Effects of Climate Change on the Medicinal Plant *Portulaca oleracea*: Focus on Antioxidant production

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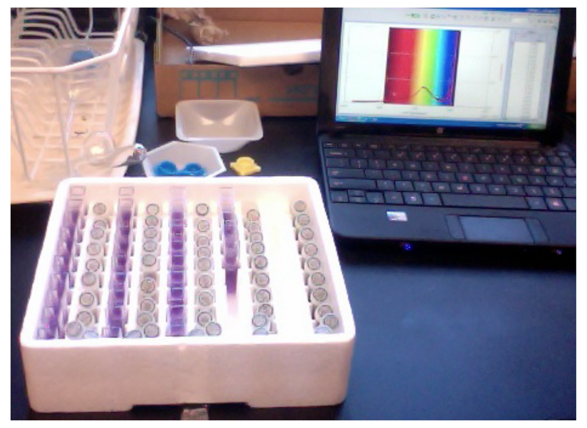
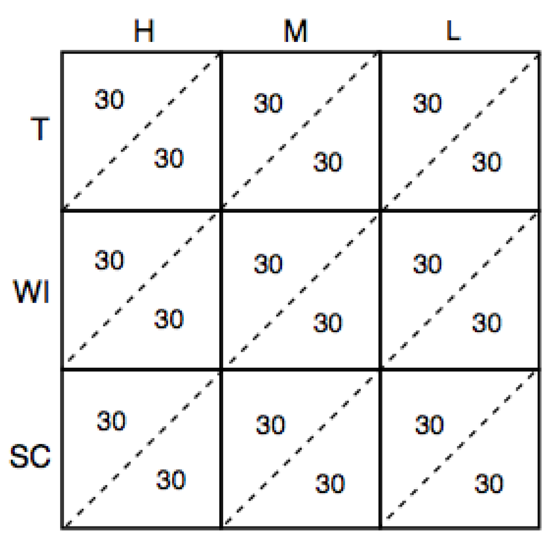
Introduction

Portulaca oleracea, commonly referred to as purslane, is a weedy medicinal plant known to withstand extreme environmental conditions (see picture below top). Like all plants, purslane is immobile and can therefore only form chemical responses to stressful conditions. These chemicals are referred to as secondary metabolites and can perform a number of functions: deterring herbivores or sequestering heavy metals. In the case of purslane, high levels of these secondary metabolites allow purslane to thrive in conditions far too hostile for many other plants, including areas of very little water or very high salinity. Our project examined antioxidant levels in three different varieties of *Portulaca oleracea* using two different stress conditions. We expected to see an overall increase in antioxidant levels as a response to increased stress. We also expected some varieties to have differential responses to the stressful stimuli. These tests will help us understand how plants respond during times of extreme heat, rising sea levels and drought brought on by climate change.



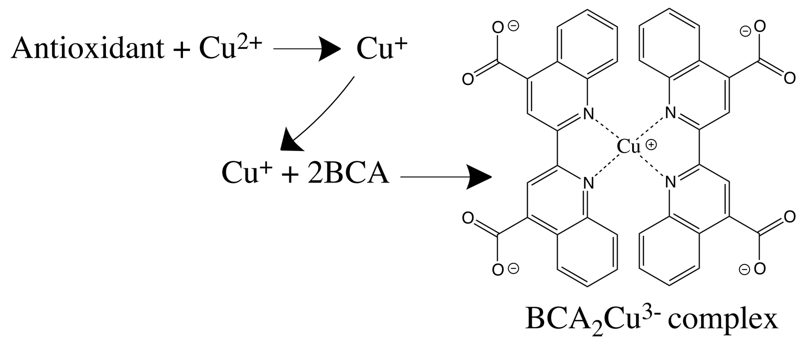
Experimental Design

1,080 plants of 3 purslane varieties (T, WI, and SC) were planted at the beginning of the summer. Each variety received 3 different treatments (High, Medium, and Low) of both salt and water. Samples were analyzed at an intermediate stage (weeks 2 and 3), and a final stage (weeks 5 and 6).

