



# Osmotic & Ionic Stress in Purslane

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## Background

Purslane (*Portulaca oleracea*) is a succulent, robust medicinal plant, distributed in temperate and tropical regions world-wide. Purslane has been investigated extensively for bioactive compounds. The most noteworthy are the  $\omega$ -3 fatty acids, and in particular,  $\alpha$ -linolenic acid (ALA). We have identified purslane as a suitable model system for exploring plant responses to stress. Stress in plants takes various forms, such as increased temperature, increased salinity, and decreased water supply. Each of these stressors is expected to be a problem with climate change, so understanding how plants respond to such stressful environments may be very useful in improving crops for warmer, drier, and saltier futures.



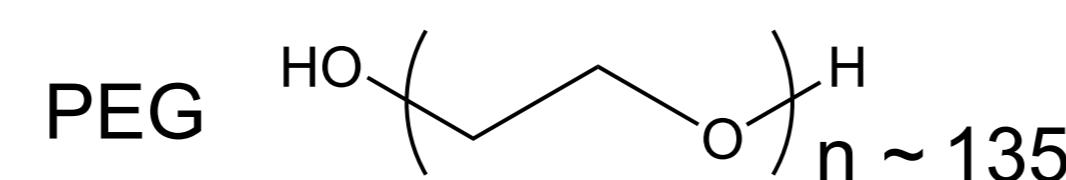
Our previous work investigated the responses of two varieties of purslane to saline stress. In particular, we investigated whether some varieties exhibit greater plasticity than others with respect to saline stress, and whether functional traits such as biomass, flower production, and concentration of secondary metabolites, such as betalains and the amino acid proline, respond to saline stress in a coordinated fashion. These molecules are of interest for their role as anti-oxidants, which are important in the response to stress. In the work reported here, we added an additional stressor, 7.5% polyethylene glycol or PEG. PEG denies the plant water via osmotic stress. This complements the treatment with NaCl which causes both osmotic stress and toxicity due to the increased  $\text{Na}^+$  ions.

## Experimental Design

We grew 120 plants of two different varieties, WI-9 and T-16. These plants were subjected to three different treatments: water, 200 mM NaCl, and 7.5% polyethylene glycol (PEG). We chose these treatment options, because they both cause osmotic stress, and NaCl is also toxic. This allows us to isolate the effect of the sodium toxicity. The 7.5% PEG treatment was employed, because it produces the same osmotic pressure as 200 mM NaCl.

