

Developmental Responses of Wheat cv. Norin 61 to Fluence Rate of Green Light

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Abstract : Wheat (*Triticum aestivum* L. cv. Norin 61) plants were grown under five different photon flux densities obtained by modulating the number of green light-emitting diodes (LEDs) under white fluorescent lamps. The experiment was conducted under continuous irradiation at a constant temperature of 20°C to clarify the developmental responses of wheat to the fluence rate of green light. The higher the photon flux density of green light, the shorter the number of days from emergence to heading. The earliest heading was observed in the plants grown under 496 green LEDs, 32.0 days after emergence. A significant logarithmic function could fit the relationship between the fluence rate of green light and developmental rate. In this report, principal component analysis (PCA) was adopted to analyze the confounding of green light versus photosynthetic photon flux density (PPFD) with 17 developmental and morphological traits. The eigenvalues explained were 5.64, 3.20, and 2.61, respectively, for the first, second and third principal components (PCs). The first PC was assumed as the factor related to the isometric growth, and the third PC was assumed as the factor related to the developmental rate and culm elongation. Therefore, it was supposed that the first and third PCs were affected by the PPFD and the photon flux density of green light, respectively. The results suggested that the fluence rate of green light affects the development of wheat as a signal source. Furthermore, the development of wheat was promoted by the green light independent of PPFD.

Key words : Development, Fluence rate, Green light, Light-emitting diode (LED), Morphology, Principal component analysis (PCA), Wheat.

Many crop physiologists have been interested in the interaction between photoperiod and temperature to predict the days to heading of wheat in developmental physiology. Theoretically, development of wheat can be affected by photoperiod, temperature, and genotype (Wallace and Yan, 1998; Miralles and Slafer, 1999). In practice, however, the spectral quality and intensity of light play an important role in plant development through the regulation of phytochromes (Fitter and Hay, 2002).

In our previous study (Kasajima et al., 2007), we analyzed the effective spectral ranges for the developmental rate of wheat using broad-spectrum lighting sources, and pointed out that the green light and red light may have a promoting effect on the developmental rate. The action spectrum for floral promotion of wheat exhibited the action maxima at 660 and 716 nm (Carr-Smith et al., 1989). However, the effect of green light on the developmental rate is complex, because a large amount of green light is usually reflected by the leaf canopy.

There are several reports on the physiological responses to green light. For example, the blue light-stimulated phototropism of *Arabidopsis* and lettuce was observed (Steinitz et al., 1985). Reversal of blue light-stimulated stomatal opening by green light has been

reported in a diversity of plant species (Frechilla et al., 2000; Talbott et al., 2002). A novel phytochrome-like photoreceptor showing reversible photoconversion between blue and green-absorbing forms was detected in a cyanobacterium (Yoshihara et al., 2004). However, there are few reports on the effect of green light on the development of wheat. A photoreceptor for green light in higher plants is also unknown. From these reports, there is a possibility that green light may have a function that regulates the development of wheat. Therefore, we hypothesized that green light affects the development of wheat through a photoreceptor specific to green light. Studies on the qualitative effect of green light on the development of wheat as a signal source should be given priority. In this respect, there is a need to examine the developmental response to the fluence rate of green light, which is the focus of the present study. Fluence rate is the photon number per unit area and unit time; it is an index of light intensity in photobiological research (Konjevic et al., 1989; Somers et al., 1998).

Light-emitting diodes (LEDs), are good light sources in the present experiment compared with fluorescent lamps, because it is easy to modulate the photon flux density of green light. In addition, LEDs have a pronounced peak wavelength in monochromatic

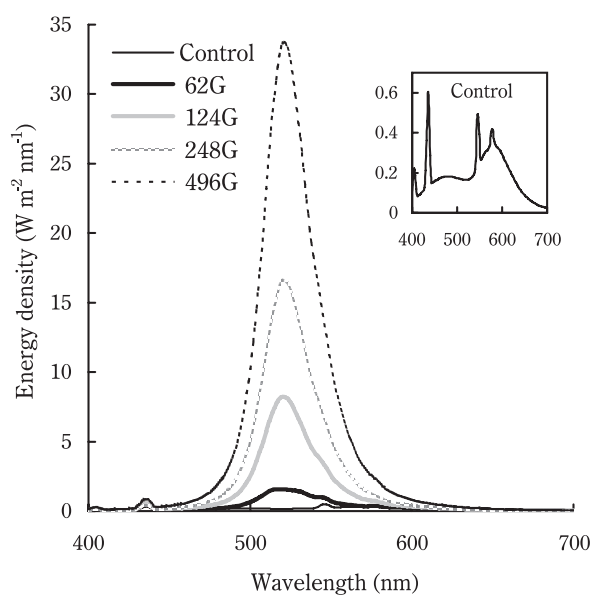


Fig. 1. Emission spectra of five kinds of light used in the present experiment. See Table 1 for composition of light.

