A Genetic Screen Identifying Mutations Which Suppress the Phenotype of a Red Light Hypersensitive Mutant.

Gavin Sunde[1], Jordan Montpetit[1], Timothy D. Lauer[1], Matthew Christians[2], Richard D. Vierstra[2], Derek J. Gingerich[1]
[1]Department of Biology, University of Wisconsin-Eau Claire [2]Department of Genetics, University of Wisconsin-Madison

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).

We have found that that two highly similar genes *LIGHT RESPONSE BTB1 (LRB1)* and LIGHT RESPONSE BTB2 (LRB2) have a role in the red light-response pathway in Arabidopsis. 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). *LRB1* and *LRB2* are predicted to encode proteins with a BTB (Bric-a-Brac, Tramtrack, Broad Complex) domain. One well characterized function of these domains is to link BTB proteins to Cullin 3 (CUL3) proteins in BTB/CUL3 E3 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 *LRB1* and LRB*2* 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 *LRB*s 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.

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

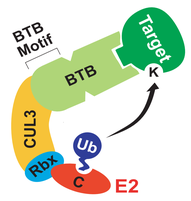
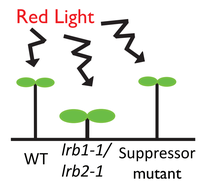


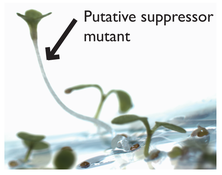
Figure 2. Predicted structure of BTB/CUL3 E3 ubiquitin ligase complexes. The E3 binds the target and ubiquinates it. This ubiquination typically leads to degradation of the target. *LRB1* and *LRB2* are predicted to encode BTB proteins.

Suppressor/Enhancer Screen Methods

Strategy:

- Mutagenize population of *lrb1-1/lrb2-1* 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 (M2) under red filtered light; identify individuals that have reduced or increased red light sensitivity compared to the *lrb1-1/2-1* double mutants.





- Approximately 30,000 M2 generation plants were screened.
- 109 individuals with putative suppressor phenotypes were identified. 3-4 individuals with similar suppressor phenotypes were typically identified per M2 pool, therefore we estimate that we have identified 30-35 independent suppressor lines.
- M3 generation seedlings are being tested for various red light responses.

Characterization of the Suppressor Lines

Hypocotyl elongation, cotyledon expansion, and cotyledon folding in response to red light are being tested in M3 generation individuals.

Hypocotyl Length

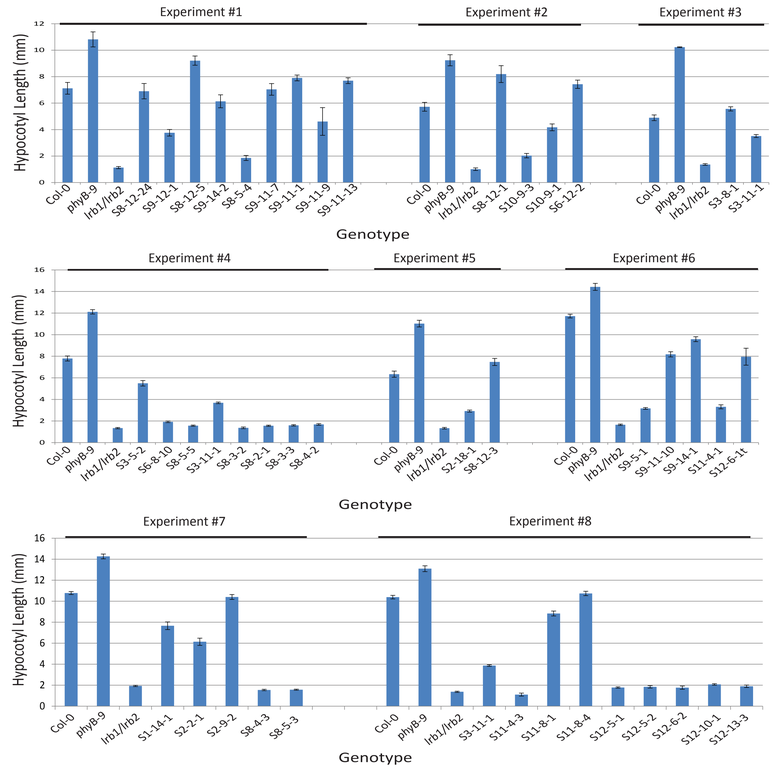


Figure 3. Mean hypocotyl lengths for 44 suppressor lines and corresponding controls grown under 10 µmol/m²/sec continuous red light. Data is from multiple experiments. The seeds were sterilized and plated on 1/2 MS media; cold treated at 4°C for four days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to 10 µmol/m²/sec red light for four days. Standard error bars are shown.

Cotyledon Folding

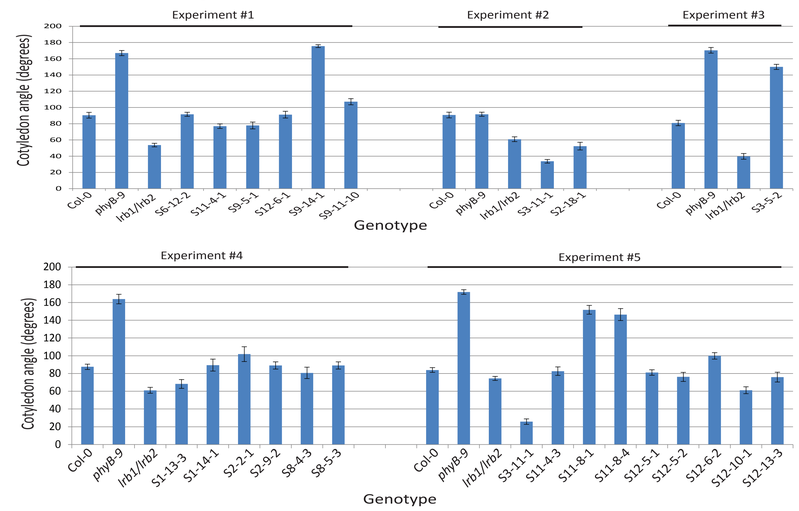


Figure 4. Mean cotyledon angles from 24 suppressor lines and controls from each experiment. The seeds were sterilized and plated on 1/2 MS media; cold treated at 4°C for 4 days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to 10 µmol/m²/sec red light for four days. Standard error bars are shown.