## Experimental Design Con't

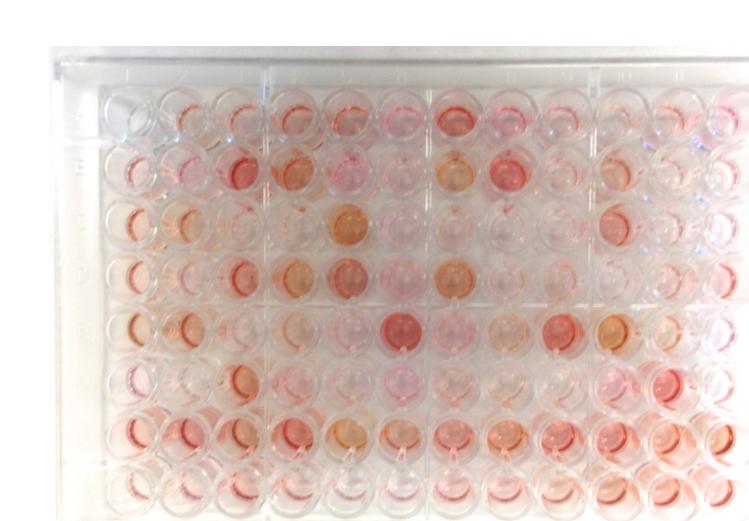
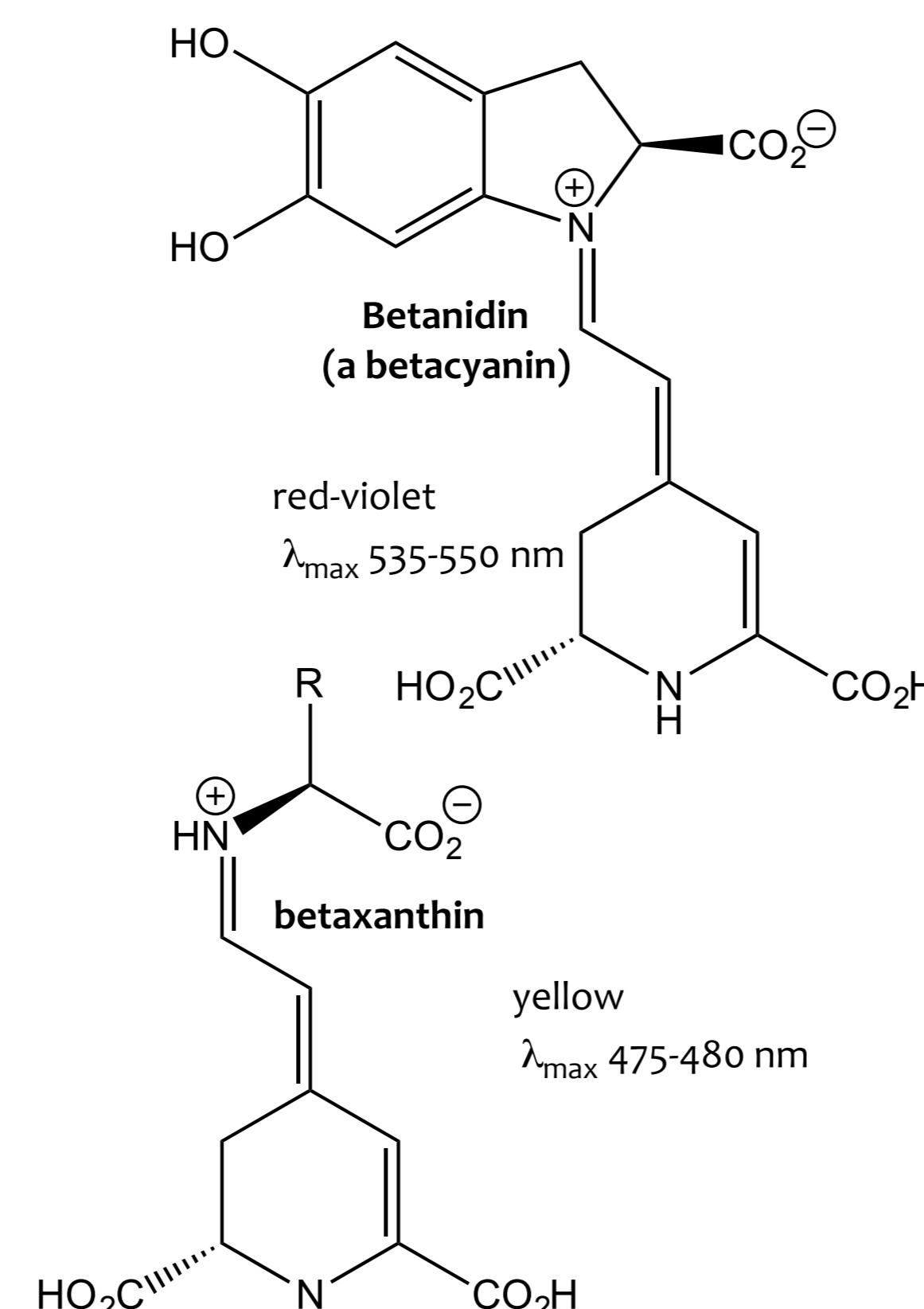
	Water	200mM NaCl	7.5% PEG
T-16	40	40	40
WI-9	40	40	40



## Methods

**Proline:** Leaf tissue was extracted using sulfosalicylic acid, and the extract was reacted with ninhydrin. After extraction into toluene, the yellow-pink reaction product was quantified using visible spectroscopy at  $\lambda_{max} = 522$  nm. A calibration curve was then used to compute the concentrations in the original leaf tissue.

**Betalain Pigments:** Leaf and stem tissues were extracted using 60% methanol in water. After bead-beating and centrifugation, the extract was analyzed using visible spectroscopy. Published indices were used to compute the concentrations in the original tissues. These compare the absorbances at several wavelengths to compensate for impurities. Representative betalain structures are shown below.



