent exposure to green light during the dark period increased the JA level in a cryptochrome-2 dependent manner. It has been reported that the effects of green light often oppose blue light responses (Folta 2004; Folta and Maruhnich 2007) and cryptochromes

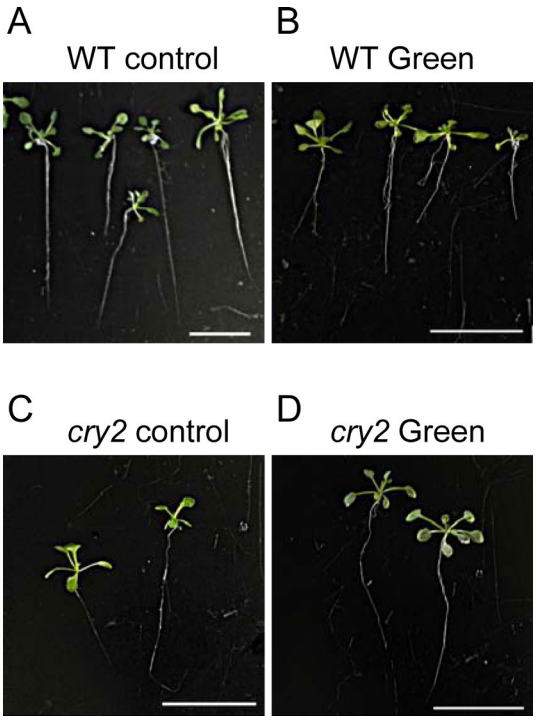

Figure 2. Effect of green light treatment during the dark period on 2-week-old WT (A, B) and *cry2* mutant (C, D) *Arabidopsis* seedlings. Plants were grown under a long-day cycle of 18 h light and 6 h dark and were exposed to green light for 2 h during the dark period on Day 2, 4, 7, 9, 11 and 14 after germination (B, D). Scale bars indicate 25 mm.

Table 2. Effect of the intermittent light treatment during the dark period on the JA and SA contents of the WT and *cry2* mutant *Arabidopsis* plants.

		JA			SA		
		Control[a]	Green[b]	Blue[c]	Control[a]	Green[b]	Blue[c]
		(ng g^{-1} FW)			(ng g^{-1} FW)		
WT	Col-0	16.5±0.3	52.0±4.7**	26.7±0.3*	63.5±1.6	95.6±2.5**	54.4±0.8
Mutant	*cry2*	38.9±0.3	37.0±0.5	52.2±0.6**	87.9±2.5	105.3±2.9**	102.9±1.8**

Values are mean±SE (N=3). Significant differences with respect to the control are indicated by * (p<0.05) and ** (p<0.01), according to Tukey's test. JA and SA contents in whole plants, including shoots and roots, were simultaneously determined using LC-MS/MS. [a]Control plants were grown under a long-day cycle of 18 h light and 6 h dark for 2 weeks. No illumination was provided during the dark period. [b]Plants were exposed to green illumination for 2 h during the dark period on Day 2, 4, 7, 9, 11 and 14 after germination. [c]Plants were exposed to blue illumination for 2 h during the dark period on Day 2, 4, 7, 9, 11 and 14 after germination.

display the interconvertible changes; the activation by blue light can be deactivated by green light (Banerjee et al. 2007; Bouly et al. 2007; Folta and Maruhnich 2007). In this study, the intermittent exposure to green light during the dark period suppressed root elongation in the wild type and *cry1* mutant plants but there was no significant difference observed in the blue light treatment (Table 1). In contrast to the suppression of root elongation, the hypocotyl elongation was suppressed by the blue light treatment similarly as the green light treatment in the wild type and *cry1* mutant plants (Table 1). These results suggest that the suppression of root elongation is specifically triggered by the intermittent exposure to green light during the dark period. The green light specific suppression of root elongation can be mediated via cryptochrome 2 but the mechanism of the green light signal perception, especially the differences from the blue light perception, remain to be elucidated.

