A Screen for Shade Avoidance Mutants in *Arabidopsis Thaliana*

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Abstract

In this study, we used the *PHYB::LUCIFERASE* promoter::reporter gene construct to screen *in vivo* for disruption of the shade response in the 7 day-old, light-grown Arabidopsis seedlings. 438 putative mutant lines were obtained after screening 30,000 M2 lines from 1800 EMS mutagenised seeds. After rescreening these putative mutant lines we have identified 28 shade avoidance-defective mutants, falling into three categories: 16 mutant lines show increased response to shade, 9 lines show decreased response and 3 lines constitutively express *PHYB::LUC* at a high level. Several interesting mutant lines also show additional physiological phenotypes associated with defects in the shade avoidance response.

Introduction

The study of shade avoidance has great agricultural importance as the response involves the relocation of reserves into elongation growth at high planting density. Light reflected from neighbouring plants is depleted of red light resulting in a low red: far red ratio. Plants have developed a strategy that initiates elongation growth and early flowering to avoid the shade after perceiving this change in red light through the photoreceptor, phytochrome (1). All of the five phytochromes in Arabidopsis, phyA-phyE, act in the shade avoidance response. Microarray analysis found that there are 301 shade responsive genes including the *PHYB* gene itself which is strongly induced by shade (2). The aim of this research is to use a mutant screen to find genes involved in the shade avoidance signalling pathway.

Materials and Methods

We used the *PHYB::LUCIFERASE* promoter :: reporter gene construct to screen *in vivo* for disruption of the shade response in the 7 day-old, light-grown EMS mutagenised Arabidopsis seedlings.
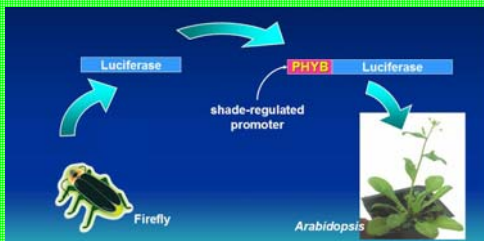

Seedlings are plated on agar medium in Petri plates and luminescence is detected by a NightOwl Molecular Imager (Berthold Technologies, UK) before and after 2 hrs simulated shade treatment and the data are analysed using the Winlight image analysis package.
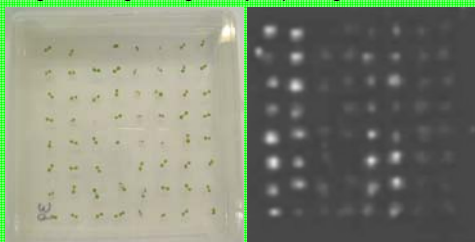
Results

1. Screening for shade Avoidance-defective Mutants

438 putative mutant lines were obtained after screening 30,000 M2 lines from 1800 EMS mutagenised seeds. After rescreening these putative mutant lines, we have identified 28 shade avoidance-defective mutants, falling into three categories: 16 mutant lines show increased response to shade, 9 lines show decreased response and 3 lines constitutively express *PHYB::LUC* at a high level.

Luminescence before and after shade treatment
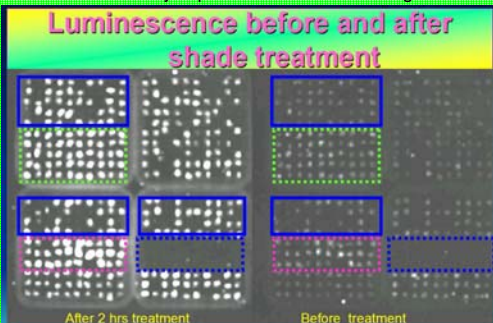
Seedlings in blue line box are wild type
Seedlings in green dash box showed increased response
Seedlings in red dash box showed increased response
Seedlings in blue dash box showed down-regulated expression and decreased response

2. Mutant characterisation

We have begun mutant characterisation by focusing on the most interesting mutant, I1-69, showing an increased response to shade.

