Characterization of an Arabidopsis thaliana Mutant Identified in a Genetic Screen for Altered Red Light Responses

Jordan T. Montpetit, Gavin Sunde, Timothy Lauer, Derek J. Gingerich
Department of Biology, University of Wisconsin Eau Claire, WI

Introduction
Perception of light is crucial for a plant’s survival. Plants can perceive red (~670nm) and far-red (~730nm) wavelengths of light via cellular light receptors known as photoreceptors. The Arabidopsis lab has observed the participation of two genes, LRB (Light-Regulating BTB) 1 and LRB2, in the red light signaling pathway. Disruption of both genes (in an lrb1/lrb2 mutant) in the model dicotyledonous plant Arabidopsis thaliana produces a red hypersensitive phenotype, one outcome of which is to make the plants shade-tolerant. The proteins encoded by these genes are part of a large protein family known as the Bric-a-Brac, Tramtrack, and Broad Complex (BTB) protein family. BTB proteins are part of protein complexes known as BTB/CUL3 ubiquitin ligases, which select target proteins for destruction (Pintard, 2004). BTB proteins function in these complexes by binding the proteins to be degraded (Pintard, 2004).

To better understand the function of the LRB1 and LRB2 genes and the red light signaling pathway, we conducted a genetic suppressor screen to identify other genes which participate in the red light pathway. This screen identified mutations which reduce the red-hypersensitive phenotype conferred by the lrb1/lrb2 mutations. Here we present one of the mutants identified in the red light pathway. This screen identified mutations which reduce the red-hypersensitive phenotype and was selected for genetic mapping.

Suppressor Screening

A population of lrb1/lrb2 double mutants (in the Col-0 ecotype background) seeds were exposed to ethyl methanesulphonate (EMS), a chemical which causes point mutations throughout the genome. These M1 seeds were germinated and the plants self-fertilized to produce M2 seed. From this screen, individuals that have reduced red light sensitivity were selected for genetic mapping.

Strategy:
- Mutagenize population of lrb1-1/lrb2-2 seeds with ethylmethanesulphonate (EMS).
- Germinate seeds and grow plants (10 plants/et), 2000 individuals total.
- Collect seed from these individuals.
- Germinate and grow this next generation (M2) under red light; identify individuals that have reduced red light sensitivity compared to the lrb1-1/lrb2-2 double mutants were identified.

Result: Line S3-5-2 was one of the mutants identified in this screen.

S3-5-2 Phenotype

Figure 2. Mean hypocotyl length for line S3-5-2 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 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 red light for four days. Hypocotyl length was measured at the end of the red light treatment. Standard error bars are shown.

Rough Mapping

Table 1. The suppressor mutation in line S3-5-2 is linked to markers C1W11 and CIW15 on chromosome 2 in the Arabidopsis genome. Markers tested by PCR in initial rough-mapping experiments using plants from the F2 mapping population are shown. Marker type [simple-sequence length polymorphism (SSLP), and insertion/deletion (InDel)] are shown. If a marker is genetically linked to the suppressor mutation then fewer Ler alleles should be found in the mapping population.

Conclusions

• lrb1 lrb2 suppressor mutant S3-5-2 displays strong insensitivity to red light
• Mapping and complementation testing show the mutation responsible for this response in mutant S3-5-2 is located near or possibly in the PHYB gene on chromosome 2
• PHYB encodes the primary red light photoreceptor in Arabidopsis
• Genetic sequencing is needed to confirm the S3-5-2 mutation in the PHYB gene

References


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