

## Shade Avoidance and the Regulation of Leaf Inclination in Rice

Juhee Shin<sup>1</sup>, and Phun Bum Park<sup>1,\*</sup>

<sup>1</sup>Department of Bioscience and Biotechnology, University of Suwon, Hwasung 445-743, Korea

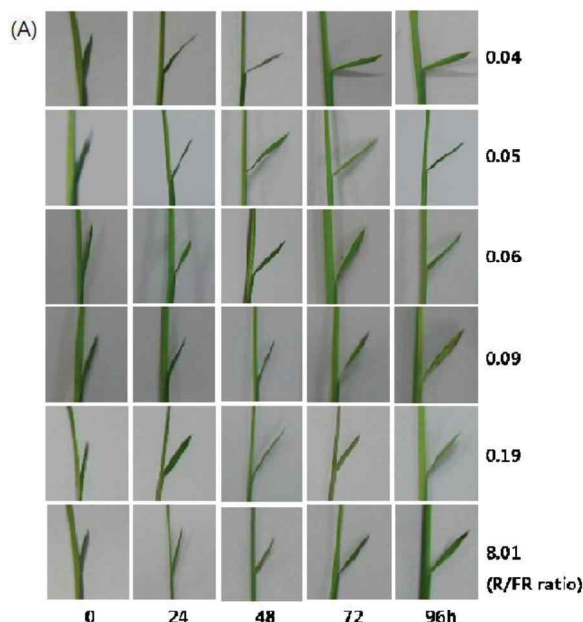
**ABSTRACT:** The shade avoidance syndrome is a morphological and physiological response when plants are exposed to shade. Recent work in *Arabidopsis* had begun to define the molecular components of the shade avoidance syndrome in dicotyledonous model plant. However, little is known about the shade avoidance response networks in agriculturally important monocotyledon crops such as rice. Here, we found that the degree of bending at the lamina joint is inversely proportional to the R:FR ratio. To elucidate which phytochrome is involved in this response, we did lamina joint inclination assay with the rice phytochrome-deficient mutants (*osphyA*, *osphyB*, and *osphyC*) and the wild type plants. Whereas the *osphyA* and *osphyC* knockout mutants bent at the lamina joint in the far-red rich condition as the wild type plants, the *osphyB* knockout mutants no longer bent at the lamina joint in the far-red rich condition. These results suggest that PHYB acts as a sole photoreceptor in the lamina joint inclination response in rice.

Plant responses to shade (the reduced ratio of red light to far-red light) referred to as the shade avoidance syndrome (SAS). The phytochrome photoreceptors perceived R:FR ratio change, then control the adaptive responses such as stem elongation, leaf expansion, seed germination, leaf inclination, and flowering time<sup>1-3</sup>. Even though PHYB is the major photoreceptor which controls SAS, physiological evidences have proven that other phytochromes act redundantly in case of flowering time (PHYD, PHYE), petiole elongation (PHYD, PHYE) in *Arabidopsis*. The reduced R/FR ratio decreases PHYB activity, and activates the auxin-related genes through phytochrome interacting factors (PIFS) leading to stem elongation, and also SAS links gibberellins, ethylene, and brassinosteroid signaling<sup>4,5</sup>. Recent studies in *Arabidopsis* have defined the molecular networks of the SAS, however little is known in agronomically important grasses such as rice<sup>6,7</sup>.

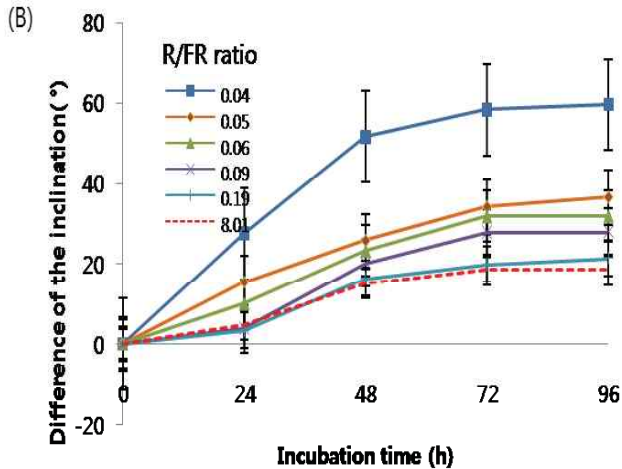
The objective of this study was to investigate SAS in rice seedlings. Eight-day old wild type rice seedlings were transferred to the 6 different R/FR ratio chambers. The lamina joint inclination of rice seedlings had measured for 4 d. The lamina joint inclination increased as the R/FR ratio decreasing (Fig 1A). The difference of the inclination between the starting point and after 4 d in red light rich condition was 18.5° (R/FR=8.01), whereas the difference was 59.5° in far-red light rich condition (R/FR=0.04) (Fig. 1B). The first leaf of rice seedling in far-red rich light condition was almost vertical. Based on the above observation, we

confirmed that the lamina joint inclination is the one of the SAS in rice seedlings.