green light (Goto, 2003). The spectral range of green light is 500–550 nm (Kasajima et al., 2007), and that of photosynthetic photon flux density (PPFD) is 400–700 nm. Therefore, the spectral ranges of green light and PPFD overlap each other. That is, PPFD levels may affect the development of wheat in addition to the fluence rate of green light. The PPFD levels are shown to affect not only crop growth and yield but also morphological traits (Inada, 1993).

Past studies have examined action spectra for photosynthesis of higher plants. It is well known that the action spectra have action maxima at blue and red ranges, but are lower in the green range (Inada, 1976). The present experiment adopted the additional irradiation of green LEDs under white fluorescent lamps as will be described later. Principal component analysis (PCA) was, therefore, performed to analyze how the overlapping of green light and PPFD affects the developmental and morphological traits of wheat.

The cultivar “Norin 61” is a major spring wheat variety in Japan and belongs to class II in the degree of winter habit (Gotoh, 1979). This variety was used, because it has few defects in main characteristics and shows low vernalization requirement (Gotoh, 1979). The objectives of this study were (1) to determine the developmental and morphological responses of wheat to fluence rate of green light; and (2) to analyze the confounding of green light versus PPFD by PCA.

Materials and Methods

1. Cultural conditions

Wheat seeds (*Triticum aestivum* L. cv. Norin 61) were sown in 4.5 cm × 4.5 cm × 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). An experiment was conducted using a 50 cm × 60 cm × 50 cm growth box (length × width × height) which was coated with aluminum film to improve the efficiency of irradiation in each treatment. Five days after emergence, the seedlings were thinned to one plant per pot, and then grown under continuous irradiation (24/0 h light/dark photoperiod) in a room maintained at 20°C, to eliminate the effects of photoperiod and vernalization responses. The experiment used a completely randomized design with two replications.

2. Light treatments and measurements

The five light treatments were (1) three white fluorescent lamps (control); (2) 62 green LEDs with three white fluorescent lamps (62G); (3) 124 green LEDs with three white fluorescent lamps (124G); (4) 248 green LEDs with three white fluorescent lamps (248G); and (5) 496 green LEDs with three white fluorescent lamps (496G). Their spectral distributions were measured with a spectroradiometer (HSU-100S; Asahi Spectra Co., Kamijujo, Tokyo, Japan) with dimensions of $W m^{-2} nm^{-1}$ (Fig. 1). The spectra were recorded at the top of the plant canopy. For all treatments, three white fluorescent lamps (FL15N; Toshiba Co., Shibaura, Tokyo, Japan) were set parallel to each other. Green LED arrays (NSPG500S; Nichia Co., Kaminaka, Tokushima, Japan) were mounted between the fluorescent lamps at regular intervals, except for the control. We adopted additional green LED irradiation under white fluorescent lamps, because the wheat plants could not grow under monochromatic green light. Table 1 shows the light photon flux densities with dimensions of $\mu mol m^{-2} s^{-1}$ in green (500–550 nm) and red (600–700 nm) ranges and photosynthetic photon flux density (PPFD, 400–700 nm), which were obtained by calculating the spectral data from the following formula (Kadota, 2001):

$$1 W m^{-2} = 8.36 \times \lambda \times 10^{-3} \mu mol m^{-2} s^{-1},$$

where λ is the wavelength. Previously (Kasajima et al., 2007), we showed that red light increases the developmental rate of wheat. In contrast, it has been reported that blue light did not affect the growth and development of wheat (Dougher and Bugbee, 2001; Kasajima et al., 2007). The photon flux densities of the red range were nearly constant in all treatments (Table 1).

3. Examination of developmental and morphological traits

Developmental and morphological traits of plants were recorded for the nine plants that were exposed to green light from the LEDs. The traits examined in the present experiment were number of days from emergence to the first heading and 50% heading, panicle length, awn length, number of spikelets per ear, main culm length, first to fourth internode length

Table 1. Photon flux densities in each treatment.

