Effect of Red Light Irradiance on Circumnutation of Arabidopsis thaliana Inflorescence Stems

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The nutation frequency of Arabidopsis inflorescence stems gradually decreased when cultivated under dark or irradiation of weak white light (<5 μmol m⁻² s⁻¹). Re-irradiation of white, blue, red and far-red light during dark treatment had different effects on the nutation frequency. The nutation frequency of inflorescence stems increased soon after irradiation with a red light. This quick response in the nutation frequency to red light irradiation was observed in wild-type plant and phyB mutant, but not in either phyA or phyAphyB mutants. These results suggest that the light intensity and the red light photoperception via phytochrome A play important roles in the regulation of circumnutation.

Keywords: differential growth, internal oscillator, light perception, nutation frequency, phytochrome

INTRODUCTION

Elongating plant organs display elliptical or circular growth movement, usually known as a circumnutation movement (Darwin and Darwin, 1880; Johnsson, 1997). The mechanism of circumnutation is still poorly understood. One possible reason for this is that the analysis of plant organs’ movement is difficult without specific monitoring devices. Therefore, many researchers have tried to develop systems to monitoring the movements of plant organs. Recently, digital devices such as digital cameras and video have been using for acquiring plant images (Hayashi et al., 2004, Ishizuka et al., 2005, Kitazawa et al., 2005; Schuster and Engelmann, 1997; Tanabata and Shinomura, 2004). These digital devices have made progress possible in plant movement research. To analyze the circumnutation of plant organs, we have also utilized time-lapse monitoring via digital video camera (Niinuma et al., 2005; Someya et al., 2005, 2006).

It has been demonstrated that the circumnutation of plant organs is influenced by various external stimuli (Ginzo and Décima, 1995; Hatake et al., 2003; Zachariaisen et al., 1987). We previously reported a relationship between white light irradiance and circumnutation of Arabidopsis inflorescence stems (Someya et al., 2006). However, a clear relationship between other light conditions such as blue, red or far-red light and circumnutation has not been demonstrated. It is common knowledge that light intensity and quality affect the growth and development of plants (Hanyu

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and Shoji, 2000; Morelli and Ruberti, 2002). The aim of the present paper was to study the influence of different light conditions on the circummutation of *Arabidopsis* inflorescence stems using a digital video camera system.

**MATERIALS AND METHODS**

*Plant materials*

*Arabidopsis thaliana* genotype Columbia, Landsberg *erecta*, their phytochrome-deficient mutant phyA211 (Columbia background: Col), phyB9 (Col), phyA201 (Landsberg *erecta* background: Ler), phyB5 (Ler) and phyA201/B5 (Ler) were used in this study. Mutants were purchased from the *Arabidopsis* Biological Resource Center (Columbus, OH, USA). Plants were grown under continuous white light of 30 μmol m⁻² s⁻¹ as described previously (Someya et al., 2006). Photon flux was measured with a quantum photometer (model LI-250, Li-Cor, Lincoln, NE, USA) using a quantum sensor (model LI-190SA, Li-Cor, Lincoln, NE, USA). The plants were used at the age of approximately 1 month when inflorescence stems are about 50 (±10 mm) mm long.

*Plant movement measurements and analysis*

The movement of inflorescence stems was recorded by a digital video camera (DCR-TRV50, Sony Corporation, Tokyo, Japan) vertically and horizontally. Infrared imaging was employed to monitor the inflorescence stem in the dark condition, since the inflorescence stems are physiologically blind to this wavelength (Iino and Carr, 1981; Orbović and Poff, 1991). Recordings were made at 10 min intervals for 72 h, and the images were analyzed using the NIH image program (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/nih-image/). The number of nutations of the inflorescence stems around their axis during 3 h periods from 0 h until 72 h was calculated from the video images, and recorded as the nutation frequency (number of nutations per 3 h). More than 10 individual plants were examined in each experiment.

