

NATURAL GENE EXPRESSION VARIATION UNDER DYNAMIC LIGHT
IN *Arabidopsis thaliana*

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Thursday 18th April, 2013

Word Counts:
Introduction: x words
Results: y words
Discussion: z words

Abstract

This is the abstract.

Contents

1	Introduction	2
1.1	Photoprotection	2
1.2	Dynamic and Fluctuating Light	2
1.3	Transcriptional Responses to Excess Light	3
1.4	Aims	4
2	Methods	5
3	Results	6
4	Discussion	7
5	References	8
6	Appendix	11

1 Introduction

Nearly all terrestrial biomass, including human life, depends on the ability of plants to create organic material from inorganic inputs and sunlight (photosynthesis). This ability is reduced when the quality or quantity of light a plant receives is not optimal. Excess light is particularly damaging, causing both reductions in photosynthetic ability (photoinhibition) and cellular or tissue damage and death. Thus, plants have evolved mechanisms by which the detrimental effects of excess light can be minimised. These mechanisms, collectively termed photoprotection, work to dissipate excess energy, reduce the amount of light absorbed, or prevent or repair any damage caused (Niyogi, 1999). Although difficult to quantify on a global scale, the cost of the detrimental effects of excess light on primary productivity is high (Raven, 2011).

1.1 Photoprotection

Several mechanisms of photoprotection occur within the chloroplast. Non-photochemical quenching (NPQ) dissipates excess energy from excited state chlorophyll molecules as heat (Miller, Li, and Niyogi, 2001). It occurs in photosystem II, and is particularly important during rapid changes in intensity (). Cyclic electron flow occurs in photosystem I and acts by decreasing the pH of the thylakoid lumen, which in turn is thought to stabilise the oxygen evolving complex and aid NPQ (Takahashi et al., 2009). State transitions act by phosphorylation of photosystem II and light harvesting complex II proteins. State transitions reversibly alter the balance of excitation energy between photosystems I and II (Tikkanen et al., 2006). Through reversible dissociation of the light harvesting complex II and photosystem II, state transitions lower the light harvesting ability of photosystem II and prevent absorption of excess light by photosystem II (Johnson et al., 2011). The mutants *Non-photochemical Quenching 1* (*npq1*) and *npq4*, *proton gradient regulator 5* (*pgr5*) and *state transition 7* (*stn7*) and *stn8* are defective in these three mechanisms respectively.

In addition to these chloroplastic photoprotective mechanisms, plants can respond to high light on a cellular scale. Chloroplast avoidance movement, the movement of chloroplasts parallel to sunlight, decreases the amount of absorbed light (Kasahara et al., 2002). Transcriptional induction of heat shock proteins, antioxidant scavenging and photodamage repair occur following excess light, and help to minimise oxidative damage (Niyogi, 1999). Production of anthocyanins, a protective class of pigments, is induced by the production of reactive oxygen species due to excess light (Vanderauwera et al., 2005). Together, these responses serve to reduce the photodamage of cells and tissues due to excess light.

1.2 Dynamic and Fluctuating Light

The light plants receive in natural environments is dynamic, in that the intensity and spectral composition (or quality) of sunlight varies over a day, even in the absence of shading from any cloud or

vegetation. In addition, the intensity of sunlight received may fluctuate throughout the day as cloud and canopy shade intermittently shade a plant. The time scale of this fluctuation varies from minutes to hours depending on its source; clouds may pass over plants in minutes, whereas canopy shade will move over plants slowly as the sun tracks from east to west (Figure X ; ()).

Plant biologists use laboratory growth chambers to provide repeatable experimental conditions. These growth environments have allowed us to manipulate and observe plants away from the random influences such as weather and predation. However, these cabinets typically have conditions very different to those experienced in the field (Figures 1A and 1B). As both field and lab experiments have value, both have continued in parallel, leading to a gap in knowledge between the lab and field in some areas of plant biology.