Treatment	Composition of lighting	Green ¹⁾	Red ²⁾	PPFD ³⁾
		($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
Control	3 white fluorescent lamps	49	61	257
62G	62 Green LEDs + 3 white fluorescent lamps	689	43	1035
124G	124 Green LEDs + 3 white fluorescent lamps	1328	57	1813
248G	248 Green LEDs + 3 white fluorescent lamps	2608	62	3368
496G	496 Green LEDs + 3 white fluorescent lamps	5166	88	6479

^{1), 2), 3)} represent the photon flux density of 500–550, 600–700, and 400–700 nm range, respectively.

Table 2. Days from emergence to heading in each treatment.

Treatment	Days to first heading ¹⁾	Days to 50% heading ²⁾
Control	35.5 a ³⁾	37.9 a
62G	33.0 ab	34.6 b
124G	34.5 ab	35.7 ab
248G	33.5 ab	34.0 b
496G	32.0 b	33.9 b

¹⁾ Number of days from emergence to first heading and ²⁾ 50% heading, which were determined from regression lines of the percentage of heading plants plotted against days after emergence.

³⁾ Values with different letters are significantly different at the 5% level by Fisher's test.

from bottom to top, angle of blade inclination, length of blade, width of blade, length of sheath in flag and penultimate leaf. The morphological traits were measured at the late stage of grain filling (55 days after planting).

4. Statistical analysis

The mean values were calculated from nine plants per replication. ANOVA and PCA were adopted to analyze the change in developmental and morphological traits using the programs Excel (Microsoft Co., USA) and Excel Statistics (Esmi Co. Ltd., Japan). In the present study, particularly, we applied PCA to separate the growth and developmental factors by using morphological characteristics and heading time data.

Results

1. Variation of heading time in each treatment

A significant variation of heading time was observed among the treatments (Table 2). The number of days from emergence to the first heading was the lowest in the plants grown under 496G, followed by 62G. The difference in the days to the first heading between the plants grown under 496G and control was 3.5 days. The number of days to 50% heading was significantly lower in the plants grown under 62G, 248G, and 496G than in the control, and it was 34.7, 34.0, and 33.9 days

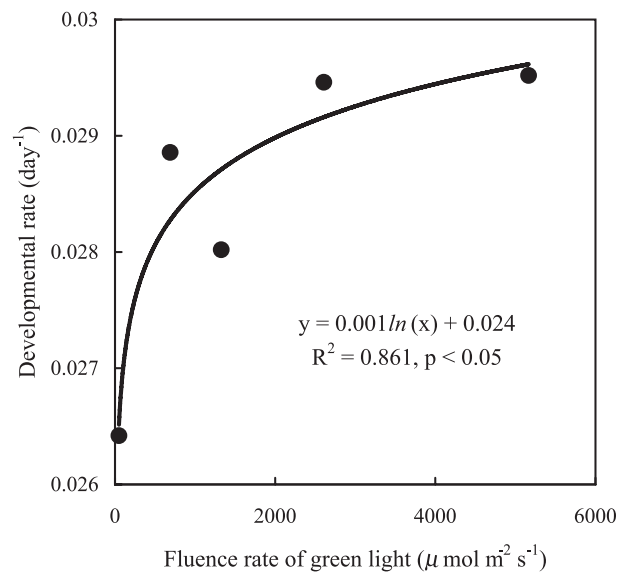


Fig. 2. Relationship between the fluence rate of green light and developmental rate. Developmental rate was obtained by calculating a reciprocal number of the days to 50% heading.

after emergence, respectively.

2. Response of developmental rate to the fluence rate of green light

The relationship between the developmental rate and the fluence rate of green light was examined (Fig. 2). We calculated the reciprocal number of days from emergence to 50% heading as an index of the developmental rate. Fig. 2 shows a significant logarithmic function at the 5% significance level. The developmental rate was affected by the fluence rate of green light.

3. Principal component analysis of developmental and morphological traits

PCA was performed on a correlation matrix using the 17 variables to analyze the covariance factor related to the fluence rate of green light. The eigenvalues explained were 5.64, 3.20, and 2.61, respectively, for the first, second and third principal components

Table 3. Factor loading in PCA.

