

**Plant Production Science** 

ISSN: 1343-943X (Print) 1349-1008 (Online) Journal homepage: https://www.tandfonline.com/loi/tpps20

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To cite this article: Shin-ya Kasajima, Naoto Inoue & Rezwanul Mahmud (2009) Response Spectrum for Green Light-Induced Acceleration of Heading in Wheat cv. Norin 61, Plant Production Science, 12:1, 54-57, DOI: 10.1626/pps.12.54

To link to this article: https://doi.org/10.1626/pps.12.54

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Published online: 03 Dec 2015.

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### [Short Report]

## Response Spectrum for Green Light-Induced Acceleration of Heading in Wheat cv. Norin 61

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**Abstract** : The response spectrum for green light induced-acceleration of heading in wheat cv. Norin 61 was investigated using narrow-bandwidth (10 nm) green light within the range of 520–550 nm. Heading was observed from approximately 30 days after emergence. The earliest heading was observed at 540 nm, which suggests the presence of a green light photoreceptor different from hitherto known photoreceptors.

Key words : Green light, Heading rate, Response spectrum, Spectral sensitivity, Wheat.

Green light (500-550 nm) is the central portion of photosynthetically active radiation (400-700 nm). In general, a large amount of green light is reflected by or passed through the leaf canopy. Thus, green light is usually considered to be relatively inefficient for photosynthesis. The inhibitory effects of green light have been demonstrated over the last 50 years. For example, tomato (Lycopersicon esculentum) seedlings grown under red and blue light had greater dry-matter production than those grown under red and blue light supplemented with green light (Went, 1957; Folta and Maruhnich, 2007). The inhibitory effect on plant tissue growth was quickly reversed when green light was removed (Klein, 1964). The phenomenon is now interpreted as an antagonistic action of green light on normal light-mediated responses (Folta, 2005).

Recent reports suggest that the heading is accelerated by green light (Kasajima et al., 2007; 2008). The effect of green light may not be involved in the photoperiodic and vernalization responses (Kasajima et al., 2008). Thus the role of green light in developmental physiology may be one of the most important challenges for future agronomic studies.

Although photoreceptors such as phytochrome and cryptochrome are well known, green light-specific photoreceptors have not yet been identified in higher plants (Devlin et al., 2007). Because phytochrome and cryptochrome can absorb some green light, it has been difficult to exclude the effects of these photoreceptors in green light responses (Folta and Maruhnich, 2007). To determine whether known photoreceptors like phytochrome are involved or not, studies on the response to restricted bandwidths of the green light spectrum should be given priority. Thus, we need to determine the response spectrum for green light-effect on developmental processes.

The response spectrum is a plot of wavelength responses that shows the rate of reaction to equal light quantum at each wavelength, which is a useful first approach for screening spectral sensitivity of plant responses (Inada, 1984; Holmes, 1997). The accuracy of the response spectrum is lower compared with an action spectrum, because the action spectrum is usually determined by examining the fluence rateresponse curve at each wavelength. On the other hand, the response spectrum can be obtained under only one light quantum for each wavelength (Inada, 1984). The response spectrum is a simplified approach for screening of green light sensitivity, because it gives similar information as a true action spectrum, unless ultraviolet spectrum, which possess considerable energy per quantum, is analyzed (Holmes, 1997). In this study, we analyzed the response spectrum of green light for screening the spectral sensitivity of the response.

We previously reported that the heading rate in wheat was significantly correlated with the ratio of energy in 500-550 nm range compared to that in the whole spectral range (Kasajima et al., 2007). However, the most effective wavelength within the 500-550 nm range is still unknown. To determine the response rates of different wavelengths, it is important to know the effect of green light on the heading rate. In this study, the response spectrum for the green lightinduced acceleration of heading was determined using narrow-bandwidth (10 nm) light at different wavelengths within the range of 520-550 nm, a spectral range of green light (Inada, 1984). The effects of narrow-bandwidth green light at different wavelengths on the heading rate were investigated under the fixed supplemental white fluorescent light.

