Characterization of *lbs* (*light BTB suppressor*) Mutants in *Arabidopsis thaliana*

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Introduction

A plant's ability to assess light quantity and quality is fundamental to maintaining healthy growth. One way that plants sense changing light conditions is via the perception of red (R~670 nm) and far red (FR~730 nm) wavelengths by a group of light receptors called the phytochromes. Phytochromes are principle mediators of shade induced elongation responses, which can negatively impact agronomic performance by increasing lodging or decreasing yield. For this reason, there has been increasing interest in engineering these responses in crop plants. However, currently the pathways for R/FR light reception are not fully understood.

We have identified two genes which act in the red light-response pathway in *Arabidopsis*: *Light Response BTB1 (LRB1)* and *Light Response BTB2 (LRB2)*. Plants with disruptions of these two genes are red light hypersensitive and have reduced hypocotyl length when grown under red light. We hypothesize that *LRB1* and *LRB2* target some component in the phytochrome B pathway for degradation.

In order to identify that target, or other components in red light signaling, we have conducted a genetic suppressor screen, identifying mutations which relieve red light inhibition of hypocotyl elongation in the *lrb1/lrb2* double mutant. This screen is ongoing, but thus far we have identified and confirmed four individuals with moderate to strong suppressor phenotypes. We are currently conducting detailed phenotypic characterization of these suppressor lines to determine the effect of these mutations on other red light-regulated processes. Here we describe these suppressors in terms of hypocotyl length grown under both dark and red light conditions, and verify their *lrb1-1/lrb2-1* alleles.

Suppressor Mutant Screen Strategy

Mutagenize population of *lrb1/lrb2* seeds with ethylmethanesulfonate (EMS).

Germinate seeds and grow plants (10 plants/pot), 2000 individuals total.

Collect seed from these individuals.

Germinate and grow this next generation under red light; identify individuals which have reduced red light hypersensitivity. (These *lbs* mutants possess longer hypocotyls).

Description of Suppressors

lbs1

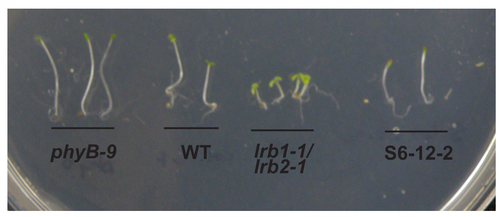

Figure 1. A. *lbs1* (S6-12-2), double mutant (*lrb1-1/lrb2-1*), wild type Columbia ecotype (WT), and phytochrome B mutant (*phyB-9*) individuals grown for 5 days under 1 µM/m²/sec. red (670 nm) wavelength light. **B.** Quantitative analysis of *phyB-9*, WT, *lrb1-1/lrb2-1*, and *lbs1* individuals grown for 5 days under 10 µM/m²/sec. red light (green bars) or in the dark (black bars). The *phyB-9* mutant has a disruption of the phytochrome B red-light photoreceptor and is mostly red light insensitive. Values for n are located above the standard error bars.

lbs2

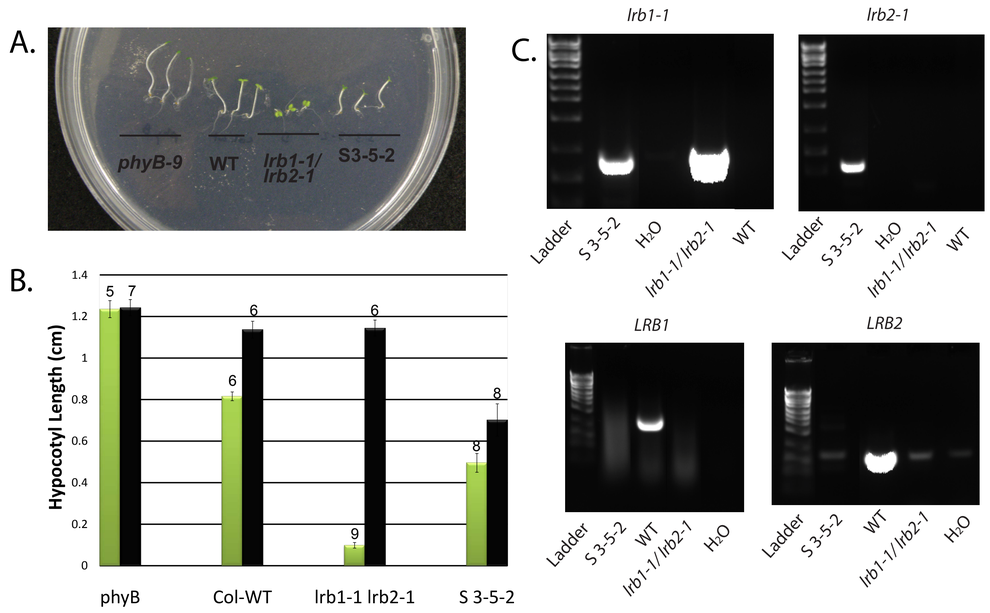

Figure 2. A. *lbs2* (S3-5-2), double mutant (*lrb1-1/lrb2-1*), wild type Columbia ecotype (WT), and phytochrome B mutant (*phyB-9*) individuals grown for 5 days under 10 µM/m²/sec. red (670 nm) wavelength light. **B.** Analysis of *phyB-9*, WT, *lrb1-1/lrb2-1*, and *lbs2* individuals grown for 5 days under 10 µM/m²/sec. red light (green bars) or in the dark (black bars). Values for n are located above the standard error bars. **C.** Gel electrophoresis analysis of *lbs2* polymerase chain reaction (PCR) products, which verify the presence of the T-DNA disruptions in *LRB1* and *LRB2*. WT, *lrb1-1/lrb2-1*, and water were used as control reactions. Top left image shows a PCR product verifying the presence of the *lrb1-1* T-DNA insert within the *LRB1* gene. Top right image shows a PCR product verifying the presence of the *lrb2-1* T-DNA insert within the *LRB2* gene. Bottom left image shows a PCR product only in the presence of a WT *LRB1* gene. Bottom right image shows a PCR product only in the presence of a WT *LRB2* gene.