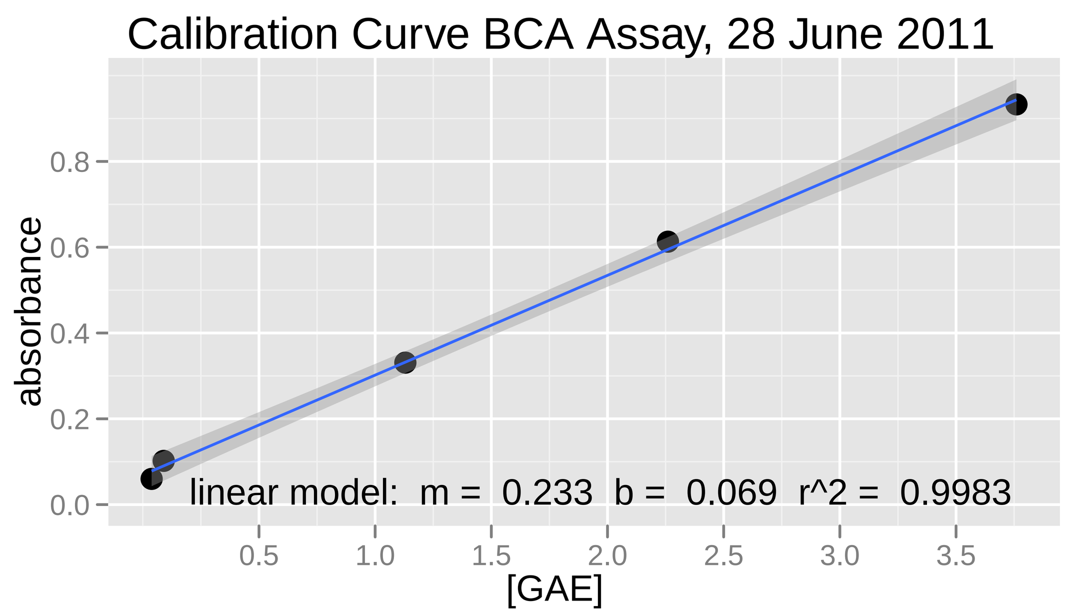


BCA Assay

The BCA assay quantifies total antioxidants within a plant sample. Plant samples in buffered ammonium acetate (pH 7) are combined with a solution of copper (II) ions and bicinchoninic acid (BCA).The antioxidants in the sample reduce the copper (II) to copper (I) which then forms a complex with BCA (see figure below) displaying a purple color. We analyzed the solution in the cuvette using UV-vis spectroscopy to track the purple complex (558 nm). We used Beer's law to calculate a concentration value.

