Investigating the function of LRB-interacting proteins in *Arabidopsis thaliana*

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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 phytochromes. The genome of the model flowering plant *Arabidopsis thaliana* encodes five different phytochromes. Phytochrome B (phyB) modulates red light responses, including a set of physiological responses known as shade avoidance syndrome (SAS). The ratio of red to far-red light changes as light filters through a canopy, and this is sensed primarily by phyB which induces SAS, allowing the plant to grow out of the shade. SAS is characterized by responses such as elongation of the stem and petiole and decreased leaf area.

The Gingerich lab has found that *Arabidopsis thaliana* contains two proteins, LRB1 and LRB2 which also act in red light signaling. Arabidopsis plants with both of these genes disrupted are overly sensitive to red light (and therefore shade tolerant). The LRBs are part of protein family known as the Bric-a-Brac, Tramtrack, and Broad Complex (BTB) proteins. BTB proteins are part of protein complexes known as BTB/CUL3 E3 ubiquitin ligases, which select proteins for destruction (Figure 1). BTB proteins function in these complexes by binding the proteins to be degraded (Pintard, 2004). We surmise that LRB1 and 2 act in the molecular signaling pathway through which red-light activated phyB modulates shade responses.

In attempt to better understand LRB1 and LRB2's function, we conducted a type of protein-protein interaction screen known as a yeast two-hybrid screen with these two proteins. This screen identified more than a dozen different proteins which may be able to bind LRB1 and/or LRB2 in plant cells.

These LRB-interacting proteins themselves may have roles in red light signaling. In order to determine this, we identified mutant *Arabidopsis thaliana* plants homozygous for T-DNA insertions in genes At5g14000, At5g22570 and At4g36980, which encode three of these LRB interactors. Plant lines with T-DNA insertions were provided by the SALK institute in LaJolla, CA, and Syngenta Biotechnology Incorporated. Mutant plants were screened for using PCR and agarose gel-electrophoresis techniques. Seed from mature plants homozygous for T-DNA insertions in the At5g14000 and At5g22570 genes was collected and grown under varying intensities of red light to determine if disruption of these genes altered growth responses.

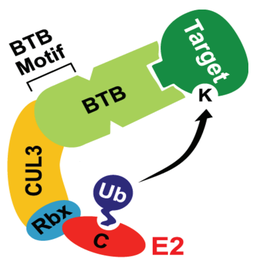

Figure 1. Structure of the BTB/CUL3 E3 ubiquitin ligase complex. The E3 binds the target and ubiquinates it. This ubiquination leads degradation of the target.

Identifying T-DNA Insertion Mutants

Arabidopsis seedlings were grown in petri dishes on growth media. After approximately 7 days, leaf samples were taken from individual plants for DNA extraction. PCR (polymerase chain reaction) was used as a diagnostic to determine if T-DNA insertions were present in one or both copies of the target gene in an individual. PCR was performed with primers that specifically match sequences in the target gene (RP and LP), as well as primers that match a sequence within the T-DNA insertion is itself [LBa1 for the SALK lines, and LB1 for the SAIL (Syngenta) lines]. PCR products were subjected to agarose gel electrophoresis and imaged under ultraviolet light. The presence of a T-DNA insertions and hence the genotype of an individual was determined by the appearance of DNA bands of known molecular weight in the images.

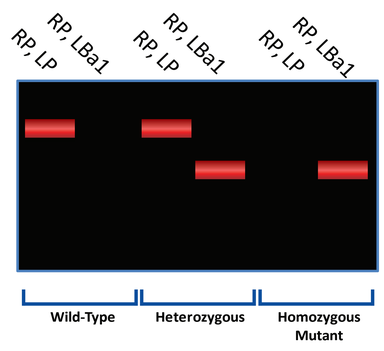

Figure 2. A diagram showing expected PCR genotyping results for the three possible genotypes. Wild-type plants have two undisrupted copies of the gene. Heterozygous plants have one of two copies of the gene disrupted. Homozygous plants have both copies of the target gene disrupted. These plants likely lack function of this gene.