Cotyledon Area

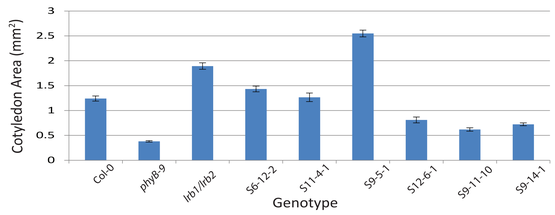


Figure 5. Mean cotyledon area from 6 suppressor lines and controls. The seeds were sterilized and plated on 1/2 MS media; cold treated at 4°C for 4 days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to 10 µmol/m²/sec red light for four days. Standard error bars are shown.

Suppressor Mutant 11-8-1

The S11-8-1 line has hypocotyl elongation that is constant under red light but increases in dark. The cotyledon folding is similar to that of the *phyB-9* mutant.

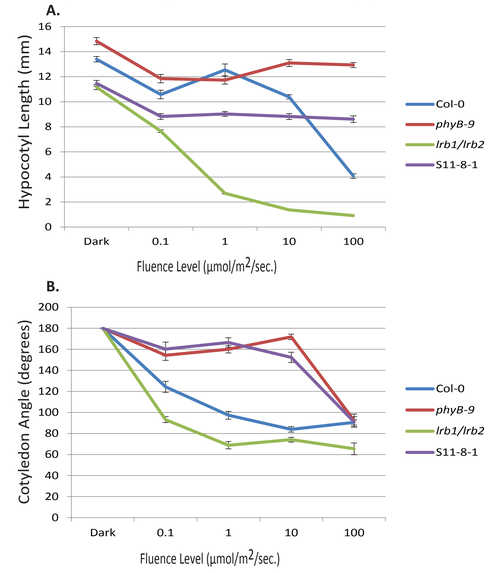


Figure 6 A. Mean hypocotyl length for line S11-8-1 and controls at 4 red light fluence levels and in the dark. The seeds were sterilized and plated on 1/2 MS media; cold treated at 4°C for four days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to red light for four days. Standard error bars are shown. **B.** Mean cotyledon angles of S11-8-1 and controls at four red light levels and dark. The seedlings were grown as above. Standard error bars are shown.

Suppressor Mutant 9-11-10

In this line red light inhibition of hypocotyl elongation is similar to WT, but cotyledon folding and expansion responses are intermediate between those seen in WT and *phyB-9*.

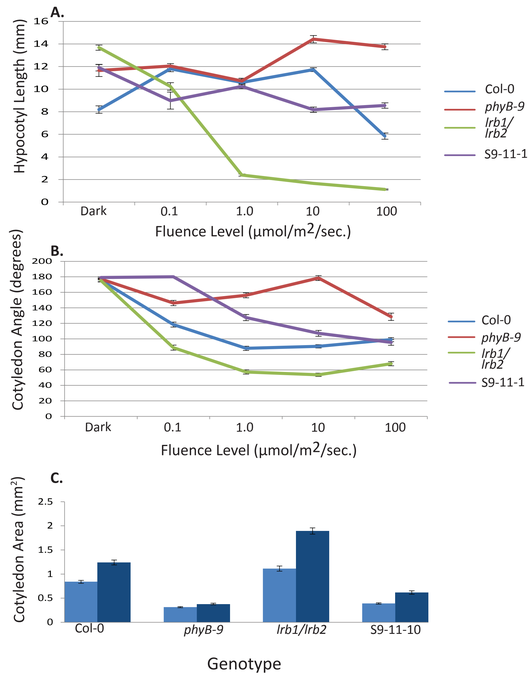


Figure 7 A. Mean hypocotyl length of S9-11-10 and controls at 4 red light levels and in the dark. Seeds were sterilized and grown on 1/2 MS media; cold treated at 4°C for four days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to dark, 0.1, 1.0, 10, or 100 µmol/m²/sec red light for four days. Standard error bars are shown. **B.** Mean cotyledon angles of S9-11-10 and controls at dark and four red light levels. Seedlings were grown as above. Standard error bars are shown. **C.** Mean cotyledon area of S9-11-10 and controls grown at 2 red light levels. Seedlings were grown as above. Standard error bars are shown.

Conclusions

- Our suppressor screen successfully identified mutants which reduce or enhance the phenotype of a red light hypersensitive mutant.
- Analysis of red light responses of a subset of our suppressor mutants shows that we have a pool of mutants with varying degrees of red light sensitivity.
- Based on the results from the characterizations, we will select a number of lines and map the location of the mutations in the genome.

This work was funded by a National Science Foundation-Research in Undergraduate Institutions (RUI) grant (#0919678), and University of Wisconsin - Eau Claire Differential Tuition through the UWEC Office of Research and Sponsored Progran s.