Trait	PC1	PC2	PC3
Days from emergence to 50% heading	0.253	-0.170	0.472
Panicle length	-0.850	0.135	-0.006
Awn length	0.232	-0.605	0.207
Number of spikelet per panicle	-0.800	0.024	-0.181
Main culm length	-0.056	0.297	0.929
Internode length			
I	-0.162	-0.615	0.638
II	-0.124	-0.128	0.696
III	-0.311	0.617	0.543
IV	0.268	0.701	0.349
Flag leaf			
Angle of blade inclination	-0.557	0.317	-0.212
Length of blade	-0.343	-0.770	0.017
Width of blade	-0.857	-0.113	-0.011
Length of sheath	-0.904	-0.147	0.113
Penultimate leaf			
Angle of blade inclination	-0.456	0.509	-0.198
Length of blade	-0.653	-0.621	-0.033
Width of blade	-0.754	0.22	-0.047
Length of sheath	-0.870	0.116	0.192
Eigenvalue	5.637	3.202	2.609
Cumulative contribution (%)	33.2	52.0	67.3

(PCs) (Table 3). The cumulative contribution of the total three PCs was 67.3%, which could explain approximately 70% of the total variability. Table 3 shows the factor loading of each PC related to the different morphological and developmental variations. The first PC had higher negative correlations with panicle length, number of spikelets per panicle, angle of blade inclination in flag leaf, length of blade in penultimate leaf, width of blade in flag and penultimate leaf, and length of sheath in flag and penultimate leaf. Therefore, the first PC indicated the size factor relating to the isometric growth. The second PC showed higher positive correlations with third and fourth internode length and angle of blade inclination in penultimate leaf. Conversely, higher negative correlations were observed in awn length, length of blade in flag and penultimate leaf. Therefore, the second PC indicated the shape factor relating to the allometric growth. The third PC showed higher positive correlations with number of days from emergence to 50% heading, main culm length, and first and second internode lengths. Therefore, the third PC indicated the factor related to development and culm elongation.

In Fig. 3, the main culm length was plotted against the number of days from emergence to 50% heading in each treatment, because the third PC suggested

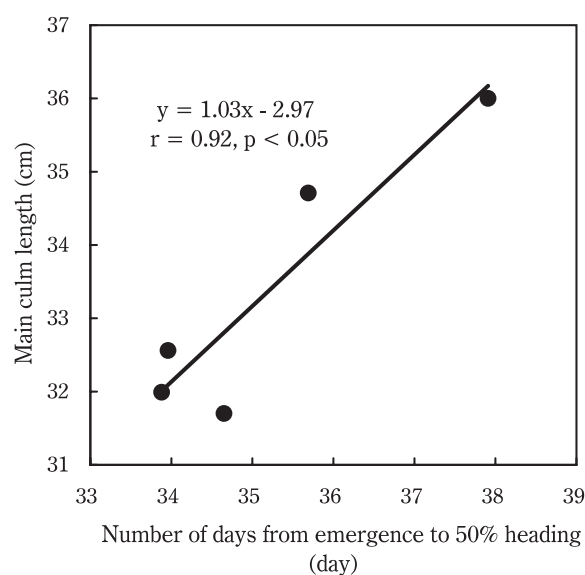


Fig. 3. Relationship between the number of days from emergence to 50% heading and the main culm length.

the covariance between culm elongation and days to heading. There was a significant correlation between number of days from emergence to 50% heading and main culm length at the 5% significance level. Compared with the plants grown under control

Table 4. Effect of fluence rate of green light on the length of internode and main culm.

Treatment	Internode length (cm)				Main culm length (cm)
	I	II	III	IV	
Control	12.0 ab	9.8 a	8.0 a	6.1 a	36.0 a
62G	10.4 ab	9.5 a	7.2 a	4.7 b	31.7 b
124G	12.0 a	9.5 a	8.4 a	4.5 b	34.7 ab
248G	10.4 ab	9.5 a	7.3 a	4.8 b	32.6 b
496G	9.8 b	9.0 a	8.2 a	4.7 b	32.0 b

Values with different letters are significantly different at the 5% level by Fisher's test.

conditions, the main culm length was significantly shorter in the plants grown under 62G, 248G, and 496G. Similar tendencies were observed in each internode length (Table 4).

Fig. 4 shows the scatter diagram of scores in each treatment of the first and the third PCs obtained by the PCA of 17 traits. No correlation was observed between the first and third PCs.

Discussion

The variations in heading time of wheat as affected by the fluence rate of green light were observed even under continuous light at a constant temperature. The data (Table 2) were consistent with a previous paper that reported the heading time was affected by the light qualities, independent of photoperiod and vernalization responses (Kasajima et al., 2007). Yamazaki (2003) also reported that flowering of *Perilla flutescens* B. was promoted by the green light from green fluorescent lamps. In the present study, also, the higher the photon flux density of green light, the shorter the number of days from emergence to heading. These results show a promoting effect of green light on the development of wheat.