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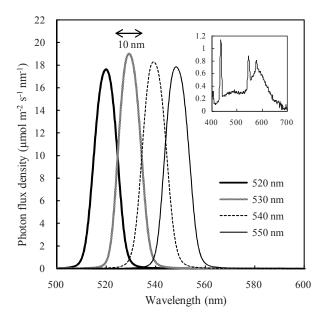


Fig. 1. The emission spectrum of the restricted green light used in each treatment. The diagram in the upper right corner of this figure shows the emission spectrum of the white fluorescent lamps used.

#### **Materials and Methods**

Wheat seeds (Triticum aestivum L. cv. Norin 61) were sown in 4.5×4.5×5.0 cm plastic pots (length×width ×height, two seeds per pot) filled with commercial garden soil (Taihei-Engei-Baido, Taihei Product Co., Hachobori, Tokyo, Japan). N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O contents in the soil were 0.35, 1.5 and 0.35 g kg<sup>-1</sup>, respectively. Fifty pots were arranged in a  $5 \times 10$  configuration, of which nine pots  $(3 \times 3)$  were used in the present experiment. The cultivar "Norin 61" was used because it has few defects in its main characteristics and has low vernalization requirement (Gotoh, 1979). An indoor experiment was conducted using a  $50 \times 60 \times 50$  cm growth box (length×width×height), which was coated with aluminum film to improve the efficiency of illumination during each treatment. The seedlings were thinned to one plant per pot five days after sowing (planting density=400 plants per square meter), and then grown under continuous light and maintained at 20°C temperature, to eliminate the effects of photoperiod and vernalization responses. Plants were irrigated two times a day with tap water without any fertilizer application.

The experiment had four light treatments with a narrow-bandwidth in the spectral range of 520–550 nm. A 10 nm bandwidth was used for each light treatment as shown in Fig. 1. This restricted bandwidth of green light was provided by an optical fiber using visual mirror module and band-pass filter (LAX-102; Asahi Spectra Co., Kamiujo, Tokyo, Japan). A rod lens (RLQ-2; Asahi Spectra Co., Kamiujo, Tokyo, Japan)

was attached to the optical fiber to obtain sufficient irradiation area and uniform light distribution. The light intensity of the narrow-bandwidth green light used in each treatment was adjusted to an equal photosynthetic photon flux density (PPFD) of 200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> (Fig. 1). For all treatments, four white fluorescent lamps (FL15N; Toshiba Co., Shibaura, Tokyo, Japan) were set parallel to each other, and the optical fiber was mounted between the fluorescent lamps (Fig. 1). We used the white fluorescent lamps (100  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> of PPFD) in addition to green light, because it is difficult to complete the life cycle of wheat under monochromatic green light as previously reported (Kasajima et al., 2008). The optical fiber and the fluorescent lamps were suspended at a height of approximately 40 cm above the pot top in the growth box. The spectral distributions were recorded at the top of the seedlings immediately after the start of light treatment using a spectroradiometer (HSU-100S; Asahi Spectra Co., Kamijujo, Tokyo, Japan).

The lighting for all treatments was maintained at  $300 \ \mu \text{mol} \ \text{m}^{-2} \ \text{s}^{-1}$ , whose  $200 \ \mu \text{mol} \ \text{m}^{-2} \ \text{s}^{-1}$  were green photons from the fiber, which was calculated from the spectral data as previously described (Kasajima et al., 2008). PPFD levels for each treatment were equalized by adjusting the height of the fiber.

The number of headed plants was recorded every day after the first heading in each treatment. In the present experiment, each plant developed one panicle without any differentiation of tillers in all treatments. We calculated the reciprocal number of mean value of days from emergence to heading as an index of the heading rate. The experiment used a completely randomized design with two replicates. One growth box was repeatedly used in the present experiment. The mean values were calculated from nine plants per replicate. All data were subjected to a one-way analysis of variance (ANOVA) with Tukey's multiple range test for comparison between the mean values.