## Acknowledgments

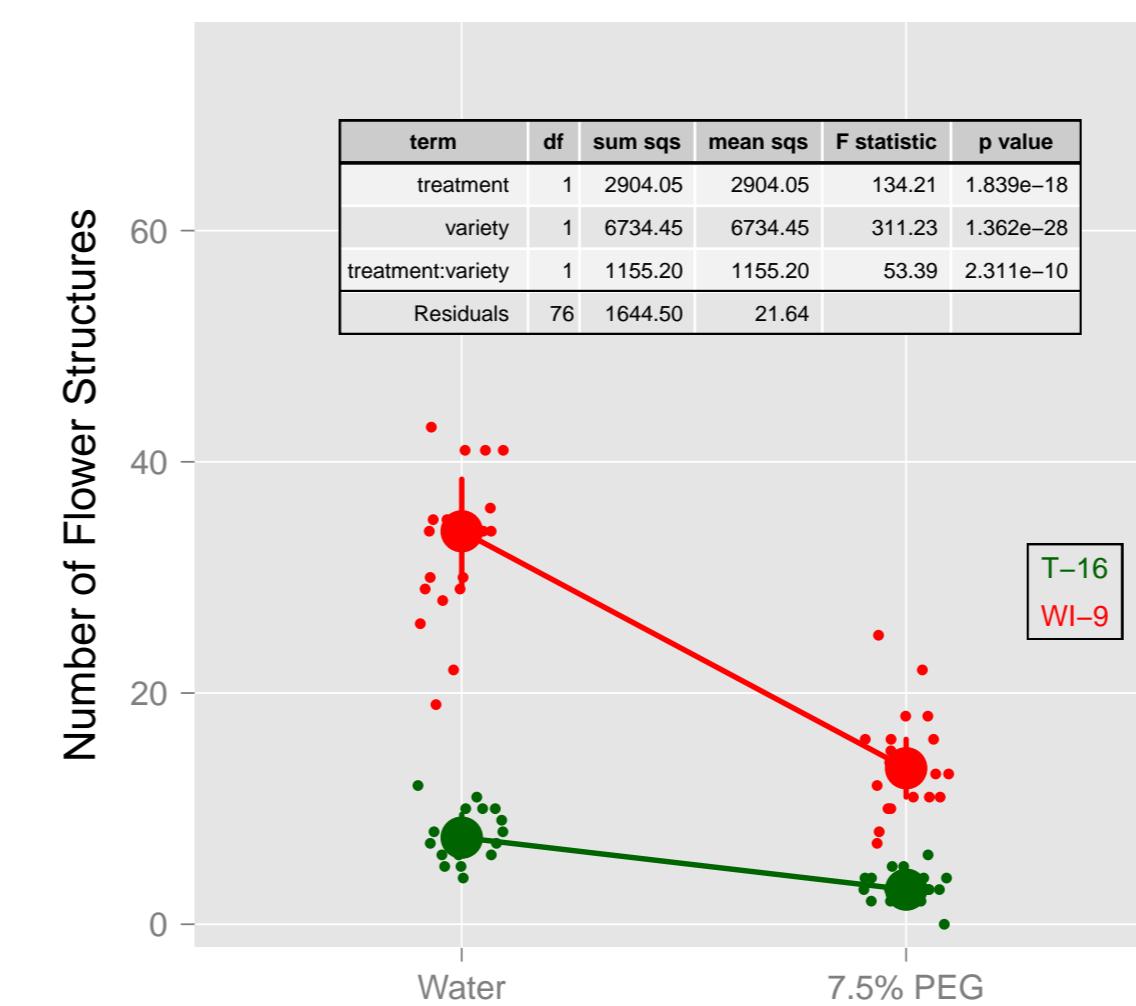
This research was supported by an Environmental Education Grant from The Andrew W. Mellon Foundation. We would like to thank them for their support.