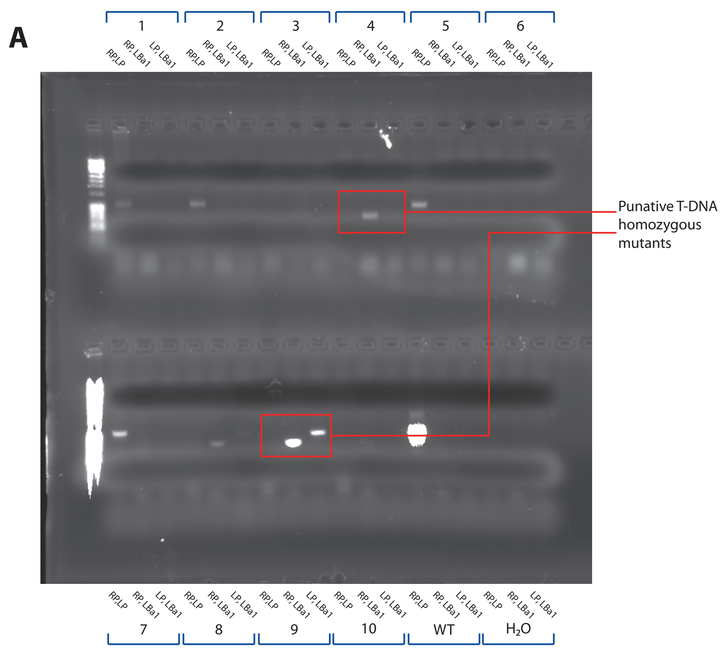

Figure 3. A. PCR anaylsis of 10 individual seedlings that may contain T-DNA insertions in gene At4g36980. Wild-type DNA (WT) and water (no DNA in the PCR reactions) were used as controls. B. A diagram of gene At4g36980 displaying exons as boxes and introns as lines. Putative location of the T-DNA insertion is represented by the triangle.

Identifying T-DNA Insertion Mutants (cont.)

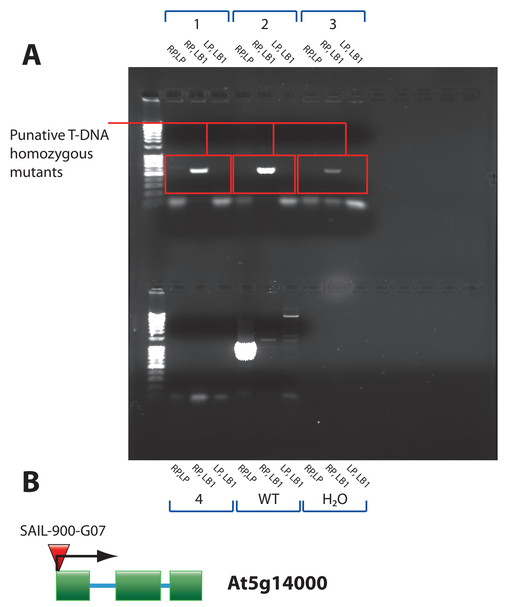

Figure 4. A. PCR Anaylsis of 4 individual seedlings that may contain a T-DNA insertion in gene At5g1400. Wild-type DNA (WT) and water (no DNA in the PCR reactions) were used as controls. B. A diagram of gene At5g14000 displaying exons as boxes and introns as lines. Putative location of the T-DNA insertion is represented by the triangle.

