The Effect of Stress on *Portulaca oleracea* (Purslane): Proline Concentration Levels

Polly Haight*, Matthew Kukurugya, Vince Guzzetta & Bryan Hanson

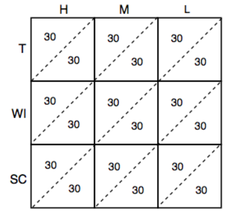
Dept. of Chemistry & Biochemistry, DePauw University, Greencastle Indiana USA

Introduction

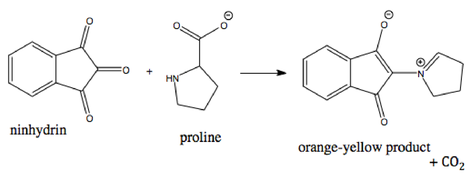
Purslane is a weedy medicinal plant known to withstand extreme environmental conditions. Under stress, it is expected that plants will increase production of compounds, such as betalains and other phenolic antioxidants, that are associated with the protection of metabolic functions. We have been investigating the response of purslane to both salinity and drought stress in order to chemically and morphologically quantify the effects that harsh environments have on the plant. Our hope is that this will lead to a prediction as to how plants in general will react to climate change. More specifically, plants are known to increase production of proline under stress. Proline is an amino acid that acts as an antioxidant – it reduces free radicals that could potentially be harmful to the plant. Its production is a self-defense mechanism. A plant's proline levels are an indicator of both the harshness of the environment and the plant's response.

Experimental Design

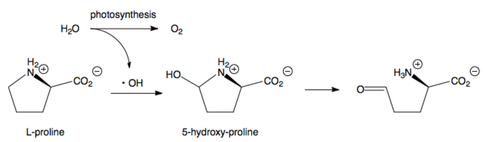
1,080 plants of 3 purslane varieties were planted at the beginning of the summer. Half of the total plants received a drought treatment, while the other half received a salinity treatment (this guaranteed both drought stress and salt stress results for each genotype). Samples were collected at an intermediate stage (weeks 2 and 3), and a final stage (weeks 5 and 6). The graphic below portrays the experimental design of the project.



Chemistry of Proline



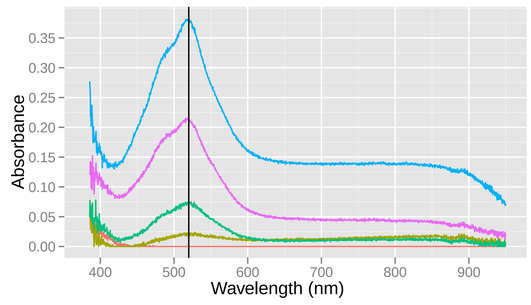
To analyze proline levels in each plant, we took advantage of the reaction of amines with ninhydrin. According to protocol we reacted ninhydrin with proline (a secondary amine) in the leaf tissue to form an orange-yellow compound (as shown above). We could then quantitatively measure the amount of the colored product that was formed via UV/Vis Spectroscopy - a higher absorbance of colored compound at 520 nm directly correlated with a higher proline concentration.



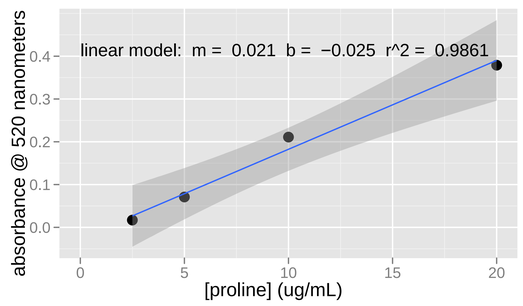
The scheme above shows how proline can act as an antioxidant.

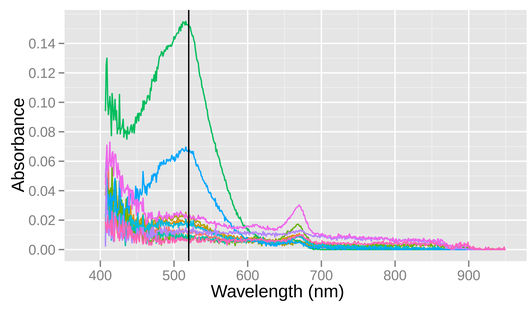
Proline Assay

A calibration curve was created each day that samples were analyzed. The figure below shows typical spectra from a calibration curve. Concentration values of 0.625, 1.25, 2.5, 5 and 10 ug of proline per mL were used as standards.



The figure below is a typical calibration curve derived from the spectra above. This calibration curve was applied to the sample data in order to calculate proline concentrations.

