**Suppressor Screen of LRB (Light Response BTB) 1 and LRB2 Mutants in Arabidopsis thaliana**

**Introduction**

The ability to sense and respond to changing light conditions is fundamental to plant growth and development. One way that plants respond to light is via wavelengths. The signaling pathways in the cell that are involved in this response are not well understood.

Dr. Gingerich has identified two redundant genes which act in the red light response pathway in the model plant Arabidopsis thaliana: LRB (Light Response BTB) 1 and LRB2. Disruption of both of these genes results in a plant which is hypersensitive to red light.

In order to better understand the roles of LRB1/2 in red light signaling we conducted a genetic suppressor screen to identify other components of the LRB-modulated red light pathway. We generated a population of LRB1/2 double mutants with additional mutations in the genome and screened this group for individuals that lack the red light hypersensitive phenotype. We identified more than 30 putative suppressor mutants and are currently confirming/quantifying the phenotypes in the offspring of these individuals. Identification of the genes disrupted in these suppressor mutants should clarify the role of LRB1/2 in red light signaling and reveal previously unknown components of these pathways.

**lrb1/lrb2 mutant Arabidopsis plants are hypersensitive to red light**

- Red light inhibits hypocotyl elongation

**Suppressor Mutant Screen Strategy**

1. Mutagenize population of lrb1/lrb2 seeds with ethylmethanesulfonylate (EMS)
2. Germinate seeds and grow plants (10 plants/pot), 2000 individuals total
3. Collect seed from these individuals
4. Germinate and grow this next generation under red light; identify individuals which have reduced red light hypersensitivity (lbs mutants)

**Identification of putative lbs mutants**

**Results/Conclusions**

- Preliminary results indicate that the EMS mutagenesis population of lrb1/lrb2 individuals has been successful in producing multiple suppressor mutants of varying suppression strength.

- The individuals may carry mutations in genes involved in the LRB1/LRB2-mediated red light signaling pathway.

**Whats Next?**

- After the suppressor screen is complete, we will perform a series of genetic analyses to identify the total number of genes we have mutated (multiple suppressor mutants may actually have mutations in the same gene).

- The suppressor mutants will be crossed with other mutants known to be affected in red light signaling to determine epistatic interactions and their relative location in the red light signaling pathway.

- We will determine if any of the mutations are in the gene for the phyB red light photoreceptor (a strong possibility).

- We are beginning the process of mapping the mutations to identify the genes disrupted.

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**Figure 1.** lrb1/lrb2 double mutant Arabidopsis plants are hypersensitive to red light. Seedlings were germinated and grown for four days under varying fluence levels of LED-generated red wavelength light.

**Figure 2.** Illustration of phenotypes displayed in respective individuals exposed to red light.

**Figure 3.** Examples of putative suppressor mutants from original population screen.

**Figure 4.** Examples of mutants identified in the phyB-9/lrb2-1 suppressor screen. M3 seed collected from M2 individuals originally identified in the red light screen, along with phyB-9, lrb1-1/lrb2-1, and WT seed, were germinated under white light for 24 hrs. and then grown under continuous red-filtered light for 5 days. Two examples of mutant lines (lbs1 and lbs2) with strong suppression and one example of a mutant line (lbs3) with weak suppression of the phyB-9/lrb2-1 red light hypersensitive phenotype are shown.

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