## Morphological Changes

Morphological changes in plants under stress were dramatic for both varieties. The upper photo shows T-16 plants, the lower photo shows WI-9 plants. Treatments from left to right: control, 200 mM NaCl, 7.5% PEG



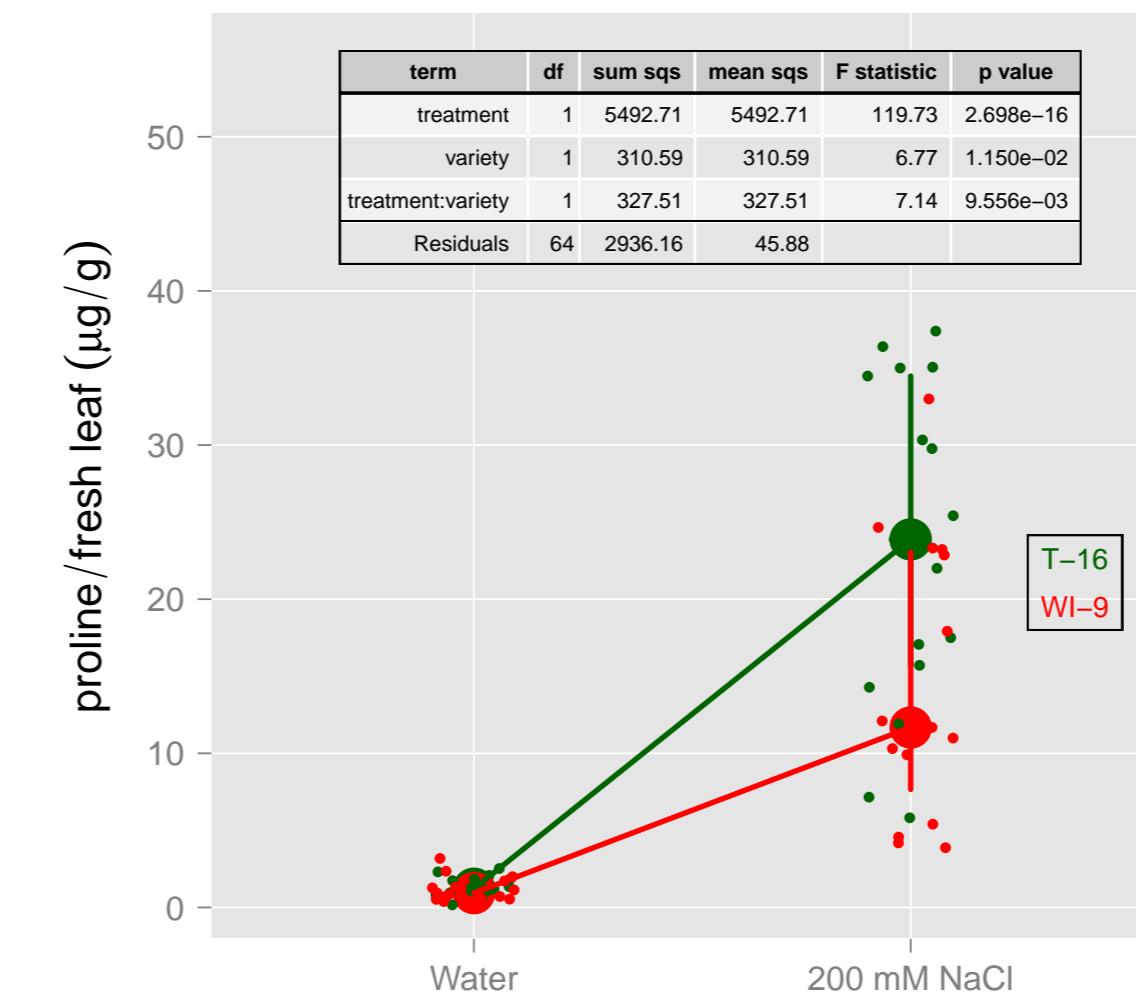
## Flowers in PEG Stressed Plants



Both varieties of purslane treated with PEG had fewer flower structures than the control group. Results similar to this were seen in the plants treated with salt.