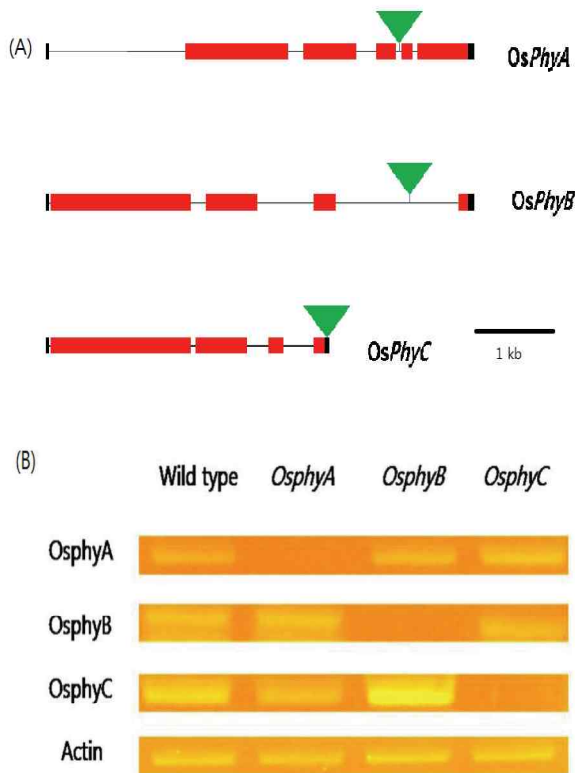
Because SAS is largely mediated by the phytochrome family of photoreceptors, we examined the rice seedling inclination of mutants deficient in individual phytochromes to determine which phytochrome is involved in this lamina joint inclination. The seeds of T-DNA insertional mutants of *OsPhyA*, *OsPhyB*, and *OsPhyC* gene were provided from Dr. An in Kyung Hee University. In seedling stage, we examined the expression level of gene of phytochrome family in T-DNA insertional mutants and wild type plants (Fig. 2A). In wild type seedling, *OsPhyA*, *OsPhyB*, and *OsPhyC* gene was expressed, however in T-DNA insertional mutants seedling, each phytochrome gene was not expressed at all (Fig. 2B). Fifteen-day old rice seedlings were transferred to the far-red rich chamber (R/FR=0.04) and the red-rich chamber (R/FR=8.01), and the lamina joint inclination had measured for 4 d. As in the wild type, the lamina joint inclination increased as the R/FR ratio decreasing in *osphyA* and *osphyC* mutant seedlings (Fig. 3ABD). However, the angle of the lamina joint was approximately 120° in the red-light rich condition, and the lamina joint inclination did not increase in the far-red light rich condition in case of *osphyB* mutant seedlings (Fig. 3C). Thus, the far-red light rich condition did not affect the lamina joint inclination in the *osphyB* plants. Because the expression of brassinosteroid-inducible genes were enhanced and the expression of brassinosteroid-biosynthetic genes were decreased in *osphyB* mutants, the angle of the lamina joint was 120° compared with 40° for the wild type seedling in the red light rich condition<sup>8</sup>.



\*To whom correspondence should be addressed.  
E-mail: pbpark@suwon.ac.kr



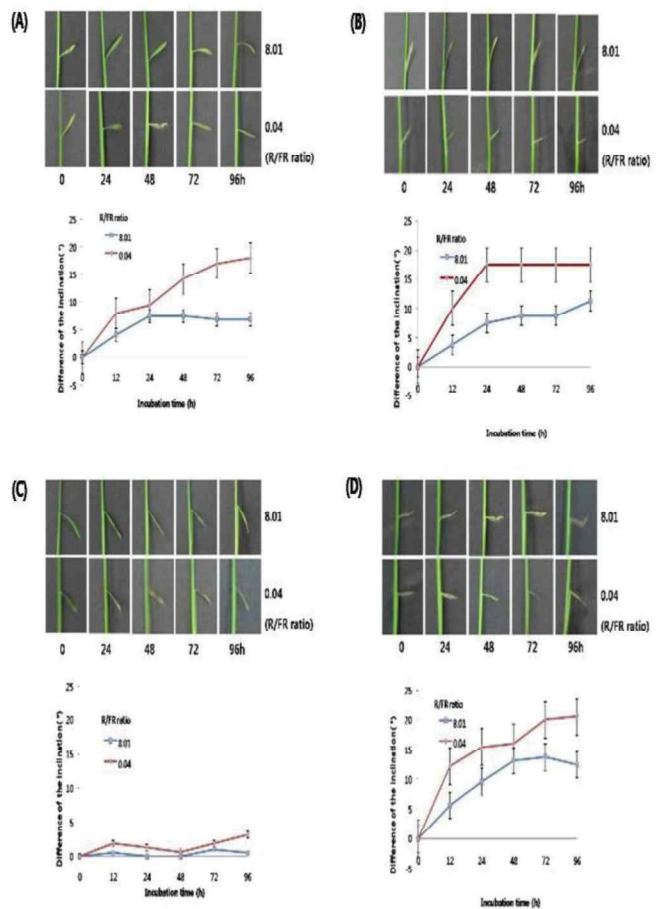
**Figure 1.** Effect of light quality on the lamina inclination. Rice seedlings were grown under the continuous white light ( $10.5 \mu\text{molm}^{-2}\text{s}^{-1}$ ) for 8 d. Rice seedlings were then transferred to the designated FR-rich shade conditions and grown for 4 d (A). Lamina joint assays were carried out under the continuous white light and the designated FR-rich shade conditions (B). Data are the means of the results from 20 plants. The error bars indicate the standard error (triplicates).



