Metabolomic Analysis of *Portulaca oleracea* Cuticular and Developmental Response to Heat Stress

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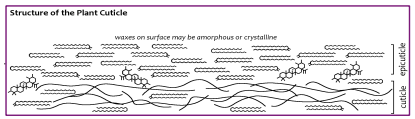
INTRODUCTION

HOW DO PLANTS RESPOND TO HEAT STRESS?

In plants, heat stress may interfere with photosynthesis, respiration, development, hormones, and metabolites. In order to cope with the diverse effects of heat stress, a plant will experience anatomical, physiological, and biochemical modifications. Because plants' reactions to heat stress are so complex, a metabolomic approach is ideal for holistically understanding the interaction between heat stress and plants. This approach investigates changes among plants' metabolites in response to specific environmental conditions. For focus, we will examine the relationship between heat stress and a plant's cuticle.

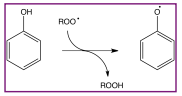


The cuticular surface of leaves serves as the chief barrier to the environment. It protects against excess light, water deficit, pathogens, and other potential stressors. A plant's cuticular surface is a hydrophobic layer composed primarily of triterpenes and long-chain esters and ethers.

WHY USE *PORTULCA OLERACEA* AS THE STUDY SPECIES?

Nearly ninety percent of *Portulaca oleracea*, or common purslane, is comprised of water. Partly for this reason, purslane is highly resistant to drought and other environmental stressors. It can therefore be subjected to elevated temperatures without jeopardizing its ability to germinate. With the threatening emission of greenhouse gases leading to global warming, knowledge of how plants like purslane react to temperature stress is valuable for understanding the interaction between plants and temperature.

Purslane is a stress tolerant plant that thrives in temperate and tropical climates.



Purslane is medically interesting because of its high concentration of antioxidants. Antioxidants inhibit oxidative processes, like the formation of cancer-causing free radicals. In the illustration to the left, the phenolic antioxidant neutralizes the free radical (ROO-) by donating an electron. The oxidized free radical is stable.

QUESTIONS

Q. DOES TEMPERATURE AFFECT THE CUTICLE
Compared infrared (IR) and reflectance spectra from two purslane varieties cultivated under two temperature conditions

Q. DOES TEMPERATURE AFFECT LEAF ANTIOXIDANT CONCENTRATION?
Used the Folin-Ciocalteau (FC) antioxidant assay to measure the total concentration of phenolic compounds in a leaf

Q. DOES TEMPERATURE AFFECT DEVELOPMENT?
Quantified the plants' nodes, branches, and biomass

Q. IS THERE AN INTERACTION BETWEEN TEMPERATURE AND VARIETY?
All tests were performed on two distinct varieties, tall green and golden, of purslane.

EXPERIMENTAL DESIGN

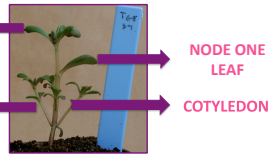
GENOTYPE (G)

ENVIRONMENT (E)

	TALL GREEN	GOLDEN
22°/ 20° C CONTROL TREATMENT	 n = 32	 n = 16
35°/ 33° C EXPERIMENTAL TREATMENT	 n = 39	 n = 35

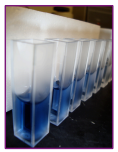
A 2 x 2 experimental design representing a hypothetical genotype by environment (G x E) effect was created. The sample sizes varied based on germination success. Plants were cultivated for three weeks in a temperature- and light-controlled growth chamber as seen at right. All plants were grown in a constant volume of water.

DISSECTION OF PURSLANE



Instrumental Techniques and Chemical Measurements: One leaf from node one was used for IR spectroscopy and the other for reflectance spectroscopy. One leaf from node two was selected for the FC antioxidant assay.

Developmental Traits: The number of nodes, branches, and an entire plant's dry mass was assessed for every plant.

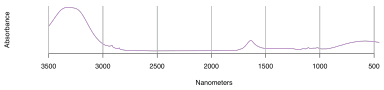


FC ANTIOXIDANT ASSAY

Phenolic compounds were extracted and their antioxidant concentrations determined through a colorimetric assay. The more richly blue the solution, the higher the concentration of antioxidants in a leaf.

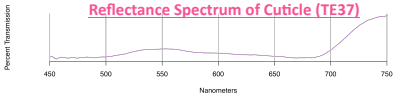
SAMPLE SPECTRA

Infrared Spectrum of Cuticle (TE37)



Different types of bonds and functional groups absorb IR radiation at distinct wavelengths. Thus, spectra of a leaf's cuticular surface can be compared to detect unique chemical differences in the cuticle. The spectra were edited to remove regions characterized by noise and those without significant peaks.