Field-grown plants tend to demonstrate reduced survival, reproduction and altered transcriptional responses compared to those grown in comparable lab conditions. For example, in *Arabidopsis thaliana* the reduction in reproductive success caused by the *npq1* and *npq4* mutants was severe in the field but had no impact under constant light in the lab (). Photoinhibition, physiological symptoms of abiotic stress and expression of high light-induced transcripts were more severe in field-grown *Solidago altissima* than those grown in comparable lab conditions (Barua and Heckathorn, 2006). Light quality is particularly important in studies of photodamage, as the extent of photosystem II damage is not consistent across the visible spectrum (Takahashi et al., 2010). Photodamage is relatively more severe under light of wavelengths between 580-620nm than in the remainder of the visible spectrum, which overlaps with a density peak in the spectral power density of fluorescent lamps (Figure 1A).

Recent advances in light emitting diode (LED) technology, and recent investments by the ANUs Research School of Biology, have lead to the provision of computer-controllable growth chambers and multi-spectral LED arrays capable of simulating natural conditions with greater accuracy compared to traditional growth cabinets. The light quality of the new LED arrays is more consistent across the visible spectrum compared to fluorescent lamps (Figure 1A). These cabinets are also able to control temperature and humidity on a diurnal cycle, enabling study of interactions between light and cold temperatures (such as those observed by Armstrong, Wardlaw, and Atkin (2007)).

1.3 Transcriptional Responses to Excess Light

Transcriptomics, or the global study of gene expression, is one method which can be used to study the response of plants to excess light. By studying how, when and to what extent each gene in the genome is expressed, we can gain insight into the response to any perturbation to a plant's environment. To study global expression, two approaches have commonly been used: microarrays and RNAseq. Microarrays quantify expression as fluorescence from fluorophore-tagged cDNAs complementary to specific small sections of the transcriptome. More modern experiments use RNAseq, the high-throughput sequencing of cDNA libraries, and rely on the number of sequence reads being proportional to the concentration of a particular transcript within the library. RNAseq allows not only quantification, but provides the actual sequence of each transcript, important for analyses of sequence variation such as allele specific expression.

The rapid response of the *Arabidopsis* transcriptome to changes in lighting has been demonstrated. Rossel, Wilson, and Pogson (2002) demonstrated 185 of 6000 genes were differentially expressed after one hour of high light exposure, including transcripts implicated in response to drought, pathogen or oxidative stress as well as hormone response. As existing leaves acclimate to excess light conditions, the steady-state level of expression of several classes of genes are altered. In the cyanobacterium

Synechocystis sp. PCC 6803, some genes involved in light capture are down-regulated and some homologues of heat-shock proteins up-regulated after 15 hours of excess light treatment (Hihara et al., 2001). In rice, similar transcriptional down-regulation of light harvesting and up-regulation of photoprotection after 24 or 72 hours of excess light treatment has been observed (Murchie et al., 2005). Using quantitative PCR, Gordon et al. (2013) have shown that high light induced expression was dependent on light quality in *Arabidopsis*, and that repeated high light treatments lead to acclimation and reduced induction of high light responsive transcripts.

1.4 Aims

The overall goal of this project is to determine if the differential expression of transcripts in previous experiments with artificial light conditions is similar to the differential expression observed under more natural conditions. Additionally, I aim to determine if the loci which regulate response to excess dynamic light are similar to those which have been found to regulate transcriptional response to artificial high light. I will generate RNAseq data collected from *Arabidopsis* exposed to novel dynamic light growth conditions, and then mine this dataset to:

1. Determine the response of *Arabidopsis* gene expression to novel dynamic light conditions.
2. Examine the extent of genetic variation in gene expression under these dynamic light conditions, and elucidate gene regulation networks controlling gene expression.
3. Explore the effect of genotype-environment interactions on gene expression under dynamic light conditions.