In the present experiment, the response curve of developmental rate to fluence rate of green light was characterized as a hyperbolic curve. This result indicated that the low fluence rate of green light affects the developmental rate of wheat. These observations supposed that the green light promotes the developmental rate as a signal, not as an energy source for growth rate, because the relationship between the fluence rate of green light and the developmental rate did not show a linear regression. Recently, several fluence rate-response curves were characterized in relation to stomatal and phototropic responses (Lascève et al., 1999; Eisinger et al., 2000). The present study provides the first response curve of developmental rate to fluence rate of green light in wheat. Further studies on the green action spectrum for developmental rate are necessary to determine the mechanisms of the reception of green light in plant cells.

In the present study, PCA was performed to analyze the covariance factor related to the fluence rate of

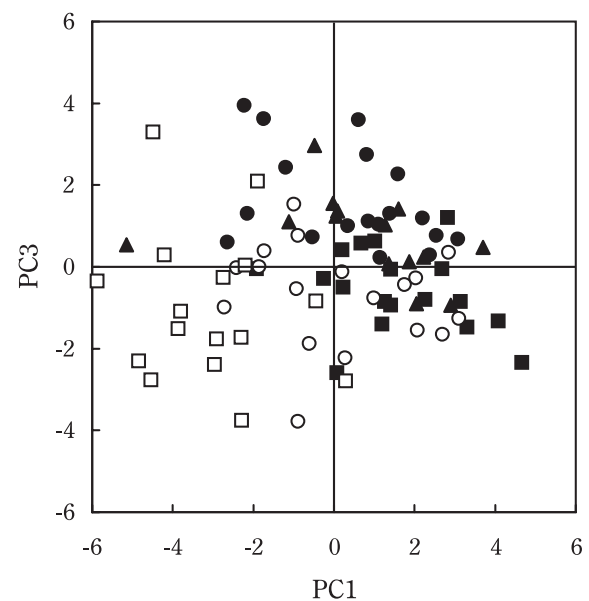


Fig. 4. Scatter diagram of the first and third PCs in the response to fluence rate of green light. The symbols in this figure represent each treatment as follows, and each dot indicates one plant. ●, control; ■, 62G; ▲, 124G; ○, 248G; □, 496G.

green light. From the results of factor loading, the first PC showed the factor related to the general size, namely, isometric growth, and the second PC shows the factor related to the plant shape, namely, allometric growth. In addition, the third PC showed the factor related to the days to heading and culm elongation. Thus, the first and third PCs are suggested to be affected by PPF and green light, respectively. The second PC might be affected by PPF but not by green light, because the third PC was separated from the first and second PCs. These observations suggest that the developmental rate of wheat was promoted by the green light independent of the PPF. This is supported by the result that no correlation was found between the first PC and the third PC in the scatter diagram of scores in each treatment of the first and third PCs (Fig. 4).

The third PC suggested the covariance between culm elongation and days to heading, because the number of days from emergence to 50% heading was

significantly correlated with main culm length (Fig. 3). Under the canopy, the proportions of blue light and red light are decreased; those of green and far-red light are slightly increased (Smith, 1982; Kasajima et al., 2006). When plants sense the low red:far-red ratio (R:FR) by dense plant canopies, they elongate more rapidly and accelerate development to produce seeds early for survival (Goto, 2003). In the present study, increased green light under the dense canopies might function to regulate the development and culm elongation. We will conduct further experiments on the ecological significance of green light under different canopy densities, because there are few reports on the role of green light under the canopy.

In *Arabidopsis*, a photoperiod-independent light-quality pathway that regulates the flowering time, has been proposed (Ausín et al., 2005). The results of the present experiment under continuous light at a constant temperature may support the existence of a metabolic pathway that is independent of either photoperiod or vernalization response. Such a pathway might be related to the genotype, but further studies on varietal difference are needed to clarify the relationship.

In conclusion, the present study suggested that the developmental process is influenced by the photon flux density of green light. The green light affects the developmental rate of wheat independent of PPFD, which suggests that green light regulates the development of wheat through the action of an unknown photoreceptor. These results may be relevant to the ecophysiological understanding of the developmental responses to green light, and to the expression of a novel photobiological function in relation to green light.

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* In Japanese.