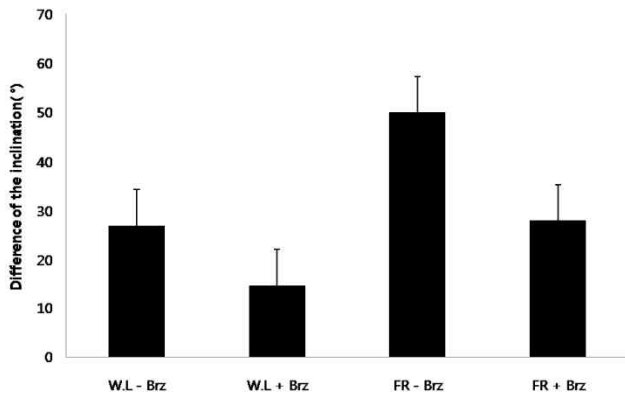
**Figure 2.** T-DNA insertional mutants of rice phytochrome genes. Schematic diagrams of *OsPhyA*, *OsPhyB*, and *OsPhyC* genes and T-DNA insertional sites (A). Boxes are exons and lines are introns. Filled boxes are coding regions. RT-PCR analysis of *OsPhyA*, *B*, *C* gene expression in wild type and T-DNA insertional mutants of rice phytochrome genes. Actin transcripts served as an internal control.

Because the lamina joint inclination is linked with the brassinosteroid signaling, we treated the brassinosteroid biosynthesis inhibitor, the brassinazol to the rice seedlings and checked the leaf inclination<sup>9</sup>. The difference of inclination in red-light rich condition (R:FR=8.01), was 27°, while the difference decreased to 14.8° in the presence of brassinazol. In case of far-red light rich condition (R:FR=0.041), the difference of leaf inclination was 50°, and the difference decreased to 28° in the presence of brassinazol (Fig. 4). Thus, these results suggest that brassinosteroid acts positively in this SAS (leaf inclination).

In conclusion, leaf inclination in far-red light rich condition is one of the SAS in rice seedlings, and PHYB is responsible for this SAS. The leaf inclination is linked with the brassinosteroid signaling. The transcripts of brassinosteroid-inducible and brassinosteroid-biosynthetic genes will be checked in the far-red light rich condition in near future.



**Figure 3.** Lamina joint inclination of phytochrome mutants. Rice seedlings were grown under the continuous white light ( $10.5 \mu\text{molm}^{-2}\text{s}^{-1}$ ) for 15 d. Rice seedlings were then transferred to the designated FR-rich shade conditions and grown for 4 d. Lamina joint assays were carried out under the continuous white light and the designated FR-rich shade condition. Data are means of the results from 20 plants. The error bars indicate the standard error (triplicates). A; wild type B; *OsphyA* mutant seedlings C; *OsphyB* mutant seedlings D; *OsphyC* mutant seedlings.



**Figure 4.** Effect of brassinazole on the leaf inclination. Wild type rice seedling were grown under the white light and FR-rich shade condition for 10 days with or without 5  $\mu$ M brassinazole prior to the measurement of leaf inclination. Data are means of the results from 20 plants. The error bars indicate the standard error (triplicates).

**KEYWORDS:** Shade avoidance syndrome, Lamina joint inclination, Phytochorme B, Rice

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#### REFERENCES

1. Smith, H. *Annu. Rev. Plant Physiol.* **1982**, *33*, 481-518.
2. Smith, H.; Whitelam, G. C. *Plant Cell Environ.* **1997**, *20*, 840-844.
3. Mullen, J. L.; Weinig, C.; Hangarter, R. P. *Plant Cell Environ.* **2006**, *29*, 1099-1106.
4. Devlin, P. R.; Patel, S. R.; Whitelam, G. C. *Plant Cell* **1998**, *10*, 1479-1487
5. Devlin, P. R.; Robson, P. R. H.; Patel, S. R.; Goosey, L.; Sharrock, R. A.; Whitelam, G. C. *Plant Physiol.* **1999**, *119*, 1479-1487.
6. Kebrom, T. H.; Brunell, T. P. *J. Exp. Bot.* **2007**, *58*, 3079-3089.
7. Casal, J. J. *Annu. Rev. Plant Biol.* **2013**, *64*, 403-427
8. Jeong, D.-H.; Lee, S.; Kim, S. L.; Hwang, I.; An, G. *Plant Cell Environ.* **2007**, *30*, 590-599.
9. Chung, Y.; Maharjan, P. M.; Lee, O.; Fujioka, S.; Jang, S.; Kim, B.; Takatsuto, S.; Tsujimoto, M.; Kim, H.; Cho, S.; Park, T.; Cho, H.; Hwang, I.; Choe, S. *Plant J.* **2011**, *66*, 564-578.