#### **Results and Discussion**

The heading date of wheat varied with the wavelength of the narrow-bandwidth green light which was given under the same supplemental white fluorescent light (Fig. 2). Previously, we confirmed that the confounding effect of green light due to the light intensity could be statistically separated by principal component analysis (Kasajima et al., 2008). The results in the present study more clearly suggest that the effect of green light on the heading date is independent of the PPFD level. The average values of leaf number, main culm length, and shoot dry weight at heading were 7.8, 35.6 cm, and 0.47 g, respectively. No significant difference in dry-matter production was observed among plants or treatments. Therefore, there is little possibility that the difference in growth rate affected the heading date.



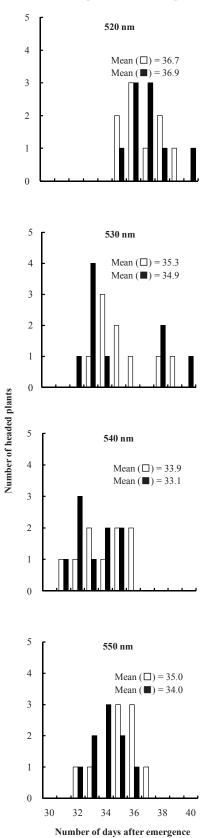


Fig. 2. Frequency distribution of the number of days to heading after emergence with number of headed plants in both first and second replicates (n=9 per replicate) in each treatment.

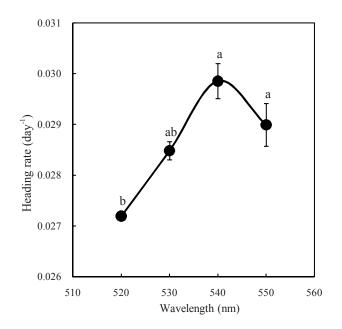


Fig. 3. The response spectrum for green light-stimulated heading rate. Heading rate was shown by calculating the reciprocal number of days to heading. Vertical bars represent the standard errors of the means of two replicates. Symbols with the same letter are not significantly different (P<0.05) according to Tukey's multiple range test.

Fig. 2 shows the frequency distribution of the number of days to heading after emergence in both first and second replicates of each treatment. In all treatments, heading was observed from approximately 30 days after emergence. The mean heading date was earliest in the plants grown under 540 nm (33.9 and 33.1 ds). Conversely, it was latest in the plants grown under 520 nm (36.7 and 36.9 ds). It appears that heading date varied with the wavelength of narrow-bandwidth green light.

Fig. 3 shows the response spectrum for green light-influenced heading rate. A peak response was observed at 540 nm. The heading rate at 520 nm was significantly lower than that at 540 nm and 550 nm. According to Inada (1984), the spectral range from 520–550 nm is a range of green light. In this study, the maximum response was observed at 540 nm, suggesting the existence of a chromoprotein that shows an absorption peak at 540 nm. This result suggests that phytochrome and cryptochrome could be excluded from the primary photoreceptors for green light effect.

The specific influences of green light on plant growth and photomorphogenesis have been reported (Folta, 2004; Kim et al., 2004; Mullen et al., 2006). In *Arabidopsis*, green light stimulates hypocotyl elongation, even in mutants lacking phytochromes and cryptochromes (Folta, 2004). These reports suggest the presence of green-light absorbing photoreceptors, which are neither phytochrome nor cryptochrome. In the present study, the response spectrum for the green light-induced acceleration of heading suggested the presence of an unidentified green light photoreceptor in wheat. However, it is necessary to determine the action spectrum for the heading rate to reveal whether a green light photoreceptor is indeed involved in the photoresponse. Moreover, it has been demonstrated that the green light responses may be caused by light-dependent regulation of abscisic acid (ABA) biosynthesis (Mullen et al., 2006). Thus, further studies are needed to clarify the effects of green light and ABA regulation on the heading rate of wheat.

The present findings indicate that the effect of green light on the heading of wheat has a peak at around 540 nm. These results suggest the presence a response to green light, which differs from the response mediated by known photoreceptors such as phytochromes and cryptochromes. This unique phenomenon will be relevant to understanding the role of green light in the developmental physiology of wheat.

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