The activation or repression of immune responses is tightly controlled by two plant hormones, JA and SA (Caarls et al. 2015). The balance between growth and defense is important for resource investment; the activation of defense responses against insect herbivores or microbial pathogens is a cost to the plant because there is a reduction in the resources that would normally be allocated to growth. There is a tradeoff between the defense responses and the shade avoidance that is needed to induce rapid growth. In *A. thaliana*, the JA and SA signaling pathways are independent (Pieterse and Dicke 2007) and are often antagonistic (Caarls et al. 2015). Because both JA and SA are induced by the green light stimulus (Table 2), green light-exposed plants are thought to be under an antagonism of JA-responsive gene expression (Caarls et al. 2015). Further studies are necessary to precisely characterize the gene expression patterns in the SA/JA crosstalk in response to green light exposure and this can be a clue to understand the mechanisms of the effect of green light on pathogen and insect resistance.

The exposure to monochromatic illumination during the night is an artificial light condition that disturbs plants physiologically and may trigger responses that do not occur naturally. As a second step for the comprehensive understanding of the effects of green light on the model plant *A. thaliana*, functional genomics analysis will reveal the green light response genes, some of which may overlap with the JA- and SA-mediated defensive response genes. Pest management through the manipulation of light is an attractive goal that has commercial benefits, especially in plant factories. Further basic studies are necessary to determine the practical applications of artificial monochromatic illumination techniques.

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References

Banerjee R, Schleicher E, Meier S, Viana RM, Pokorny R, Ahmad M, Bittl R, Batschauer A (2007) The signaling state of *Arabidopsis* cryptochrome 2 contains flavin semiquinone. *J Biol Chem* 282: 14916–14922

Bouly J-P, Schleicher E, Dioniso-Sese M, Vandenbussche F, van der Straeten D, Bakrim N, Meier S, Batschauer A, Galland P, Bittl R, et al. (2007) Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. *J Biol Chem* 282: 9383–9391

Caarls L, Pieterse CMJ, van Wees SCM (2015) How salicylic acid takes transcriptional control over jasmonic acid signaling. *Front Plant Sci* 6: 170

Chory J (1997) Light modulation of vegetative development. *Plant Cell* 9: 1225–1234

Folta KM (2004) Green light stimulates early stem elongation, antagonizing light-mediated growth inhibition. *Plant Physiol* 135: 1407–1416

Folta KM, Maruhnich SA (2007) Green light: a signal to slow down or stop. *J Exp Bot* 58: 3099–3111

Franklin KA, Whitelam GC (2005) Phytochromes and shade-avoidance responses in plants. *Ann Bot (Lond)* 96: 169–175

Hata N, Hayashi Y, Ono E, Satake H, Kobayashi A, Muranaka T, Okazawa A (2013) Differences in plant growth and leaf sesamin content of the lignan-rich sesame variety 'Gomazou' under continuous light of different wavelengths. *Plant Biotechnol* 30: 1–8

Hidaka K, Dan K, Imamura H, Miyoshi Y, Takayama T, Sameshima K, Kitano M, Okimura M (2013) Effect of supplemental lighting from different light sources on growth and yield of strawberry. *Envrion Control Biol* 51: 41–47

Johkan M, Shoji K, Goto F, Hahida S, Yoshihara T (2012) Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. *Environ Exp Bot* 75: 128–133

Kudo R, Ishida Y, Yamamoto K (2011) Effects of green light irradiation on induction of disease resistance in plants. *Acta Hortic* 907: 251–254

Pieterse CMJ, Dicke M (2007) Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends Plant Sci* 12: 1360–1385

Segarra G, Jáuregui O, Casanova E, Trillas I (2006) Simultaneous quantitative LC-ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress. *Phytochemistry* 67: 395–401

Taiz L, Zeiger ES (2010) *Plant Physiology.* Sinauer Associates Inc. Publishers. Sunderland, Massachusetts USA

Terashima I, Fujita T, Inoue T, Chow WS, Oguchi R (2009) Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. *Plant Cell Physiol* 50: 684–697

von Arnim A, Deng X-W (1996) Light control of seedling development. *Annu Rev Plant Physiol Plant Mol Biol* 47: 215–244

Wang Y, Folta KM (2013) Contribution of green light to plant growth and development. *Am J Bot* 100: 70–78

Zhang T, Maruhnich SA, Folta KM (2011) Green light induces shade avoidance symptoms. *Plant Physiol* 157: 1528–1536