lbs3

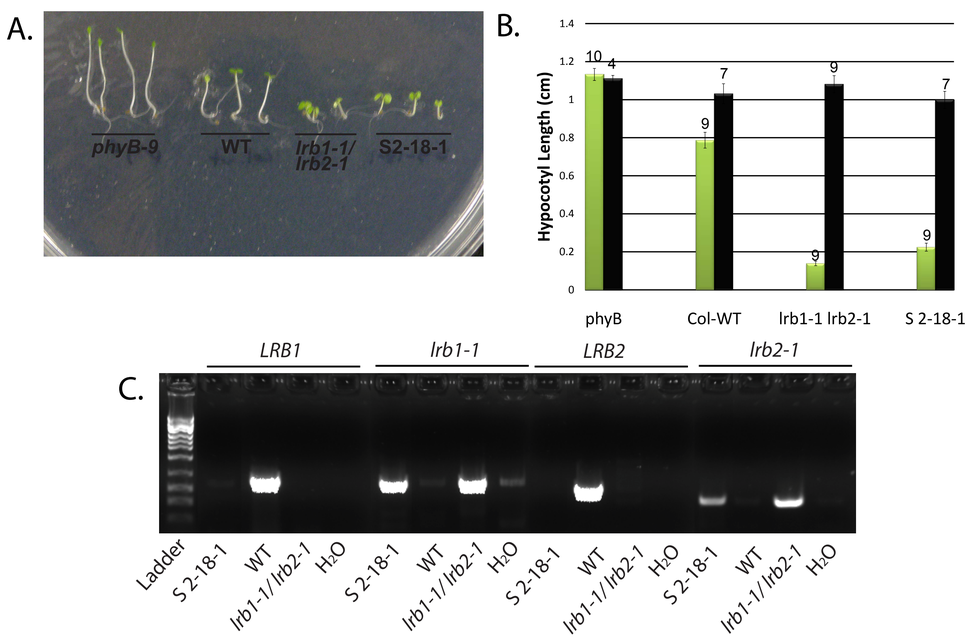

Figure 3. A. *lbs3* (S2-18-1), double mutant (*lrb1-1/lrb2-1*), wild type Columbia ecotype (WT), and phytochrome B mutant (*phyB-9*) individuals grown for 5 days under 10 µM/m²/sec. red (670 nm) wavelength light. **B.** Analysis of *phyB-9*, WT, *lrb1-1/lrb2-1*, and *lbs2* Individuals grown for 5 days under 10 µM/m²/sec. red light (green bars) or in the dark (black bars). Values for n are located above the standard error bars. **C.** Gel electrophoresis analysis of *lbs3* polymerase chain reaction (PCR) products, which verify the presence of the T-DNA disruptions in *LRB1* and *LRB2*. WT, *lrb1-1/lrb2-1*, and water are control reactions. Starting from the left, gel lanes underneath the horizontal *LRB1* line (above the image) show a PCR product only in the presence of a WT *LRB1* gene. Gel lanes underneath the horizontal *lrb1-1* line show a PCR product verifying the presence of the *lrb1-1* T-DNA insert within the *LRB1* gene. Gel lanes underneath the horizontal *LRB2* line show a PCR product only in the presence of a WT *LRB2* gene. Gel lanes underneath the horizontal *lrb2-1* line show a PCR product verifying the presence of the *lrb2-1* T-DNA insert within the *LRB2* gene.

lbs4

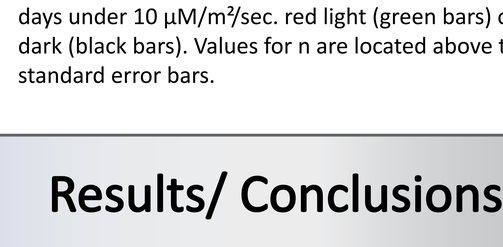

Figure 4. A. *lbs4* (S3-11-1), double mutant (*lrb1-1/lrb2-1*), wild type Columbia ecotype (WT), and phytochrome B mutant (*phyB-9*) individuals grown for 5 days under 10 µM/m2/sec. red (670 nm) wavelength light. **B.** Analysis of *phyB-9*, WT, *lrb1-1/lrb2-1*, and *lbs4* individuals grown for 5 days under 10 µM/m²/sec. red light (green bars) or in the dark (black bars). Values for n are located above the standard error bars.

Results/ Conclusions

- The suppressor mutations in the *lbs1-4* individuals repress the red-light hypersensitive phenotype caused by disruption of the *LRB1* and *LRB2* genes to varying degrees.
- This may suggest that we have identified multiple genes that have roles in red light signaling.
- Furthermore, dark grown *lbs2* hypocotyl length is similar to red light grown hypocotyl length under red treatment, suggesting that hypocotyl elongation has been uncoupled from light regulation in this mutant.

What's Next?

- We have begun perform a series of genetic analyses to identify the total number of genes we have mutated (multiple suppressor mutants may actually have mutations in the same gene).
- The suppressor mutants will be crossed with other mutants known to be affected in red light signaling to determine epistatic interactions and their relative location in the red light signaling pathway.
- We will determine if any of the mutations are in the gene for the phyB red light photoreceptor (a strong possibility).
- We are beginning the process of mapping the mutations to identify the genes disrupted.

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