## Ionic Stress

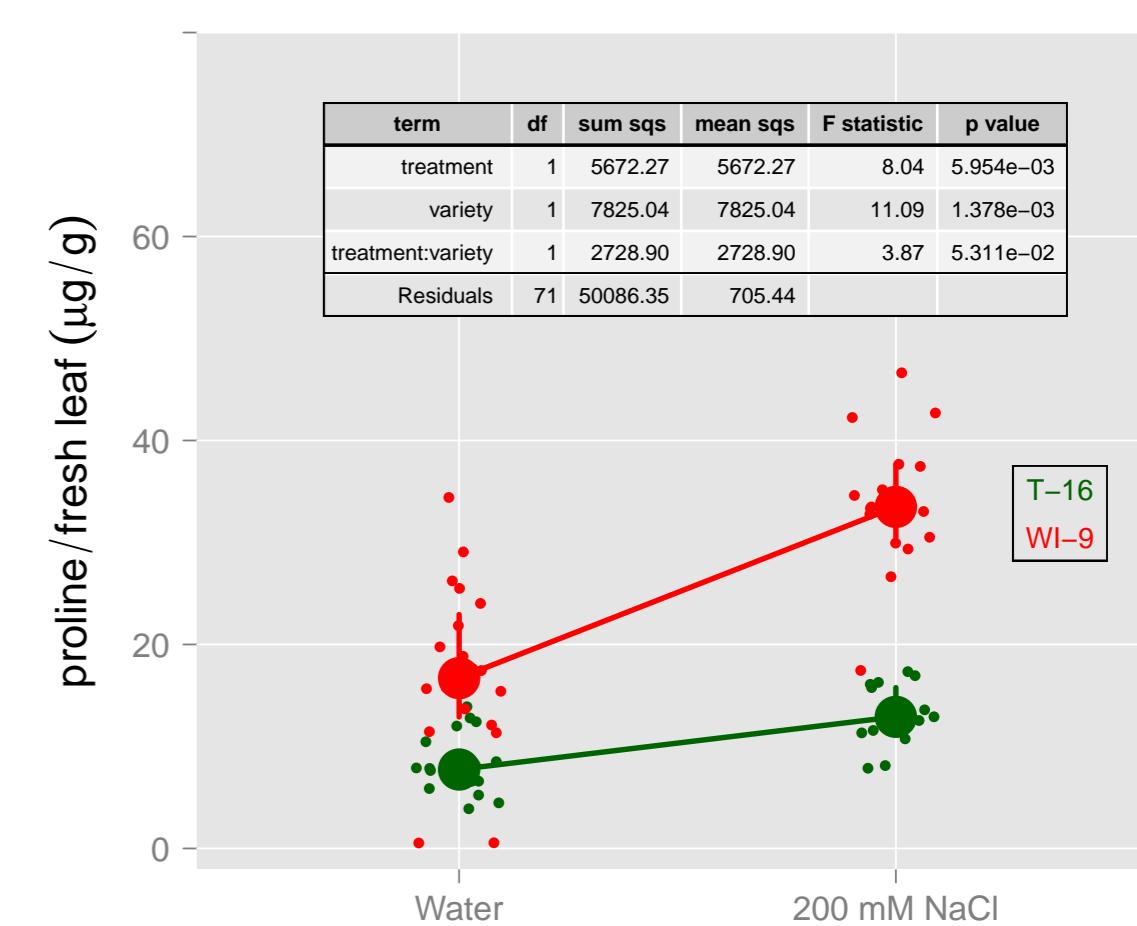
### Proline in Salt Stressed Plants



Plants of the T-16 variety treated with salt produced more proline than the WI-9 variety. All terms were statistically significant.

