A new Proline assay for studying plant drought stress

GH Report No 1767

27th April 2018 - HM, TA, JL.

Executive Summary:

The goal of this project was to obtain the first molecular proof that Seasol treatment can reduce drought stress in tomato plants, using a new spectrometer nominated for purchase.

This study concludes that application of Seasol Commercial Seaweed Concentrate improved tolerance to plant drought stress.

This conclusion is based on the findings that drought stressed tomato plants treated with Seasol Commercial Seaweed Concentrate had reduced levels of proline and display reduced wilting symptoms under drought stress.

These results support the hypothesis and purchasing the spectrometer used in this experiment.

Aim: The aim of this work is to use the plant stress molecular marker Proline to prove (for the first time) that plants treated with Seasol Commercial Seaweed Concentrate use molecular responses to reduce tomato plant drought stress symptoms.

Hypothesis: Seasol reduces plant drought stress symptoms by triggering molecular responses.

Introduction:

Proline is an amino acid that is used as an indicator of abiotic stress in plants (Claussen 2005). This association is supported by an extensive number of scientific publications demonstrating the different biological ways Proline reduces symptoms of plant stress. Proline is an important osmolyte, metal chelator, and antioxidant which are all important properties for maintaining plant cell turgor, osmotic balance, stabilising electrolyte leakage and suppressing the buildup on harmful reactive oxygen molecules (see review Hayat et al, 2012).

During abiotic stresses such as drought, plants produce more proline compared to non-stressed plants. However, to use Proline as a biological marker for plant drought stress studies, the R&D department requires:

- ***Establishing a working Proline test method***:
    - A proline testing assay can be sourced from published scientific papers and is not a barrier (to establishing this assay in the Seasol R&D laboratory).

- ***The availability of a suitable spectrometer to measure the proline levels in drought stressed plants:***
    - This is not available and needs to be purchased to overcome this limitation. As a temporary effort, a spectrometer was supplied by a manufacturer for trialing.

Recent R&D work has shown that the application of Seasol Commercial Seaweed Concentrate can reduce wilting symptoms in drought stressed tomato plants (*see GH Report No 1762, 17.04.2018, Effect of Seasol Commercial Seaweed Concentrate on Drought Stressed Tomato Plants*). However, the way this phenotype is achieved and the biological mechanisms utilized are unknown.

In this experiment, because a spectrometer was available, we now have the first evidence that Seasol Commercial Seaweed Concentrate reduces tomato plant drought stress symptoms at the molecular level by reducing the accumulation of proline.

Overall, this work using the proline assay and spectrometer supports claims that Seasol Commercial Seaweed Concentrate helps to reduce drought stress in plants.

Proline content determination

This proline content testing method is a colorimetric method which requires a spectrometer (see left side of picture below). This method uses a chemical reaction to colour the proline which can then be measured (see right side of picture below). The colour intensity of the labelled proline is measured with a spectrometer and content calculated using a proline calibration curve.

Left: Spectrometer used for colour testing. Right: Prepared Samples in container ready for colour testing.

The leaf samples (frozen) used for proline testing are from tomato plants exposed to two drought stress events (*see GH Report No 1762, 17.04.2018, Effect of Seasol Commercial Seaweed Concentrate on Drought Stressed Tomato Plants*). The proline testing method used is described by Bates et al. with modifications described by Shabnam et al(Bates et al. 1973; Shabnam et al. 2016).

In brief, 0.5g of frozen crushed leaves were put in Eppendorf tubes. 2 mL of 3 % (w/v) aqueous sulfosalicylic acid was added and using a pestle the leaves ground for 1 minute to get the leaf extract. The mixture was centrifuged at 10,000 rpm for 5 min. 0.5 mL of the supernatant was withdrawn and added to 1 mL of ninhydrin reagent (2.5 g ninhydrin in 100 mL of a solution containing glacial acetic acid). The samples were put in a boiling water bath for 60 min, followed by cooling in a water bath at room temperature for 5 min. The absorption was measured at 508 nm using BMG Omega plate reader. The proline concentration was determined from a standard curve derived from solutions containing known amounts of L-proline (50-300µM) and calculated on a fresh weight basis (µmol proline/g fresh weight leaves).

Result

Drought phenotype in Seasol vs water control: The image below shows that Seasol Commercial Seaweed Concentrate treated plants show less leaf wilting symptoms relative to the water control. All the plants were grown in premium soil containing fertiliser and had access to either treatment via the bottom containers for 3 weeks. The plants were exposed to two periods of drought stress and leaf tissue was collected on the final day of drought testing.