2 Methods

3 Results

4 Discussion

5 References

Bibliography

- Armstrong, Anna F., Kristen D. Wardlaw, and Owen K. Atkin (2007). “Assessing the relationship between respiratory acclimation to the cold and photosystem II redox poise in *Arabidopsis thaliana*”. en. In: *Plant, Cell & Environment* 30.12, 1513–1522. DOI: 10.1111/j.1365-3040.2007.01738.x.
- Barua, Deepak and Scott A. Heckathorn (Jan. 2006). “The interactive effects of light and temperature on heat-shock protein accumulation in *Solidago altissima* (Asteraceae) in the field and laboratory”. en. In: *American Journal of Botany* 93.1, pp. 102–109. DOI: 10.3732/ajb.93.1.102.
- Gordon, Matthew J. et al. (2013). “Systemic and local responses to repeated HL stress-induced retrograde signaling in *Arabidopsis*”. In: *Frontiers in Plant Physiology* 3, p. 303. DOI: 10.3389/fpls.2012.00303.
- Hihara, Yukako et al. (Apr. 2001). “DNA Microarray Analysis of Cyanobacterial Gene Expression during Acclimation to High Light”. en. In: *The Plant Cell Online* 13.4, pp. 793–806. DOI: 10.1105/tpc.13.4.793.
- Johnson, Matthew P. et al. (Apr. 2011). “Photoprotective Energy Dissipation Involves the Reorganization of Photosystem II Light-Harvesting Complexes in the Grana Membranes of Spinach Chloroplasts”. en. In: *The Plant Cell Online* 23.4, pp. 1468–1479. DOI: 10.1105/tpc.110.081646.
- Kasahara, Masahiro et al. (2002). “Chloroplast avoidance movement reduces photodamage in plants”. en. In: *Nature* 420.6917, pp. 829–832. DOI: 10.1038/nature01213.
- Murchie, E. H. et al. (Jan. 2005). “Acclimation of photosynthesis to high irradiance in rice: gene expression and interactions with leaf development”. en. In: *Journal of Experimental Botany* 56.411, pp. 449–460. DOI: 10.1093/jxb/eri100.
- Miller, Patricia, Xiao-Ping Li, and Krishna K. Niyogi (Apr. 2001). “Non-Photochemical Quenching. A Response to Excess Light Energy”. en. In: *Plant Physiology* 125.4, pp. 1558–1566. DOI: 10.1104/pp.125.4.1558.
- Niyogi, Krishna K. (1999). “PHOTOPROTECTION REVISITED: Genetic and Molecular Approaches”. In: *Annual Review of Plant Physiology and Plant Molecular Biology* 50.1. PMID: 15012213, pp. 333–359. DOI: 10.1146/annurev.arplant.50.1.333.
- Raven, John A. (2011). “The cost of photoinhibition”. en. In: *Physiologia Plantarum* 142.1, 87–104. DOI: 10.1111/j.1399-3054.2011.01465.x.
- Rossel, Jan Bart, Iain W. Wilson, and Barry J. Pogson (Nov. 2002). “Global Changes in Gene Expression in Response to High Light in *Arabidopsis*”. en. In: *Plant Physiology* 130.3, pp. 1109–1120. DOI: 10.1104/pp.005595.
- Takahashi, Shunichi et al. (Mar. 2009). “How Does Cyclic Electron Flow Alleviate Photoinhibition in *Arabidopsis*?” en. In: *Plant Physiology* 149.3, pp. 1560–1567. DOI: 10.1104/pp.108.134122.
- Takahashi, Shunichi et al. (July 2010). “The Solar Action Spectrum of Photosystem II Damage”. en. In: *Plant Physiology* 153.3, pp. 988–993. DOI: 10.1104/pp.110.155747.
- Tikkanen, Mikko et al. (Nov. 2006). “State transitions revisited: a buffering system for dynamic low light acclimation of *Arabidopsis*”. en. In: *Plant Molecular Biology* 62.4–5, pp. 779–793. DOI: 10.1007/s11103-006-9044-8.
- Vanderauwera, Sandy et al. (Oct. 2005). “Genome-Wide Analysis of Hydrogen Peroxide-Regulated Gene Expression in *Arabidopsis* Reveals a High Light-Induced Transcriptional Cluster Involved

in Anthocyanin Biosynthesis”. In: *Plant Physiology* 139.2, pp. 806–821. DOI: 10.1104/pp.105.065896.

6 Appendix