A Genetic Screen to Identify Mutations that Lead to Light Hypersensitivity in Arabidopsis thaliana

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Introduction

A plant’s ability to perceive and respond to the quantity and quality of light is essential for healthy growth and development. One way in which plants respond to changing light conditions is via their perception of red (~680 nm) and far red (~730 nm) wavelengths of light. To do this, plants rely on a group of photoreceptors known as phytochromes (PHYS). PHYS mediate a variety of light responses, including de-etiolation and shade avoidance responses. We have found that two genes (Light-Response BTB1 [LRB1] and Light-Response BTB2 [LRB2]) act as prominent negative regulators of the PHY-mediated red light response pathway. Arabidopsis thaliana plants with mutations of these two genes are red light hypersensitive (Figure 1), one consequence of which is increased shade tolerance. LRB1 and LRB2 are predicted to encode proteins which act as target adapters in the ubiquitin-26S proteasome protein degradation pathway (Figure 2). We hypothesize that LRB1 and LRB2 regulate, via ubiquitylation, the degradation of some component of the red light signaling pathway.

In order to find other actors in red light signaling we have conducted a genetic enhancer screen, identifying mutations which exacerbate the red light hypersensitivity conferred by the lrb1 lrb2 mutations. Such mutations will likely disrupt genes which, like LRB2 and 2, have a role in light perception or response. We have completed initial screening of our mutagenized population and here describe several of the putative enhancer mutants we have isolated and begun to characterized.

Enhancer Mutant Screening Strategy

Mutagenize population of lrb1-1/lrb2-1 seeds with ethylene methanesulfonate.

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

Collect seed from these individuals.

Germinate and grow next generation (M2) under red filtered light, identify individuals that have increased red light sensitivity compared to the lrb1-1/lrb2-1 double mutants.

Collect seeds from these individuals.

Germinate and grow next generation (M3) under a range of red-light fluence levels, identifying individuals with enhanced red light sensitivity compared to controls.

Examples of Enhancer Mutants Identified

- E2-14-3
- E10-17-2
- E10-19-8
- E11-6-4
- E12-2-2

Conclusions

- Approximately >30,000 M2 generation plants were screened.
- 410 M2 individuals with putative enhancer phenotypes were identified.
- Thus far, 85 lines have been reanalyzed in the M3 generation, and based on this screening, 11 of the 85 lines appear to be bona fide enhancer mutants.
- Line E2-1-2 is hypersensitive to red and blue light.
- Enhancer mutants identified in this screen may disrupt genes that have roles in light responses in Arabidopsis thaliana.

What’s Next?

- The second round of screening is ongoing.
- M4 individuals from potential red light hypersensitive mutants will be analyzed further, focusing on additional known red light responses including cotyledon expansion and angle opening, inhibition of petiole elongation, and delay of flowering time.
- Lines will also be tested for far-red and blue light sensitivity.

References


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