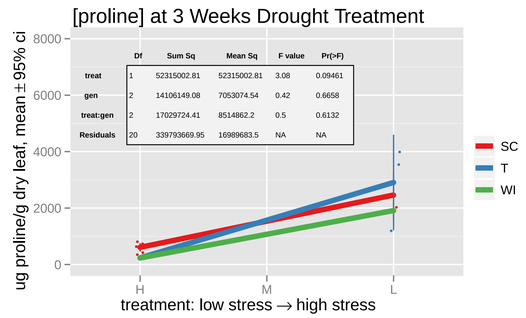




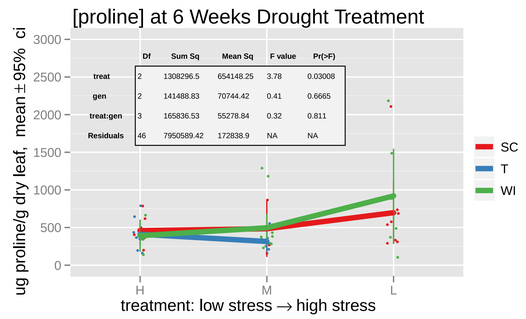
The figure above depicts a typical set of spectra from 12 tissue samples. The peak in the high 600's is due to chlorophyll a. The absorbance at 520 nm was converted to ug proline/g dry leaf for each sample.

Response to Drought Stress

Proline concentrations for all three varieties of purslane were calculated after 3 weeks of drought treatment. As can be seen in the figure below, a lack of water had the greatest effect on the tall (T) variety. However, none of the trends were significant by ANOVA.

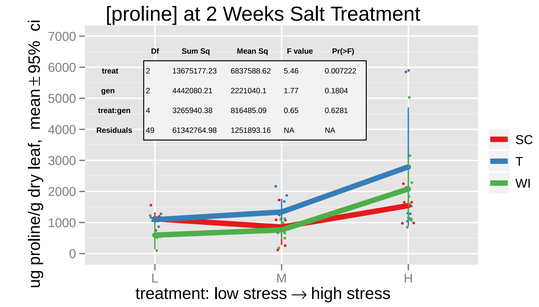


The results changed significantly after 6 weeks of drought treatment. The plants under high stress (no water) produced increased amounts of proline in all 3 genotypes. But there appears to be little change in proline production levels between the high and medium treatment plants, possibly indicating a threshold effect. Only after the plants felt a certain amount of stress was it apparent that proline levels increased. These trends are shown in the figure below. It should noted that many of the high stress (no water) plants were unable to withstand the treatment, and thus many plants died, especially the tall variety. Hence there is limited data at treatment level L.

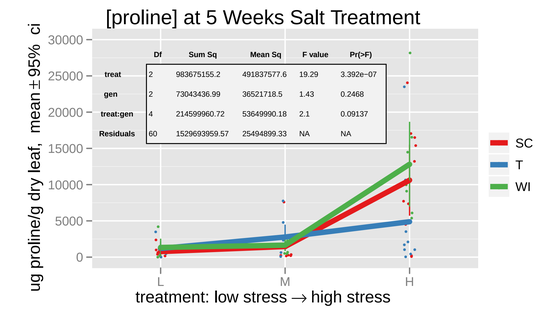


Response to Salt Stress

The first round of samples from the salt study were collected and analyzed for proline at 2 weeks. It can be seen in the next figure that proline concentration generally increased as the concentration of NaCl increased. This occurred for each genotype of purslane. However, only the treatment was significant by ANOVA.



Plants treated with NaCl were again analyzed after 5 weeks of treatment. As shown in the figure below, when the [NaCl] increased, plants produced greater amounts of proline in all 3 varieties. The trend seen here is similar to that seen at 2 weeks, but greatly enhanced. SC and WI proline levels are dramatically affected by stress, as seen in the graph below. Treatment is significant according to ANOVA. A threshold effect (similar to that seen in the 6 week drought study) is apparent here.



Conclusions

It is clear that proline concentration increases in purslane when its environment becomes more stressful. This trend was apparent in all 3 varieties under both the drought and salinity treatments. Stress caused by NaCl had the greatest effect on proline production after 5 weeks, reaching amounts of over 15,000 ug/g. There is also reason to believe that purslane undergoes a threshold effect under both NaCl and drought stress. This conclusion is apparent in the graphs showing little change between low-medium stress conditions, but a great change between medium-high stress conditions.

References

L. S. Bates, R. P. Waldren, and I. D. Teare, "Rapid determination of free proline for water-stress studies," Plant and Soil, vol. 39, pp. 205 - 207, 1973.

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

Acknowledgements

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