a) PHYB luciferase phenotype


Interesting Mutant I1-69
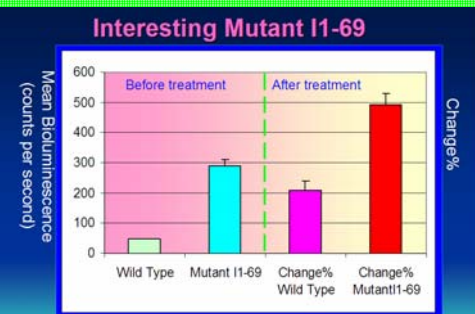
The left half of the above graph shows the significant increased expression of luciferase in the mutant I1-69 before shade treatment. The right half shows the percentage change in luciferase expression in the wild type and mutant I1-69 after shade treatment. The mutant I1-69 shows a significant greater increase than wild type.

b) Physiological phenotype

(i) Elongation growth

I1-69 mutant seedlings and their parental wild type were grown for 6 days in white light then either maintained in white or transferred to simulated shade for 2 days. The mutant I1-69 and the parental wild type showed no difference in elongation growth (hypocotyl length) in white light. Both I1-69 and the wild type showed significant increase in elongation growth after the shade treatment. Unexpectedly, the mutant I1-69 increased significantly less response than the wild type.

effect of shade on hypocotyl length
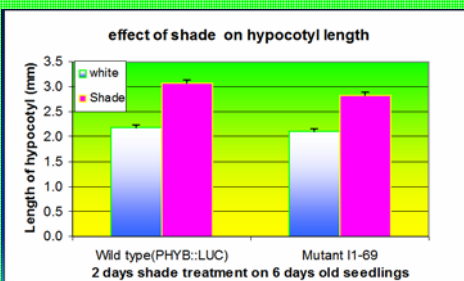
This figure shows the hypocotyl length after 2 days white light (control) or shade treatment was given to 6 day-old seedlings. Wild type showed a significant increase in length after shade treatment. The mutant I1-69 showed significantly less increase in length than wild type.

(ii) Flowering time

Experiments to look at flowering under simulated shade are currently underway. However, when I1-69 mutant seedlings and their parental wild type were grown in the greenhouse for 3 weeks, I1-69 mutant seedlings clearly flowered later than wild type.


WT   I1-69
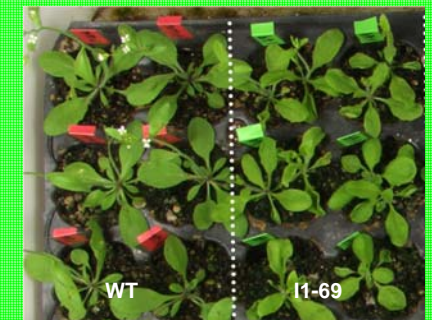
Conclusions

The I1-69 phenotype may specifically affect *PHYB* gene expression and not other aspects of shade avoidance as the elongation response is not enhanced. The reduced elongation response to shade may be a consequence of higher photoreceptor levels under shade conditions which would be expected to inhibit elongation growth. The delayed flowering time would also be consistent with high levels of the phyB photoreceptor.

Shade Signal Pathway

What are the components of the shade signal pathway? I hope to answer this question in the future through analysis of these new mutants.

What is the shade signalling pathway in *Arabidopsis Thaliana*?
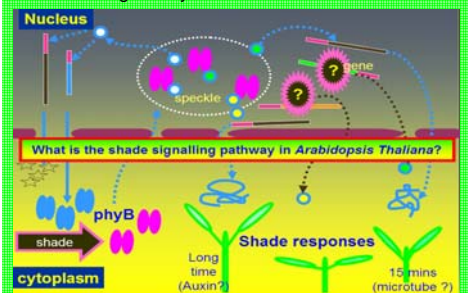
This diagram shows a possible shade signal pathway. PHYB accumulates in "speckles" in the nucleus after perceiving red light and moves back to the cytoplasm after perceiving far red light. The light signal is passed on involving protein-protein interaction and gene regulation and the plant responds to shade by showing a visible phenotype.

Future research

1. Map-based cloning of the shade mutant genes
2. Determining the role of the mutated shade signalling genes

References

1. Salter MG, Franklin KA, Whitelam GC. Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature. 2003 Dec 11;426(6967):680-3.
2. Devlin PF, Yanovsky MJ, Kay SA., A genomic analysis of the shade avoidance response in Arabidopsis. Plant Physiol. 2003 Dec; 133(4):1617-29. Epub 2003 Nov 26.

Acknowledgments

K C Wong Scholarship

THE ROYAL SOCIETY

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