Genetic Enhancer Screen to Identify Red Light Hypersensitive Mutants in the Model Plant Species *Arabidopsis thaliana.*

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Introduction

A plant's ability to perceive and respond to both quantity and quality of light is an essential component of maintaining healthy growth and development. One way in which plants respond to changing light conditions is via their perception of varying amounts of red (~660nm) and far red (~730nm) wavelengths of light[1,2]. To do this, plants rely on a group of photoreceptors known as phytochromes (PHYs)[2]. PHY mediated responses include de-etiolation (which includes reduced hypocotyl elongation as well as increased cotyledon expansion) and shade avoidance responses (which includes stem and petiole elongation)[2,4].

We have found that the two genes *Light Regulating BTB 1 (LRB1)* and *Light Regulating BTB 2 (LRB2)* participate in the red light signaling pathway in Arabidopsis. Plants with mutations in these genes are red light hypersensitive and thus display an enhanced de-etiolation response and have significant shade tolerance[3] (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 *LRB2* regulate, via ubiquitination, some component in the red light signaling pathway.

In order to determine other components, genes, or factors involved in the red light response pathway we have conducted a series of genetic enhancer screens to identify mutations which exacerbate (or enhance) the phenotypic characteristics of the *lrb1/lrb2* double mutants. Here we describe the screen and characterization of these putative light mutants.

Figure 1. Four day old seedlings grown for three days in continuous 10 μmol/m²/sec. LED-generated red light. The *lrb1/2* double mutant has signficantly shorter hypocotyls and larger cotyledons, indicating increased red light sensitivity.

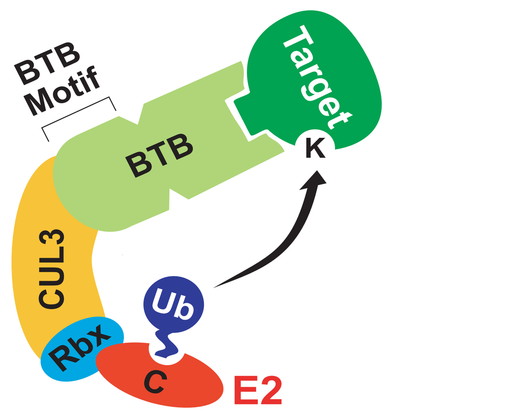
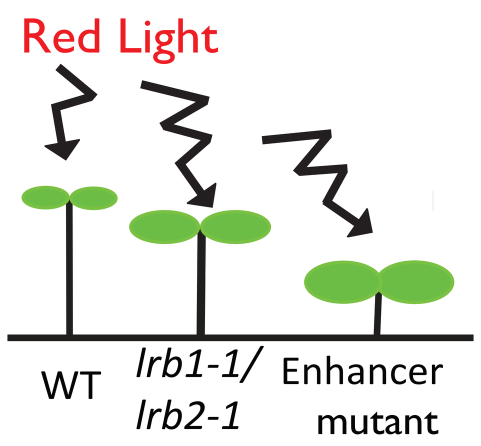


Figure 2. Predicted structure of BTB/ CUL3 E3 ubiquitin ligase complexes. The E3 binds the target and ubiquinates it. This ubiquination typically leads to degradation of the target. *LRB1* and *LRB2* are predicted to encode BTB proteins.

Enhancer Screen Method

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 increased red light sensitivity compared to the *lrb1-1/2-1* double mutants.



- Approximately 15,000 M2 generation plants were screened.
- 434 individuals with putative enhancer phenotypes were identified. 5-6 individuals with similar enhancer phenotypes were typically identified per M2 pool, therefore we estimate that we have identified 70-75 independent enhancer lines.
- To further test for and verify red light hypersensitivity, hypocotyl length of M3 seedlings is being measured following growth under low levels of red light and in the dark.

Enhancer E2-1-2 is a Putative Light Mutant

Putative enhancer mutants are those that have similar hypocotyl lengths to *lrb 1-1/2-1* in the dark but have significantly shorter hypocotyl lengths under red light conditions