## Ionic Stress Con't.

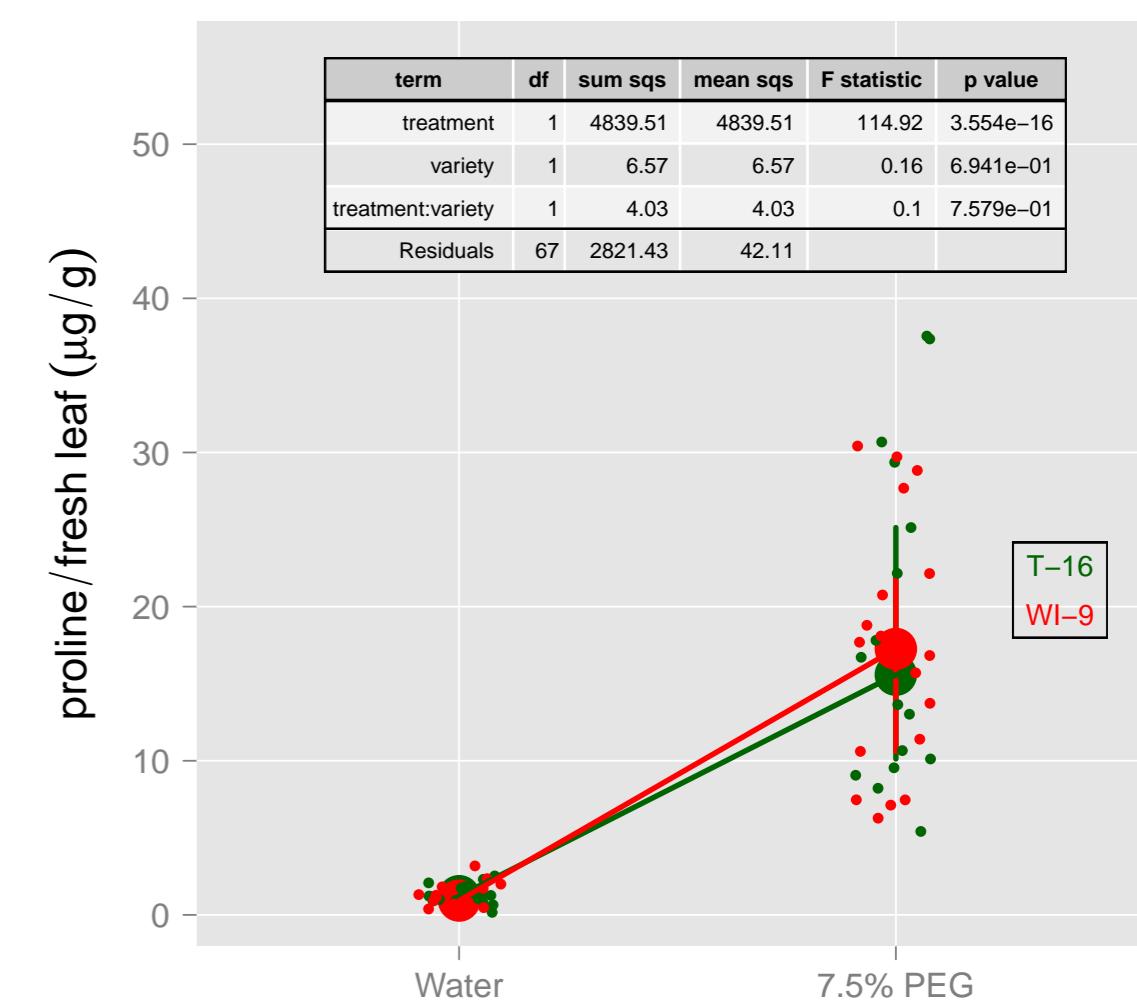
### Stem Betacyanins in Salt Stressed Plants



Plants of the WI-9 variety treated with salt produced more betacyanins than the T-16 variety. Treatment and variety were both statistically significant, but the interaction term was 0.053.

