Effects of Light Intensity on Betalain and Phenolic Antioxidant Expression in *Portulaca oleracea* (*Portulacaceae*)

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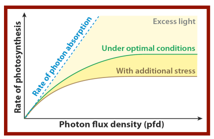
Introduction

Plants exposed to increasing intensities of light eventually reach a threshold of maximum photosynthetic efficiency. Bombardment by excess light results in the production of reactive oxygen species. This creates oxidative cell damage and leads to photoinhibition. Additional stressors, like those associated with climate change (decreased water and increased temperature), further reduce the photosynthetic capability of the plant. Two defensive responses to photo-oxidative stress are the production of antioxidants and pigments. Antioxidants are important phytochemical compounds responsible for the inhibition of free radicals within cells. Pigments often share antioxidant properties, but they can also absorb wavelengths of light that chlorophyll cannot. Pigments and antioxidants are crucial for plant survival but they are also beneficial in preventing cancer and other degenerative diseases in humans.

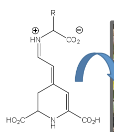


(Li, et.al, 2008)

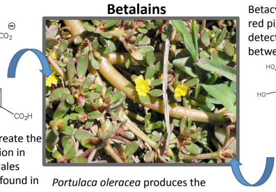
Cultivars
Left: Golden
Right: Tall Green

Portulaca oleracea is an abundant weedy plant and a member of order Caryophyllales. To study the response of this well adapted species, we examined two cultivars: tall green and golden. The experimental stressor was light intensity. **The goal of this investigation was to quantify the differential expression of antioxidants and pigments in these two genetic varieties of *P. oleracea* in two environments; and determine if there are genotype by environment (G x E) interactions.**

Betalains

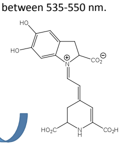


Betaxanthinscreate the yellow coloration in the Caryophyllales order and are found in a visible spectrum between 475-480 nm.



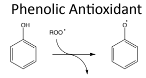
Portulaca oleracea produces the class of pigments known as betalains, which are subdivided into two distinct chemical classes.

Betacyaninsare responsible for red pigmentation and are detected in a visible spectrum between 535-550 nm.



An example of the reaction by which the active functional group of the phenol (HO- hydroxyl) is capable of reducing a reactive oxygen species. The oxidized product is very stable due to high resonance.

Quenching of a Free Radical by a Phenolic Antioxidant



Questions

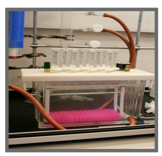
Does the stress response of *P. oleracea* vary among two cultivars and two light environments, as determined by:

- **The concentration and composition of betalains?**
- **The concentration of phenolic antioxidants produced?**
- **Morphological indicators of fitness?**

Methods

Fifty specimens of each cultivar were distributed among full sun and shade treatments. Specimens were cultivated in the treatments for four weeks, ranging from June 9th to July 7th 2009.

Nodes, flowers, branches, and dry mass were recorded as a measure of fitness.

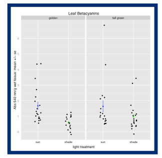


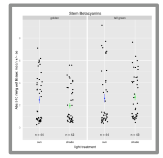
Sections of basal internode and leaf samples were removed from each specimen, macerated and soaked in 60% methanol or water. Antioxidants in the water extracts were quantified with the Folin–Ciocalteu assay. Betalains were quantified using the filtered methanol extracts .

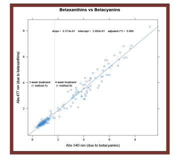
Visible spectrum was recorded and the values of interest (477 nm and 540 nm for betalains; 765 nm for antioxidants) were analyzed separately.

Results

Variation in Betalain Synthesis and Composition

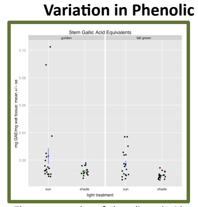


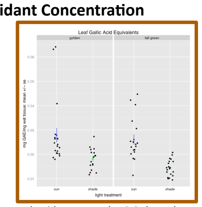




Betalain absorbance was independent of genetic and environmental factors; there was no variation among shade and full-sun plants in either cultivar.Strong correlation between the absorbance values at 540 nm and 477 nm emphasizes that later biosynthetic pathways of the two pigments might be unregulated. Clustering between methods a and b contrasts the variation in stem tissue collection.

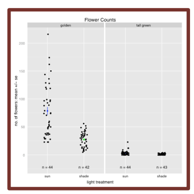
Variation in Phenolic Antioxidant Concentration

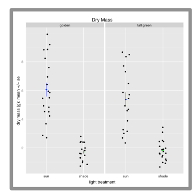




The concentration of phenolic antioxidants correlates strongly with treatment but is independent of variety. Antioxidant concentration is higher in leaf tissue.

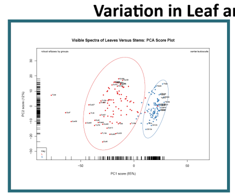
Variation in Morphology and Fitness



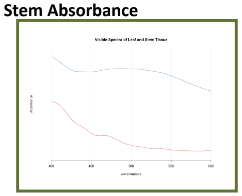


Plants cultivated in full sun produce more biomass and more flowers than shade plants. The golden variety appears to have a higher reproductive fitness based on flower production.

Variation in Leaf and Stem Absorbance



*Scores represent spectra of betalain extracts from 400 nm – 600 nm.



*Red: golden shade leaf extract; Blue: golden shade stem extract

Principal components analysis of absorbance spectra shows a sharp separation between stems and leaves regardless of light environment or cultivar, indicating organ-specific differences in betalain expression. There is a large difference in the absorbance of a peak remnant near 400 nm and after 475 nm between the tissues.

Discussion

Fitness
Morphological parameters such as flower counts serve as a measure of fitness. There was a significant genotype by environment (GxE) interaction for flower number. Based upon flower production, the Golden variety appears to have higher fitness at both light levels.

Phenolic Antioxidants
Consistent with the proposed role of antioxidants, plants in full sun show greater levels of phenolic antioxidants. Further, the levels in the leaves were consistently higher. There was no significant effect based upon genotype.

Betalain Concentration and Composition
Absorbances at 540 nm (betacyanin) and 477 nm (betaxanthin) show no significant trends explained by cultivar or light environment. This may be due to problems in consistently sampling the small number of betalain-containing cells in the fibrous stem tissues, and our protocols need further optimization. Interestingly, the betaxanthins and betacyanins track each other nearly perfectly within a particular spectrum, suggesting that any differences due to genetics or environment may occur early in the biosynthetic sequence, before the split in the pathway which leads to each structural type.

Leaf versus Stem Absorbance
PCA analysis of the visible spectrum between 400 and 600 nm (the betalain region) produced obvious clustering between stem and leaf tissues, regardless of G x E. It appears that the foothills of a large peak in the UV of the leaf samples is responsible for a large part of the clustering. This is an area for further investigation.

Future Directions

- Perform a similar experiment in more extreme light treatments, in hopes of eliciting a stronger betalain response.
- Develop a more uniform and accurate protocol for collecting and preparing the *Portulaca oleracea* stem samples.
- Perform assays investigating the composition of fatty acid concentrations in response to light stress, primarily the presence of omega 3 fatty acids.
- Experiment with a combination of stressors acting on the specimens at one time.

Literature Referenced

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Acknowledgments

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