



HOKKAIDO UNIVERSITY

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Title of Doctoral Dissertation

Effect of Circularly Polarized Light on Germination, Hypocotyl Elongation and Biomass

Production of Arabidopsis and Lettuce; Involvement of Phytochrome B

(植物における左右円偏光の発芽・胚軸伸長・成長に与える影響に関する研究)

Depending on the red light intensity and duration of light illumination studies by circular polarized lights for indicating that the Phy B molecules responsible for the seed germination and hypocotyl elongation able to sense the chirality of the red light.

The germination rates of Arabidopsis seeds in the presence of L- and R-CPL were 58.4% and 68.2%, respectively, indicating that R-CPL was more effective than L-CPL in the red light-absorbing region. Similarly, the germination rates of lettuce seeds in the presence of L- and R-CPL were 50.7% and 59.9%, respectively. Calculations showed that the germination rates of Arabidopsis and lettuce were 1.17- and 1.18-fold greater, respectively under R- than L-CPL.

Assessment of the effects of red CPL on hypocotyl elongation of Arabidopsis showed that the average hypocotyl lengths under L- and R-CPL were 6.4 mm and 5.4 mm, respectively, for 7-day-old seedlings and 7.2 mm and 6.0 mm, respectively, for 10-day-old seedlings. Similarly, the average hypocotyl lengths of 7-day-old lettuce seedlings under L- and R-CPL were 27.2 mm and 23.0 mm, respectively. Calculations showed that the hypocotyls of 7- and 10-day-old Arabidopsis seedlings and of 7-day-old lettuce seedlings were 1.18-, 1.20- and 1.18-fold longer, respectively, under L-CPL than under R-CPL. The involvement of phyB in the red CPL effect on hypocotyl elongation was assessed by measuring hypocotyl lengths in a phyB-deficient mutant of Arabidopsis (*phyB*) grown under L-CPL and R-CPL. The average hypocotyl lengths of 7-day-old wild-type seedlings under L-CPL and R-CPL were 6.7 mm and 5.3. In contrast, hypocotyls of the *phyB* mutant were longer than those of wild-type under both L- and R-CPL, being 12.5 mm and 12.6 mm, respectively, and were almost equal, suggesting that phyB is involved in the red CPL effect on hypocotyl elongation.

To study the involvement of phyB in the observed effects of CPL, UV-visible (UV-Vis) absorption spectra of a PCB-bound sensory module of Arabidopsis phyB, *AtphyB-N651*, were measured in Pr and a photostationary state between Pr and Pfr induced by saturating with red light illumination. Pfr spectra were constructed by subtracting the Pr spectrum from the spectrum of the photostationary state, so that the shoulder on the Pr spectrum disappears. The first absorption peaks of Pr and Pfr were at 650 nm and 713 nm, respectively, while their second absorption peaks were at 358 and 372 nm, respectively, with the latter having a shoulder at around 415 nm. These absorption peaks are characteristic of those

of PCB-bound cyanobacteria phytochrome 1 (Cph1) and were about 15 nm blue shifted from those of PΦB-bound Arabidopsis phyB27, with the blue shift due to the lack of a one π -electron conjugating system at the edge of the linear tetrapyrrole in PCB.

CD spectra of PCB-bound *AtphtB*-N651 were also measured in Pr and a photostationary state between Pr and Pfr induced by saturating with red light illumination. Because of the actinic effect of the strong measuring beam light of CD, the Pr spectrum during CD measurement showed formation of Pfr, as well as a decrease in Pr in the photostationary state. The UV-Vis absorption spectra measured immediately after CD measurements after illumination with saturating far-red and red light were approximated by superimposition of 89% Pr and 11% Pfr spectra and by 44% Pr and 56% Pfr spectra, respectively. Based on these fractions, the CD spectra of 100% Pr and 100% Pfr were constructed from the CD spectra. The CD spectrum of Pr has negative and positive CD Cotton effects in the regions of the first and second absorption bands, respectively, whereas the Pfr CD spectrum of Pfr has a positive Cotton effect in the region of the first absorption band and complex signals in the region of the second absorption band.

In conclusion, All these results as well as our findings, suggest that phyB is involved in the differential effects of L- and R-CPL on seed germination and hypocotyl elongation.