*Effect of different light treatments on the circummutation of Arabidopsis inflorescence stems*

The movement of the inflorescence stems of plants that had been pre-cultured under continuous white light was monitored under different light treatments: continuous light (white, blue, red and infra-red), dark-light transitions and continuous darkness (physiological darkness). Plants (Col) were monitored under continuous white light of 1, 2.5, 5, 10, 15, 20 and 30 μmol m⁻² s⁻¹ provided by white light fluorescent tubes, and under continuous blue, red or far-red light of 30 μmol m⁻² s⁻¹ provided by a light-emitting diode [LED, MIL-R18 (red), MILIF18 (far-red) or MIL-B18 (blue), Sanyo, Osaka, Japan]. In dark-light transition treatments, plants were incubated in the dark for 36 h, before re-exposure to white, blue, red, or far-red light of 30 μmol m⁻² s⁻¹. The intensity of blue and red light was measured with a quantum photometer (model LI-250, Li-Cor, Lincoln, NE, USA) using a quantum sensor (model LI-190SA, Li-Cor, Lincoln, NE, USA), and far-red light was measured with a quantum photometer using an SKR 110 sensor (Skye Instruments Ltd., Powys, UK). Phytochrome-deficient mutants of *Arabidopsis*, phyA211 (Col), phyB9 (Col), phyA201 (Ler), phyB5 (Ler) and phyA201/B5 (Ler), were incubated in the dark for 36 h before irradiance to a red light of 30 μmol m⁻² s⁻¹ provided by the LED.

**RESULTS AND DISCUSSION**

The circummutatory behavior of *Arabidopsis* inflorescence stems in genotype Col was drastically changed by dark treatment (Someya et al., 2006). An important feature of the change was a decrease in their nutation frequency upon transfer to the dark condition. Under continuous irradiance with white light, circummutation of inflorescence stems showed a uniform frequency throughout the monitoring periods. The nutation frequency was maintained under white light of 10
μmol m⁻²s⁻¹ but decreased under white light of 5 μmol m⁻²s⁻¹ (Fig. 1). When plants were transferred to continuous darkness, the nutation frequency started to decrease after about 12 h, approaching zero after about 48 h. The nutation frequency of inflorescence stems was also maintained under continuous red light of 30 μmol m⁻²s⁻¹, however the nutation frequency decreased under blue and far-red light of 30 μmol m⁻²s⁻¹ (Fig. 2). A clear relationship between light intensity and circumnutation has not been demonstrated previously. This is the first report that maintenance of the nutation frequency in Arabidopsis inflorescence stems requires at least 10 μmol m⁻²s⁻¹ of white light intensity.

After 36 h of dark treatment, which reduced the nutation frequency by approximately 50%, plants were re-irradiated with either white or red light. The nutation frequency of the inflorescence stems soon increased by approximately twofold (Fig. 3). When the inflorescence stems were irradiated with a blue light 36 h after dark treatment, the inflorescence stems showed irregular movement and this irregularity was thought to be caused by phototropism. Therefore, we could not accurately calculate the nutation frequency for blue light treatment. On the other hand, re-irradiance of far-red light did not cause the nutation frequency to recover (Fig. 3). These results suggest that red light influences the regulation of nutation frequency.

![Fig. 1](image1.png)  
**Fig. 1** The nutation frequency of Arabidopsis (genotype Columbia) inflorescence stems under continuous irradiance with white light at different light intensities. Data represents the average of each treatment (n=10).

![Fig. 2](image2.png)  
**Fig. 2** Influence of light quality on the nutation frequency of Arabidopsis (genotype Columbia) inflorescence stems. Plants were incubated under continuous white (WL), blue (BL), red (RL) and far-red (FR) light of 30 μmol m⁻²s⁻¹. Data represents the average of each treatment (n=10).
The nutation frequency of wild-type plants, which was incubated in darkness for 36 h, increased by approximately twofold within 3 h after red light irradiance (Fig. 3). However, the quick response of inflorescence stems to red light was not observed in phyA (both of Col and Ler backgrounds) and phyAphyB (Ler) mutants within 3 h after red light irradiance (Table 1). On the other hand, phyB mutants (both of Col and Ler backgrounds) showed a quick response (<3 h) soon after red light irradiance. The phyA mutants (both of Col and Ler backgrounds) did not lose the responsiveness to red light irradiance, therefore, it was considered that phytochrome A plays a role in the regulation of nutation frequency. Yoshihara and Iino (2005) reported that red light stops circumnutation of dark-grown rice coleoptiles. Our observation showed a different effect of red light on the circumnutation of Arabidopsis inflorescence stems. This variability in the effect of red light may be due to differences in the light condition. The Arabidopsis plants used for this experiment were grown under continuous light, but rice coleoptiles used in the report were grown in the dark. Here, we focus on the effect of red light perception via phytochromes against the regulation of circumnutation.