Reflectance Spectrum of Cuticle (TE37)



A reflectance spectrum indicates how much light is reflected by a leaf at visible wavelengths (450-750 nm). Differences among reflectance spectra suggest variation in the leaf color.

RESULTS

We used the "R" statistical package, particularly principal components analysis (PCA) and analysis of variance (ANOVA and MANOVA), to examine our data. PCA condenses a data set into a small number of principal components (PC) to account for any variance in the data. ANOVA and MANOVA detect response differences between G x E groups

PCA ANALYSIS

Apparatus used for IR spectroscopy

CODE	VARIETY/ TREATMENT
TC	Tall Green/ Control (22°/ 20° C)
TE	Tall Green/ Experimental (35°/ 33° C)
GC	Golden/ Control (22°/ 20° C)
GE	Golden/ Experimental (35°/ 33° C)

Apparatus used for reflectance spectroscopy

Figure 1. PCA: IR Spectra

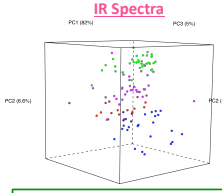


Figure 2. PCA: Reflectance Spectra

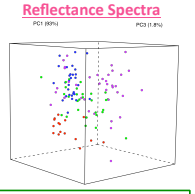


Figure 1 clearly illustrates a separation between genotypes (varieties) in different environments using IR spectroscopy- confirming that environment (temperature) and genotype both influence the cuticular surface. However, Figure 2 reveals that a similar G X E interaction for reflectance spectroscopy is not as evident. The distinction in the IR spectra may be attributed to purslane's synthesis of novel chemicals at the 35°/33°C temperature treatment. The division between G x E groups was likely not as profound in Figure 2 because reflectance spectra are defined by gentle rolling features that are not as informative as IR spectras' sharp peaks.

ANOVA AND MANOVA ANALYSIS

p > 0.05 (NONE)
p ≤ 0.05 (*)
p ≤ 0.01 (**)
p ≤ 0.0011 (***)

Response: NODES	Significance	Response: BRANCHES	Significance	Response: BIOMASS	Significance	Response: [Antioxidant]	Significance
EC	***	EC	***	EC	NONE	EC	NONE
TG	NONE	TG	NONE	TG	**	TG	NONE
EC: TG	***	EC: TG	***	EC: TG	*	EC: TG	NONE

Figure 3. Graphical ANOVA: Comparison of NODES

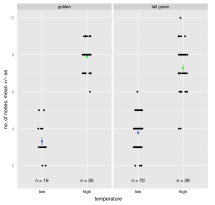
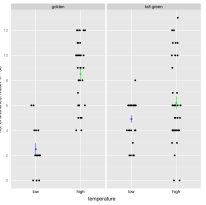


Figure 4. Graphical ANOVA: Comparison of BRANCHES



Temperature had a statistically significant effect on purslane's development. In fact, purslane generated more nodes and branches at 35°/ 33° C (Figure 3 and 4). The concentration of leaf antioxidants did not vary between G x E groups.

We found differences among varieties for some, but not all, of our response variables. Though the two varieties responded similarly to temperature (Figure 3), the golden variety exhibited a drastic increase in branching at 35°/33° C, while the tall green variety did not significantly vary its branching between temperature treatments (Figure 4).

DISCUSSION

The variation in IR spectra in response to temperature probably results from modifications to the chemical composition of the cuticular surface. For instance, to support growth at warmer temperatures, the purslane may have developed thicker cuticles to retain water. While IR spectra have distinctive peaks corresponding to a compound's functional groups, reflectance spectra are defined by more rolling peaks that are not as susceptible to alterations based on temperature.

Though 35°/33°C was intended to be the "stressful" environment, both varieties grew faster at this temperature, as indicated by the accelerated development of nodes and branches. An increase in antioxidant production, a typical response to stress, likely did not occur at 35°/33° C because purslane commonly experiences such temperatures in its natural range.

In the future, we would like to expose purslane to a truly stressful environment. This can be accomplished by cultivating purslane at extremely high temperatures (> 40° C) or by combining multiple environmental stressors, such as heat stress and water deficit. Other revisions to the experiment may include a more extensive growth period to permit flowering, more than two temperature treatments, or even a microscopic inspection of the plants' cuticles. As before, we would use spectroscopy, run the FC antioxidant assay, and quantify a plant's developmental features to further understand the effect of temperature on purslane

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