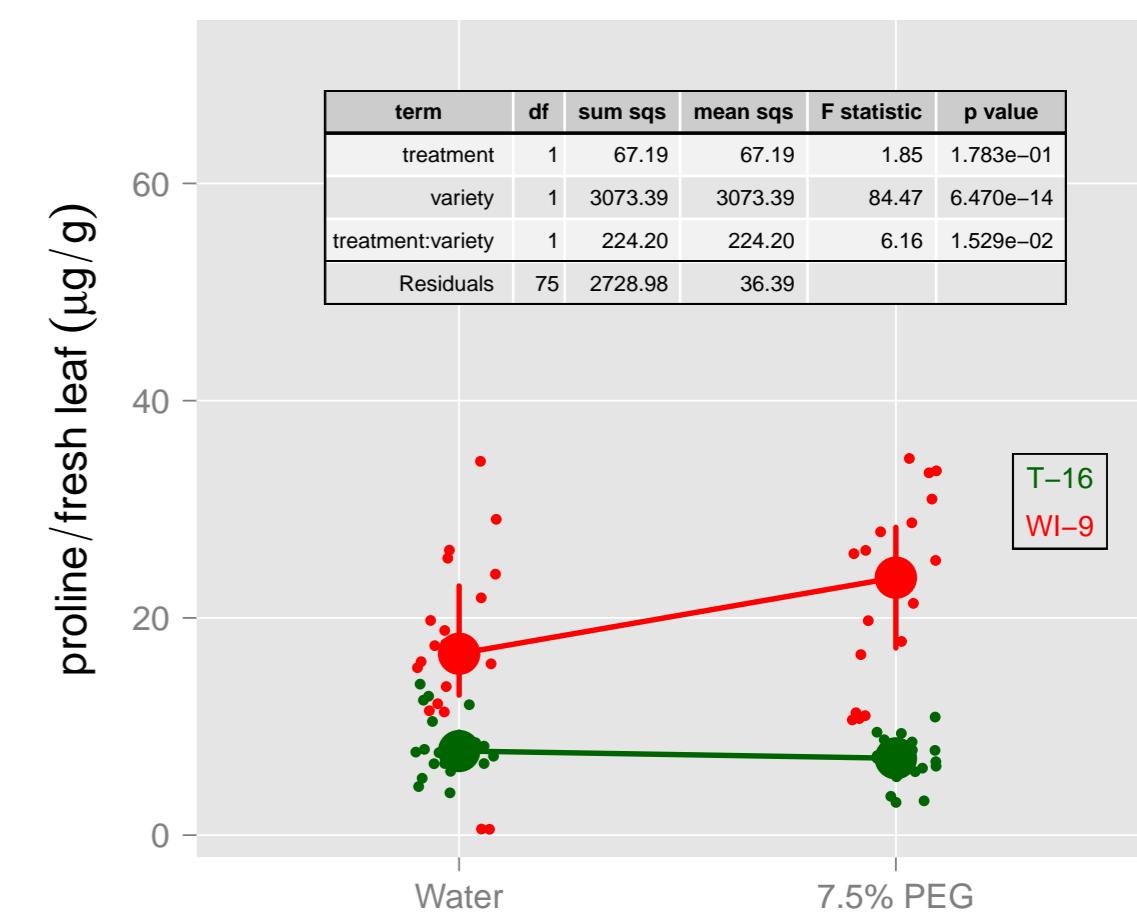
## Osmotic Stress

### Proline in PEG Stressed Plants



Plants of the WI-9 and T-16 varieties treated with PEG produced almost the same amounts of proline. Only treatment was statistically significant.