Although the meaning and the mechanism of circumnutation are still issues to be elucidated, circumnutation was thought to be regulated by an internal oscillator (Brown, 1993; Darwin and Darwin, 1880). Johnsson et al. (1999) proposed a model in which an internal oscillator and

![Graph showing nutation frequency over time.]

**Table 1** Effect of red light irradiance on the nutation frequency of inflorescence stems in Arabidopsis phytochrome mutants.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>27–30</th>
<th>30–33</th>
<th>33–36</th>
<th>36–39</th>
<th>39–42</th>
<th>42–45 (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (Col)</td>
<td>0.8±0.2</td>
<td>0.7±0.2</td>
<td>0.7±0.2</td>
<td>1.2±0.4</td>
<td>1.3±0.3</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>phyA (Col)</td>
<td>0.9±0.4</td>
<td>0.9±0.3</td>
<td>0.9±0.3</td>
<td>0.8±0.3</td>
<td>1.3±0.4</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>phyB (Col)</td>
<td>1.1±0.4</td>
<td>0.9±0.4</td>
<td>0.9±0.4</td>
<td>1.5±0.4</td>
<td>1.7±0.4</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td>WT (Ler)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2±0.1</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>phyA (Ler)</td>
<td>0.2±0.1</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>0.3±0.2</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>phyB (Ler)</td>
<td>0.4±0.1</td>
<td>0.3±0.1</td>
<td>0.3±0.2</td>
<td>0.6±0.1</td>
<td>0.7±0.2</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td>phyAphyB (Ler)</td>
<td>0.4±0.2</td>
<td>0.3±0.2</td>
<td>0.3±0.2</td>
<td>0.2±0.1</td>
<td>0.5±0.2</td>
<td>0.6±0.3</td>
</tr>
</tbody>
</table>

'Col and Ler represent ecotype Columbia and Landsberg erecta, respectively. (WT) wild type; (phyA) phytochrome A-deficient mutant; (phyB) phytochrome B-deficient mutant; (phyAphyB) phytochrome A- and B-deficient double mutant.

All plants were treated with 36 h darkness followed by red light irradiance (30 μmol m⁻²s⁻¹). Values are means±standard deviation (n=10).
gravitropism work jointly to regulate circumnutation. We do not yet know how the circumnutation of *Arabidopsis* inflorescence stems is caused. However, we observed three significant points in the present study: i) maintenance of the nutation frequency requires at least 10 μmol m⁻² s⁻¹ of white light intensity, ii) only red light irradiance can also maintain the nutation frequency, and iii) phytochrome A-deficient mutants lost their quick response in the nutation frequency to red light irradiation. We were thus able to show that a red light photoperception via phytochrome A plays an important role in the circumnutation of *Arabidopsis* inflorescence stems. The red light may control the nutation frequency by sending signals to a proposed internal oscillator that regulates the rhythm of the nutation. Moreover, we previously reported that the circumnutation of *Arabidopsis* inflorescence stems is required for at least two phytohormones, auxin and brassinosteroid (Someya et al., 2005). Tepper and Yang (1996) also reported that exogenous auxin restored circumnutation in decapitated green pea seedlings, which had not previously shown circumnutation. Because the light intensity and quality affected the nutation frequency, phytohormone degradation or both of the distribution and quantity of some phytohormones via light perception might have influenced the circumnutation of the *Arabidopsis* inflorescence stems.

REFERENCES