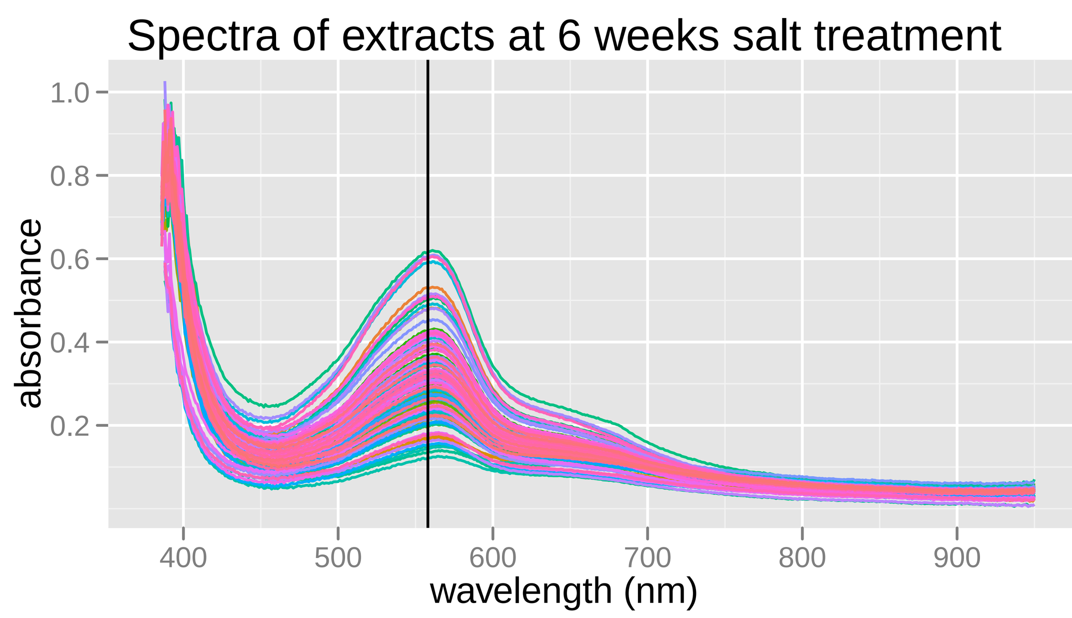


We ran a calibration curve on each day of sample analysis. The calibration curve below is an example from our 2 week salt treatment data. We analyzed solutions of 0.09, 0.38, 1.13, 2.26, and 3.76 ppm of gallic acid (a known antioxidant).



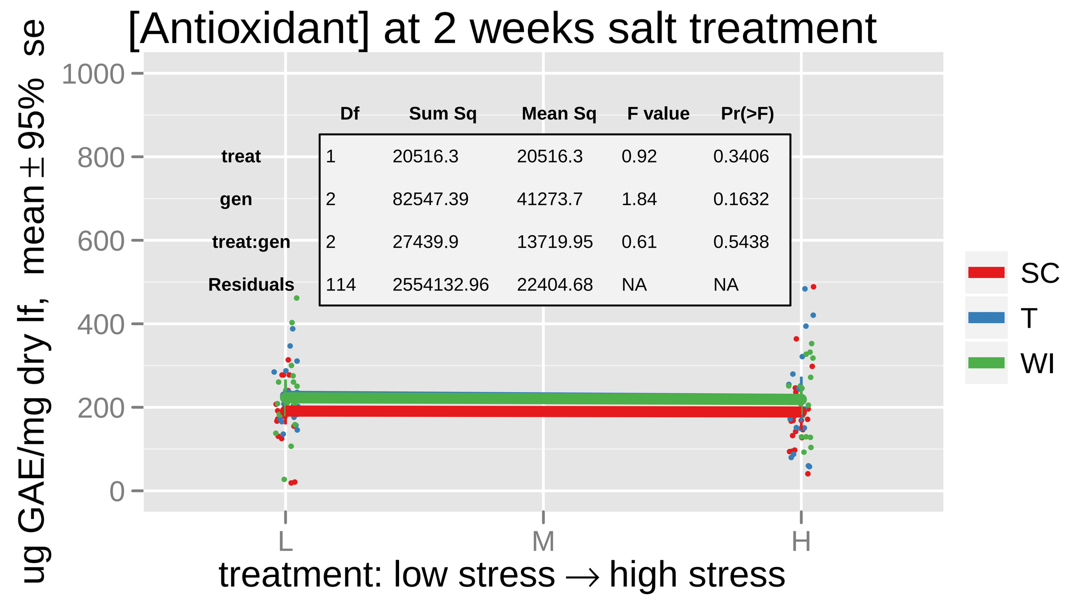
Response to Salt Stress

Below is a compilation of all of our BCA spectra for the 2 week salt trial. This graph allows us to troubleshoot spectra. The line is drawn through the peak absorbance value at 558 nm. This is the absorbance value we use to calculate concentration.



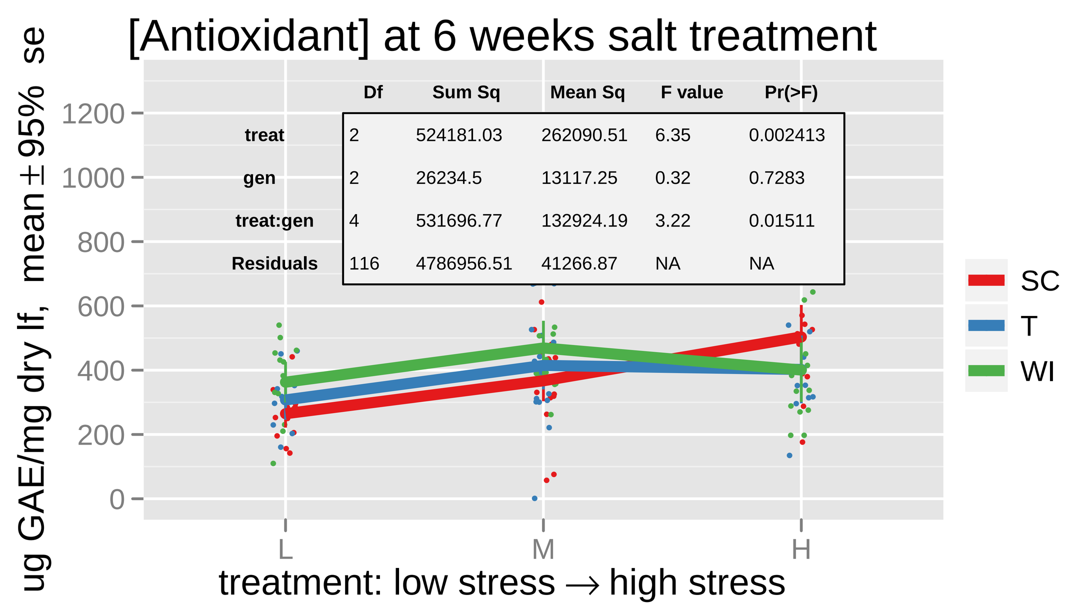
Response to Salt Stress con't.