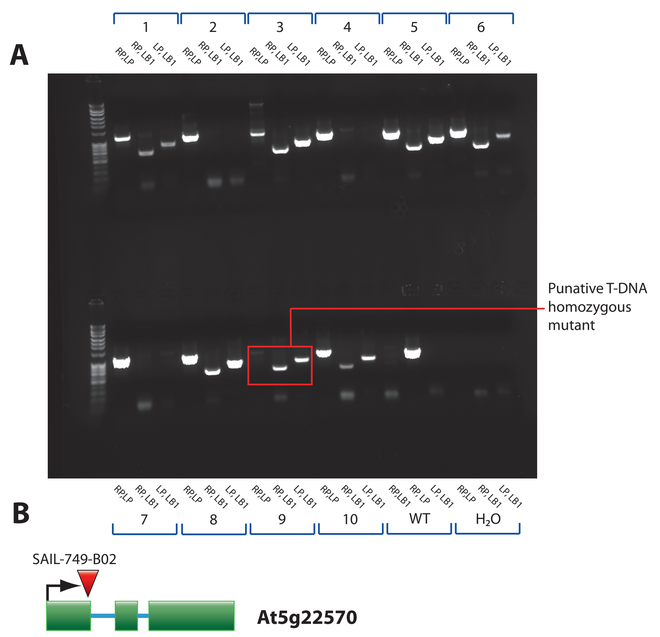

Figure 5. A. PCR Anaylsis of 10 individual seedlings that may contain a T-DNA insertion in gene At5g22570. Wild-type DNA (WT) and water (no DNA in the PCR reactions) were used as controls. B. A diagram of gene At5g22570 displaying exons as boxes and introns as lines. Putative location of the T-DNA insertion is represented by the triangle.

Red Light Growth Assays

Seed from plants homozygous for T-DNA insertions in genes At5g14000 (SAIL-900-G07) and At5g22570 (SAIL-749-B02) was white-light treated to induce germination and then grown under red light (10µmol/m^2/s and 100µmol/m^2/s) for four days. Growth responses to red light were assayed by measuring the hypocotyl length. These measurements were compared to wild-type (WT), *lrb1-1/2-1* mutant, and *phyB-9* mutant seedlings grown under the same conditions. WT seedlings display normal physiological responses to red light, *lrb1-1/2-1* mutatnts are hypersensitive to red light, and *phyB-9* mutants are insensitive to red light.

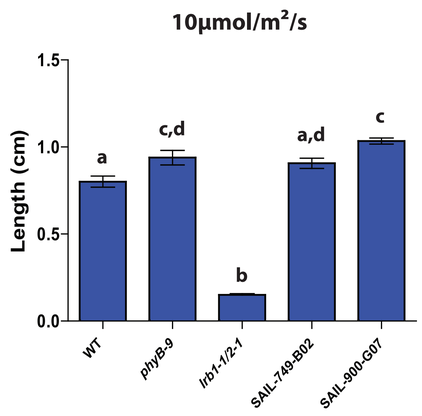

Figure 6. Hypocotyl length from 5 day old seedlings grown under 10µmol/m^2/s red light. Error bars show standard error. Means with the same letter are not significantly diffferent from each other (Tukey-Kramer test, P<0.05).

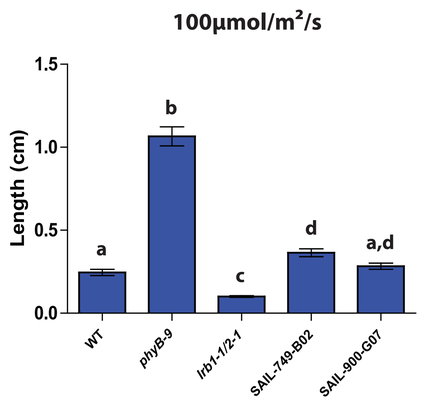

Figure 7. Hypocotyl length from 5 day old seedlings grown under 100µmol/m^2/s red light. Error bars show standard error. Means with the same letter are not significantly diffferent from each other (Tukey-Kramer test, P<0.05).

Conclusions

- We have identified individual seedlings homozygous for T-DNA insertions in genes At5g14000, At5g22570 and At4g36980.
- We tested lines with T-DNA insertions in genes At5g14000 and At5g22570 for red light sensitivity.
- Sensitivity of these lines appears to be similar to wild-type. However, based on our data, this conclusion cannot be made definitively.
- Further testing of red light responses in these lines will be performed.

References

Pintard, L., Willems, A., & Peter, M. (2004). Cullin-based ubiquitin ligases: Cul3-BTB complexes join the family. *Embo Journal*, 24(5), 1092-1092.

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