### Stem Betacyanins in PEG Stressed Plants

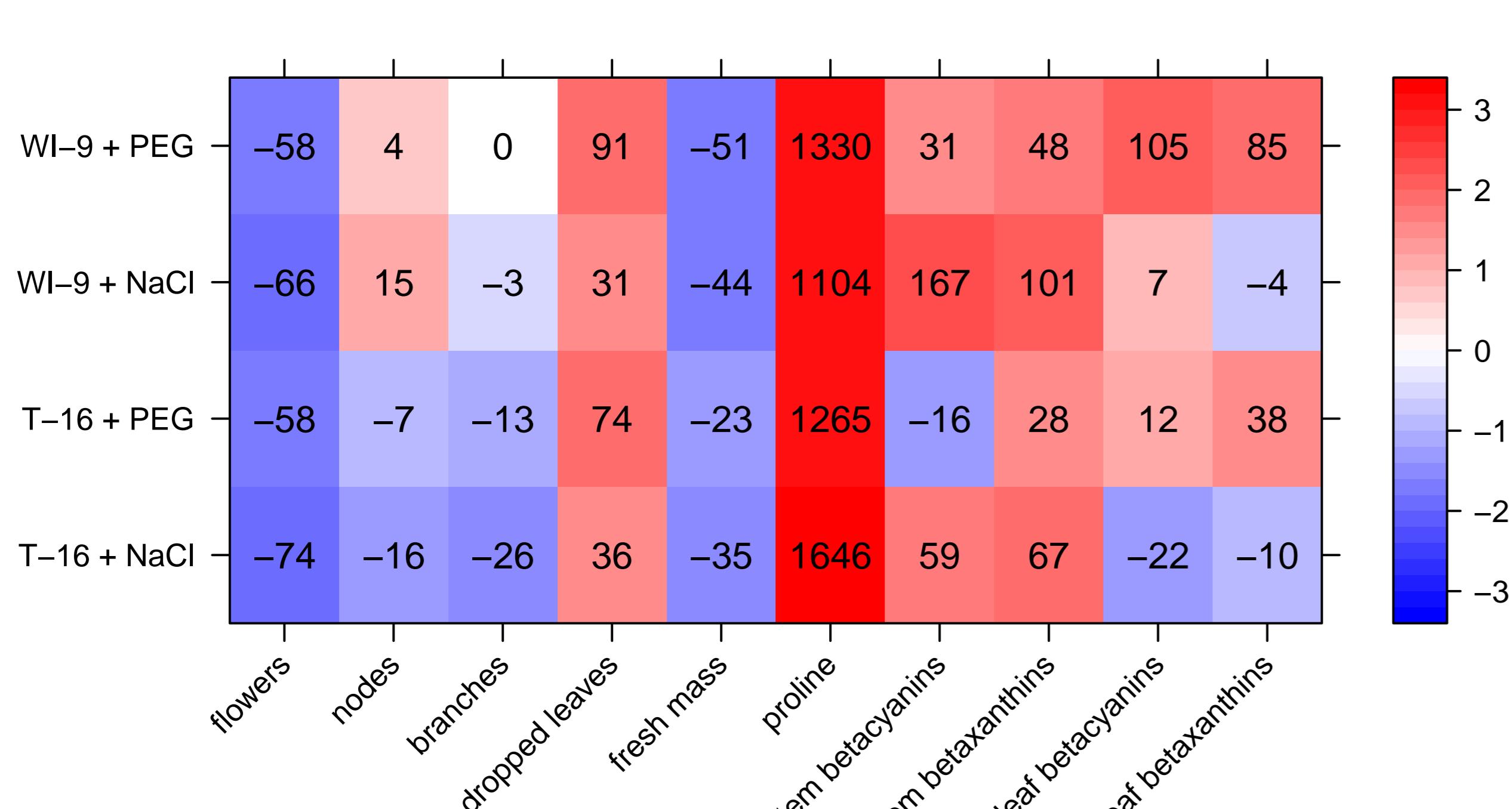


Plants of the WI-9 variety treated with PEG produced more betacyanins than the T-16 variety. Only treatment was statistically significant.

## All Results: Variety x Treatment

In addition to the results reported in detail above, all of our results are summarized in the following heat map.

Percent Changes Relative to Control (color-coded based upon log % change)



## Key Findings

- WI-9 increased node production under the salt stress, whereas T-16 decreased node production.
- Both varieties had more dropped leaves under the PEG stress than under the salt stress. WI-9 was more sensitive to PEG stress than T-16.
- Under both stresses, both varieties greatly increased their proline concentrations. However, the T-16 variety, more so than the WI-9 variety, was more sensitive to salt than PEG, increasing proline concentration in even greater amounts under the salt stress.
- Both varieties slightly decreased the amount of leaf betaxanthins under the salt stress. However, under the PEG stress, both varieties greatly increased the concentration of leaf betaxanthins, especially in the WI-9 variety.
- Under PEG stress, WI-9 increases leaf betacyanin concentration roughly 10 times more than the T-16 variety.
- Both varieties increased stem betaxanthin concentration more under the salt stress than under the PEG stress. Under either kind of stress, WI-9 invested more heavily in stem betaxanthins than T-16.
- Under the PEG stress, the two varieties shifted stem betacyanin concentrations in opposite directions. Under the salt stress, both varieties, but especially WI-9, increased the stem betacyanin concentration.