The graph below displays total production of antioxidants after 2 weeks of salt stress. The graph does not allow us to conclude anything significant about production of antioxidants in response to 2 weeks of salt stress. Furthermore, the graph shows no significant trend of differential antioxidant production among the purslane varieties in response to 2 weeks of salt stress.



	Df	Sum Sq	Mean Sq	F value	Pr(>F)
treat	1	20516.3	20516.3	0.92	0.3406
gen	2	82547.39	41273.7	1.84	0.1632
treat:gen	2	27439.9	13719.95	0.61	0.5438
Residuals	114	2554132.96	22404.68	NA	NA

This graph shows antioxidant production after 6 weeks of salt stress. It appears as though the SC genotype is producing higher amounts of antioxidants than the T and WI genotypes in response to the stressful condition. This is a significant genotype by environment response that shows that not all purslane varieties respond the same to drought stress.

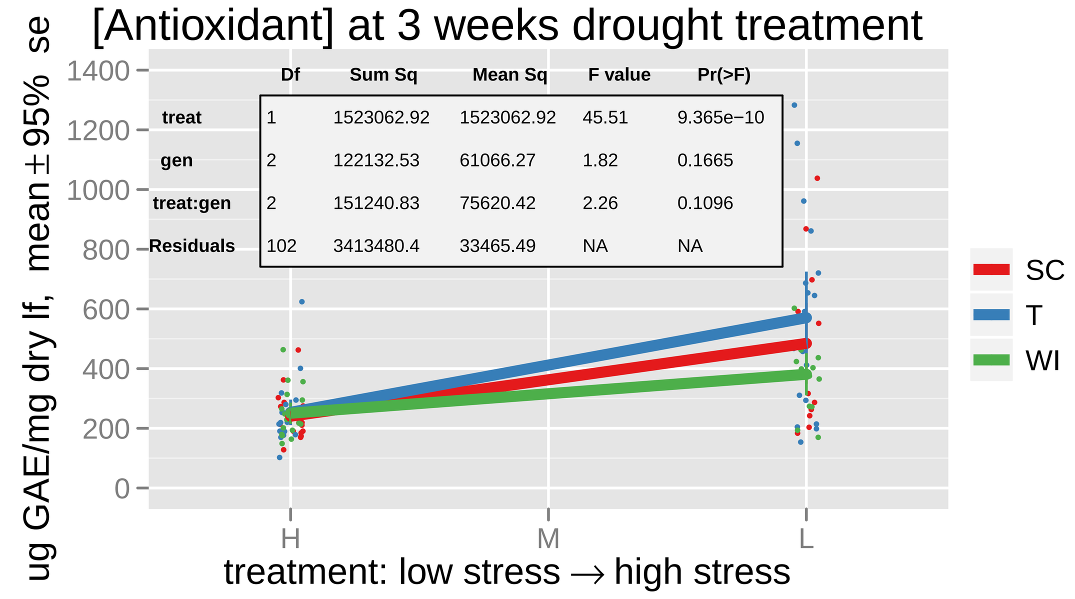


	Df	Sum Sq	Mean Sq	F value	Pr(>F)
treat	2	524181.03	262090.51	6.35	0.002413
gen	2	26234.5	13117.25	0.32	0.7283
treat:gen	4	531696.77	132924.19	3.22	0.01511
Residuals	116	4786956.51	41266.87	NA	NA

