Characterization of Lines Identified in a Genetic Screen for Genes Involved in Red Light Responses in the Model Plant 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~660 nm) and far red (FR~730 nm) wavelengths by a group of light receptors called the phytochromes (PHYS). PHY-mediated responses include de-etiolation (which includes reduced hypocotyl elongation and increased cotyledon expansion) and shade avoidance responses (which include stem and petiole elongation) (Mathews, 2006).

We have found that that two highly similar genes LIGHT RESPONSE BTB1 (LRR1) and LIGHT RESPONSE BTB2 (LRR2) act as negative regulators of PHY-mediated red light-responses in Arabidopsis (Christians et al., 2012). Plants with disruptions of these two genes are red light hypersensitive and thus display enhanced de-etiolation in response to red light and significant shade tolerance (Figure 1). LRR1 and LRR2 code proteins with a BTB (Brca1-Brat, Tramtrack, Broad Complex) domain. One well-characterized function of these domains is to link BTB proteins to Cullin 3 (CUL3) ubiquitin (Ub)-ligase complexes. In these complexes the BTB protein acts as the target adapter, recruiting the Protein(s) to be ubiquitinated by the E3 (Figure 2). We hypothesize that LRR1 and LRR2 regulate, via ubiquitination, some component in the red light signaling pathway.

In order to identify the component(s) of the red light signaling pathway that the LRRs are regulating (or other actors in the pathway) we have conducted a genetic suppressor/enhancer screen, identifying mutations which relieve or increase red light inhibition of hypocotyl elongation in the lrb1/lrb2 red hypersensitive double mutants. Here we describe this screen and characterization of these mutants.

Suppressor/Enhancer Screen Methods

**Strategy:**
Mutagenesis population of lrb1-1 lrb2-1 seeds with ethylmethane sulfonate (EMS).
Germinate and grow plants (100 plants/pot), 5000 individuals total.
Collect seed from these individuals.
Germinate and grow this next generation (M2) under red filtered light; identify individuals that have reduced or increased red light sensitivity compared to the lrb1-1/lrb2-1 double mutants.

- Approximately 30,000 M2 generation plants were screened for both suppressor and enhancer phenotypes.
- 100+ individuals with putative suppressor phenotypes and 400+ enhancer phenotypes were identified. 34 individuals with similar phenotypes were typically identified per M2 pool.
- M3 generation seedlings are being tested for various red, far red, and blue light responses.

**The lrb1-1/lrb2-1 Line Has a Mutation in the PHYB Gene**

We have identified one suppressor mutant that has a single mutation in PHYB (Figure 6A). This mutation prevents splicing of intron 1 (Figure 6B), and results in no PHYB protein being detected in extracts from seedlings with the lrb1-1/lrb2-1 mutation (Figure 6A). This suggests lrb1-1/lrb2-1 is a PHYB null mutant.

**Conclusions and Future Work**

- Our suppressor and enhancer screen successfully identified mutants which reduce or enhance the phenotype of a red light hypersensitive mutant in Arabidopsis thaliana.
- We have identified one suppressor mutant that has a single mutation in the PHYB gene preventing expression of PHYB.
- We are currently investigating more mutants, including some that have reduced or are lacking PHYB expression.
- We are beginning to identify more mutations present in our mutants using full genome sequencing.

**References**


**Image 1:** Four day old seedlings grown for three days in continuous 10 µmole/m2/sec. LED-generated red light. The lrb1/lrb2 double mutant has significantly shorter hypocotyls and larger cotyledons, indicating increased red light sensitivity.

**Image 2:** Predicted structure of BTB/CUL3 E3 ubiquitin ligure complexes. The E3 binds the target and ubiquitinates it. This ubiquitination typically leads to degradation of the target. LRR1 and LRR2 are predicted to encode BTB proteins.

**Image 3:** Mean hypocotyl length of Col-0, phyb-9, lrb1-1 lrb2-1, and the lrb1 line grown in the dark or under varying levels of R for 4 days. Each point represents ≥30 individuals with standard error bars shown.

**Image 4:** Mean hypocotyl length of Col-0, phyb-9, lrb1-1 lrb2-1, and lrb1 grown in the dark or under varying levels of FR (A) or B (B) for 4 days. Each point represents ≥30 individuals with standard error bars shown.

**Image 5:** The lrb1 line has a single mutation in the PHYB gene, changing the final nucleotide of the first intron from a G to an A. This mutation prevents splicing of intron 1 (Figure 6B). In the last nucleotide of the first intron of the PhyB mutant and discovered the mutation lbs1 PHYB gene. A small section of the genome that contained amongst others the genetic markers. These markers narrowed the region of the mutation to be in the dark (“Dark”) and seedlings that received an additional 6 hour-fluence of FR (Figure 6A). This suggests we discovered the mutation lbs1 PHYB mutation. The location of the mutation in lbs1 was determined through mapping using genetic markers. These markers narrowed the region of the mutation to be in the dark (“Dark”). Figure 6B shows the standard error bars shown.

**Image 6:** Immunoblot analysis of PHYB protein levels in crude extracts from phyb-9 mutants, wild-type (WT), lrb1/lrb2, lrb1-1 lrb2-1, and lrb1-1/lrb2-1 seedlings. Extracts were taken from seedlings grown for 5 days in the dark (“Dark”) and seedlings that received an additional 6 hour-fluence of FR. The relative levels of PHYB were visualized by immunoblotting with a PHYB antibody. The protein band corresponding to PHYB was detected in the seedlings treated with FR (Figure 6A). This result suggests improper splicing of intron 1 as the PHYB protein product produced by plants with the lrb1-1/lrb2-1 mutation is longer. Sequencing of the PHYB products confirmed that splicing of intron 1 is not spliced out in plants with the lrb1-1/lrb2-1 mutation. PME2 was used as a control.