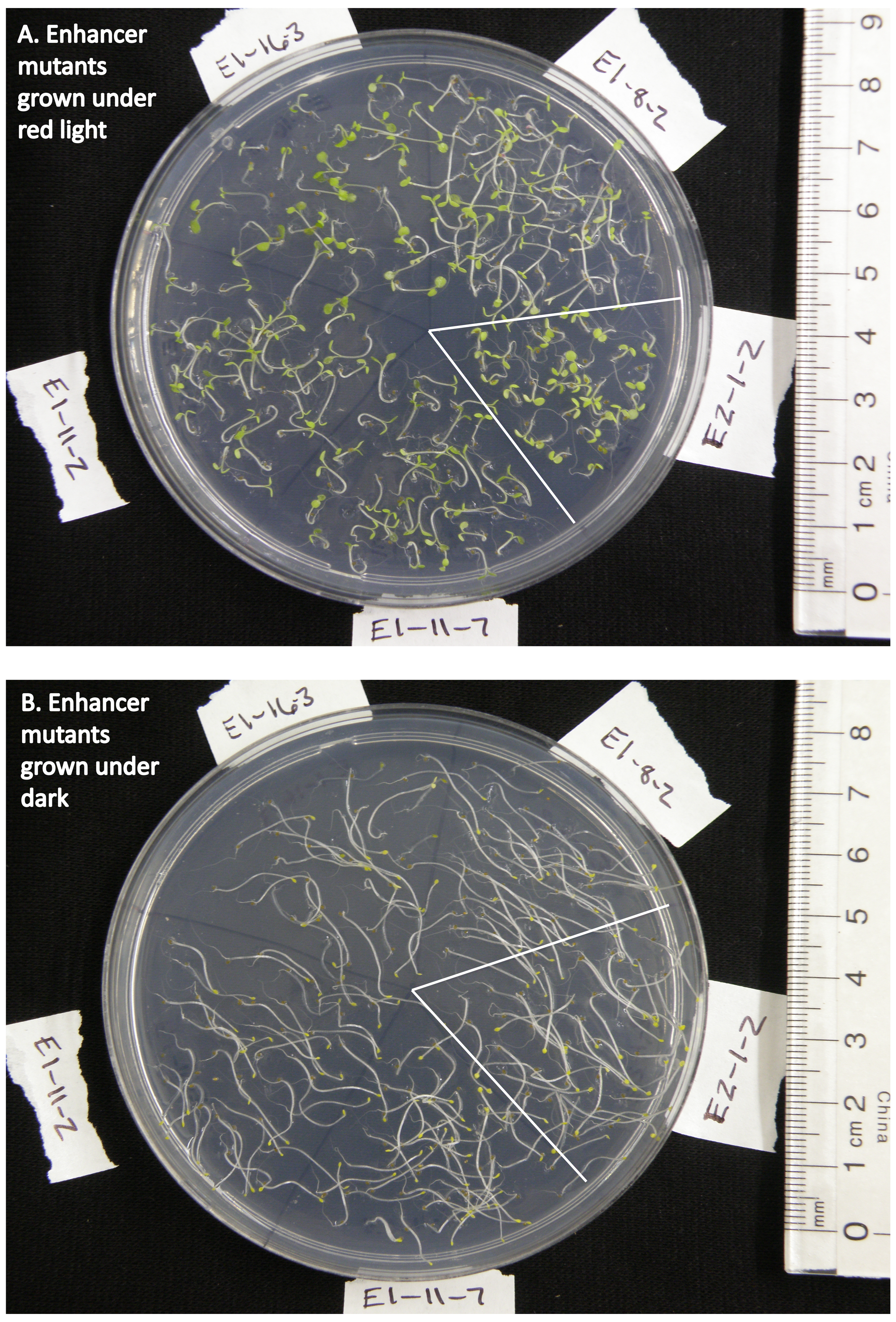


Figure 3. M3 seedlings following four days of growth under 0.10 μmol/m²/sec red light (A) and in the dark (B). The seeds were sterilized and plated on 1/2 MS media; cold treated at 4°C for four days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to 0.10 μmol/m²/sec red light and in the dark for four days.