Response to Drought

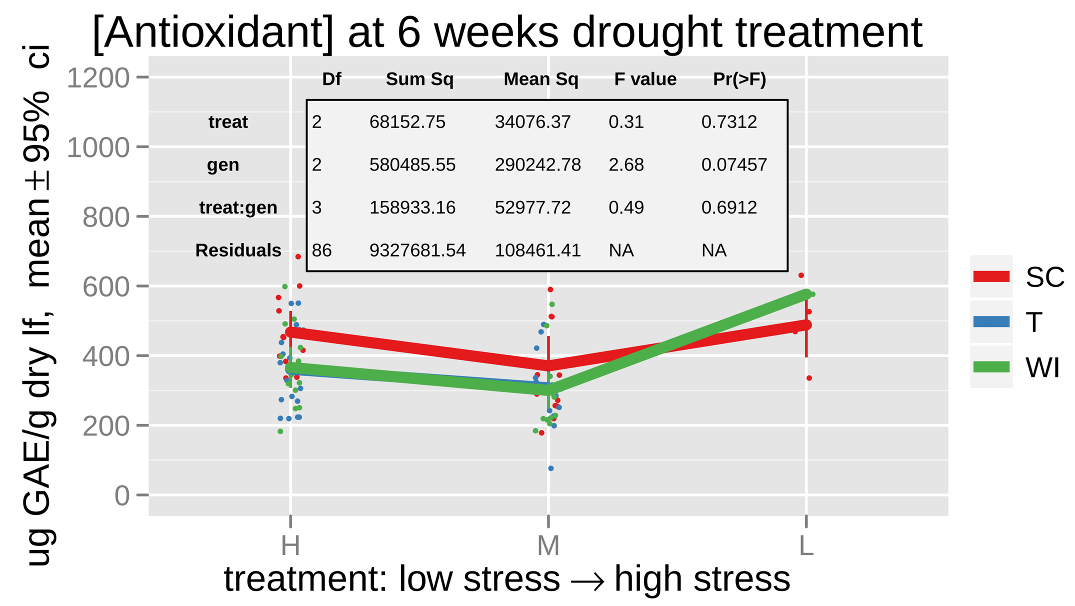
The figure at the top of the next column displays levels of antioxidants produced by plants under low water conditions after 3 weeks. There is an increase in antioxidant production in response to the drought stress and the T genotype is producing more antioxidants than the SC which in turn is producing more than the WI. ANOVA, however shows us that only the response to treatment, and not the genotype by treatment response, is statistically significant.

Response to Drought, con't



	Df	Sum Sq	Mean Sq	F value	Pr(>F)
treat	1	1523062.92	1523062.92	45.51	9.365e−10
gen	2	122132.53	61066.27	1.82	0.1665
treat:gen	2	151240.83	75620.42	2.26	0.1096
Residuals	102	3413480.4	33465.49	NA	NA

The next figure shows a slight decrease in the production of antioxidants between medium and high drought stress. We also saw a dramatic increase in antioxidant levels in the low water treatment with the WI variant producing more than the SC. Unfortunately too many of the T plants died in the low water treatment to have usable data, so we do not know how the T variety responds to drought stress.



	Df	Sum Sq	Mean Sq	F value	Pr(>F)
treat	2	68152.75	34076.37	0.31	0.7312
gen	2	580485.55	290242.78	2.68	0.07457
treat:gen	3	158933.16	52977.72	0.49	0.6912
Residuals	86	9327681.54	108461.41	NA	NA

Conclusions

Salt stress increases the production of antioxidants in *Portulaca oleracea* after at least 5 weeks of treatment. Variety SC responds to salt stress with higher production of antioxidants in comparison to T or WI varieties. Drought stress seems to produce an overall increase in antioxidants as well but no significant genotype by environment interaction was observed.

References

D.C Marino, L. Z. L. Sabino, J. A. Jr., A. D. A. Ruggiero, and H. D. Moya, "Analysis of the polyphenols content in medicinal plants based on the reduction of Cu(II)/bicinchoninic complexes," *Journal of Agricultural and Food Chemistry*, vol. 57, pp. 11061-11066, 2009.

Software: R packages ChemoSpec and HandyStuff github.com/bryanhanson

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