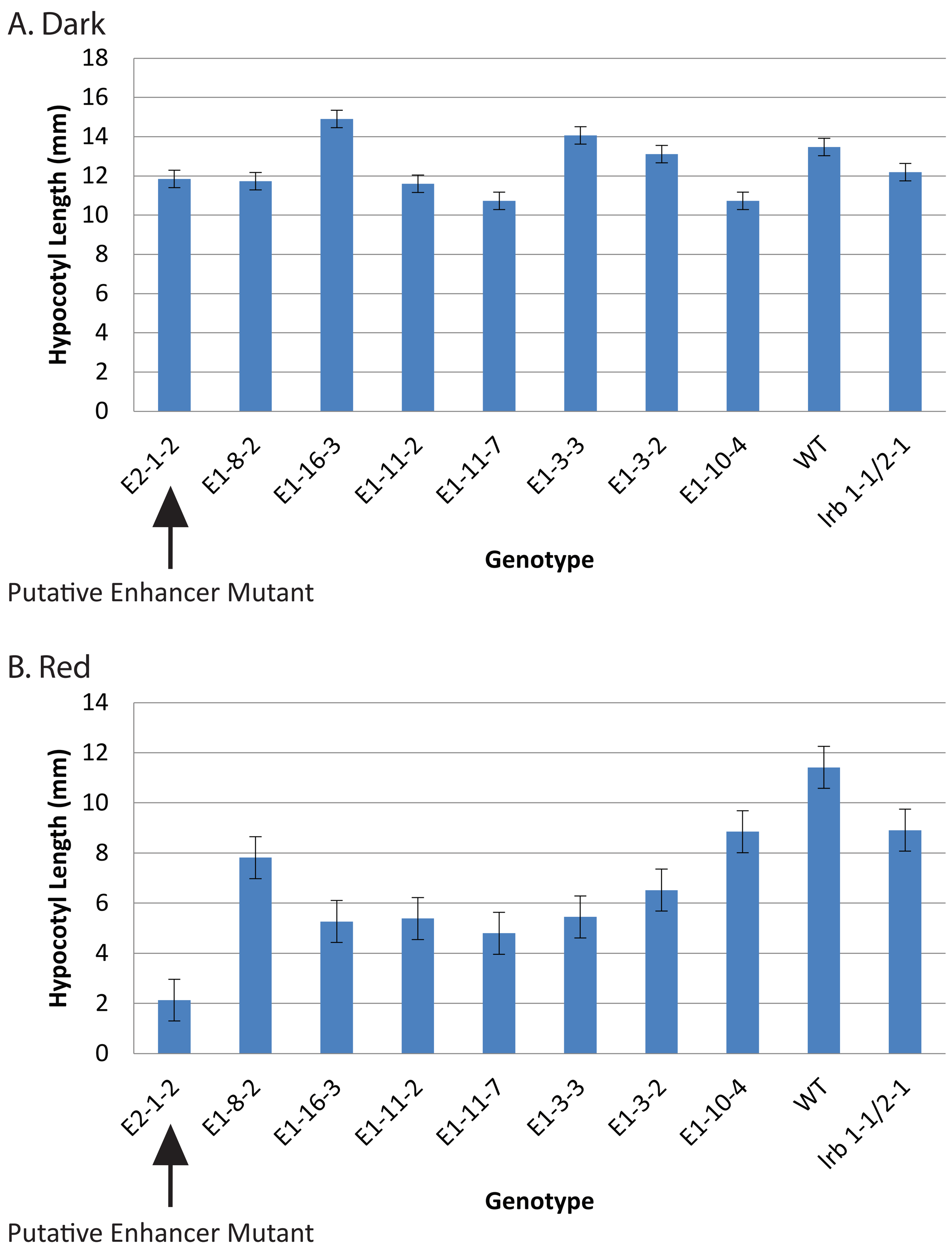


Figure 4. Mean hypocotyl lengths for 8 enhancer lines and corresponding controls grown under 0.10 μmol/m²/sec continuous red light (B) and in the dark (A). The seeds were sterilized and plated on 1/2 MS media; cold treated at 4°C for four days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to 0.10 μmol/m²/sec red light and in the dark for four days. Standard error bars are shown.

Conclusions

- Thus far we have identified one mutant line, E2-1-2 that has shown strong red light hypersensitivity but elongates normally in the dark and thus may be a *bona fide* light mutant.
- Our enhancer screen successfully identified at least one enhancer line which enhanced the phenotype of a red light hypersensitive mutant.
- Analysis of hypocotyl elongation responses of a subset of our enhancer mutants shows that we have a pool of mutants with varying degrees of red light sensitivity.
- Enhancer mutations identified in this screen may disrupt genes that have roles in light response in *Arabidopsis thaliana.*

What's Next?

- The enhancer screen is an ongoing process and we anticipate screening an additional 15,000 M2 individuals and identifying more putative enhancer lines.
- M4 lines from potential red light hypersensitive mutants will be analyzed further, focusing on additional known red light responses.
- Responses to be analyzed include but are not limited to cotyledon size, area, and opening time, petiole elongation, and flowering time.

References

1. Franklin, K.A., Larner, V.S., Whitelam, G.C., The signal transducing photoreceptors of plants. Int. J. Dev. Biol., 2005. 49(5-6): p. 653-64

2. Chen, M., Chory, J., Fankhauser, C., Light signal transduction in higher plants. Annu. Rev. Genet., 2004. 38: p. 87-117

3. Christians, M.J., Gingerich, D.J., Lauer, T.D., Vierstra, R. D., The Light-Regulating BTB 1 and 2 proteins assemble nuclear ubiquitin ligases that modify phytochrome B and D signaling in Arabidopsis. Plant Physiology, 2012. In Review.

4. Franklin, K.A., Quail, P.H., Phytochrome functions in Arabidopsis development. J. Exp. Bot., 2010. 61(1): p. 11-24.

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