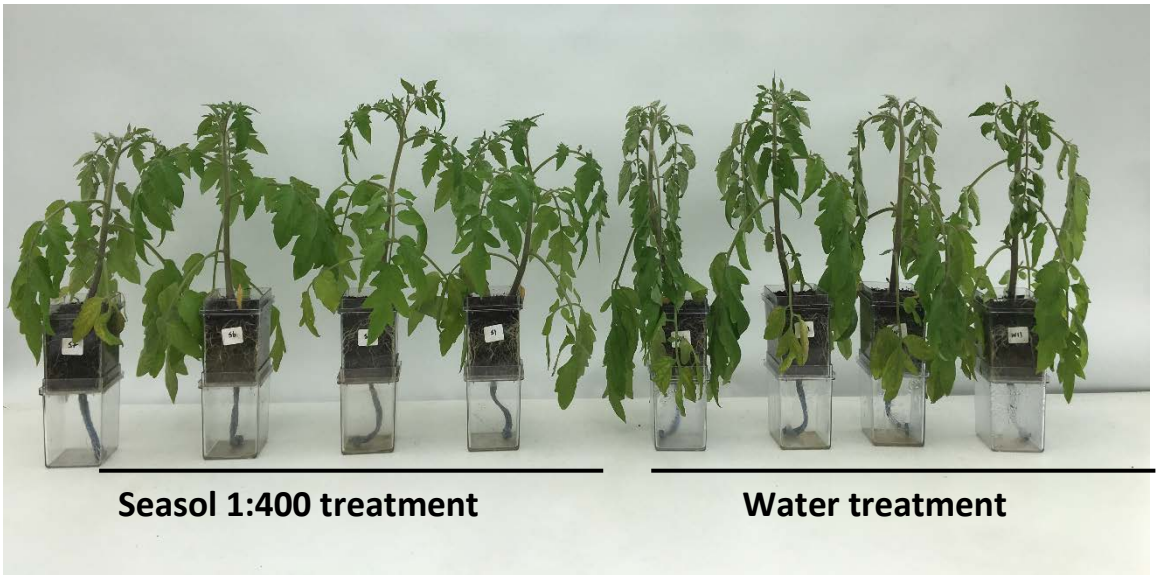

Figure 1: The image shows tomato plants showing wilting symptoms due to water deficit. The Water treatment applied on the right vs the Seasol Treatment applied to the left, as marked on underneath the magenta jars.

Proline content in Seasol treated vs Water control

The graph and the table below show that drought stressed plants treated with water produced 66% more proline relative to Seasol Commercial Seaweed Concentrate treatment at 1:400 dilution. The difference in proline content is statistically significant at 95% confidence interval. The lower level of proline accumulation indicates the Seasol Commercial Seaweed Concentrate treated plants experienced less drought stress. This is consistent with the reduced wilting symptoms observed and shown above.

Proline content (µmoles/g leaf Fresh Weight)		
Replicates	**Water**	**Seasol 1:400**
1	2.87	1.66
2	1.16	0.57
3	7.23	1.97
4	5.49	2.55
5	3.21	2.58
6	2.75	1.03
7	2.77	3.14
8	1.71	2.19
9	2.55	1.71
10	3.63	1.64
11	1.71	1.51
12	3.57	2.35
Average	**3.22b**	**1.91a**

Table 1 - Proline content in drought stressed tomato leaves

The table shows the average proline measurement over twelve replicates for each treatment. Proline content was detected in tomato leaves showing drought stress symptoms. The Seasol Commercial Seaweed Concentrate (1:400 dilution) treatment shows a lower average proline content, indicating a lower level of drought stress.

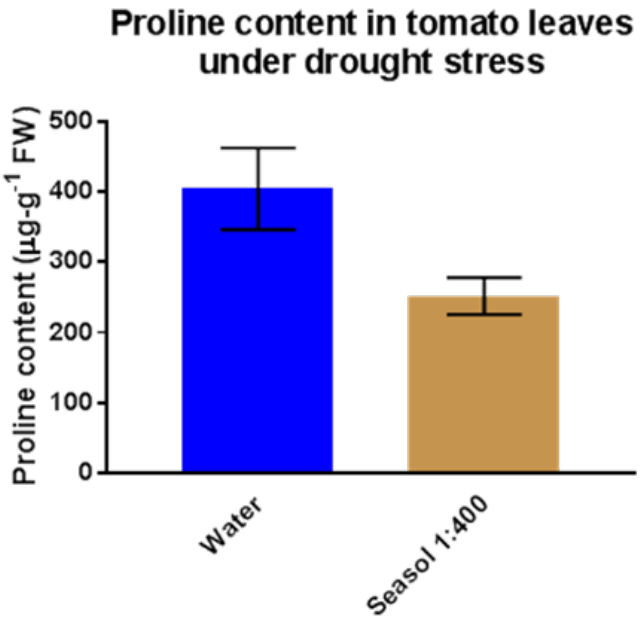

Figure 2 – Comparison of proline content across treatments

The graph shows the average proline content in the leaves of drought stressed tomato plants treated with Water or Seasol Commercial Seaweed Concentrate (1:400 dilution). The Seasol Commercial Seaweed Concentrate (1:400 dilution) treated plants showed lower proline content and the results are statistically significant at 95% confidence interval (Refer to appendix for statistics). The units have been converted to ug.g^{-1} FW to enable comparison to proline publications using seaweed extracts.

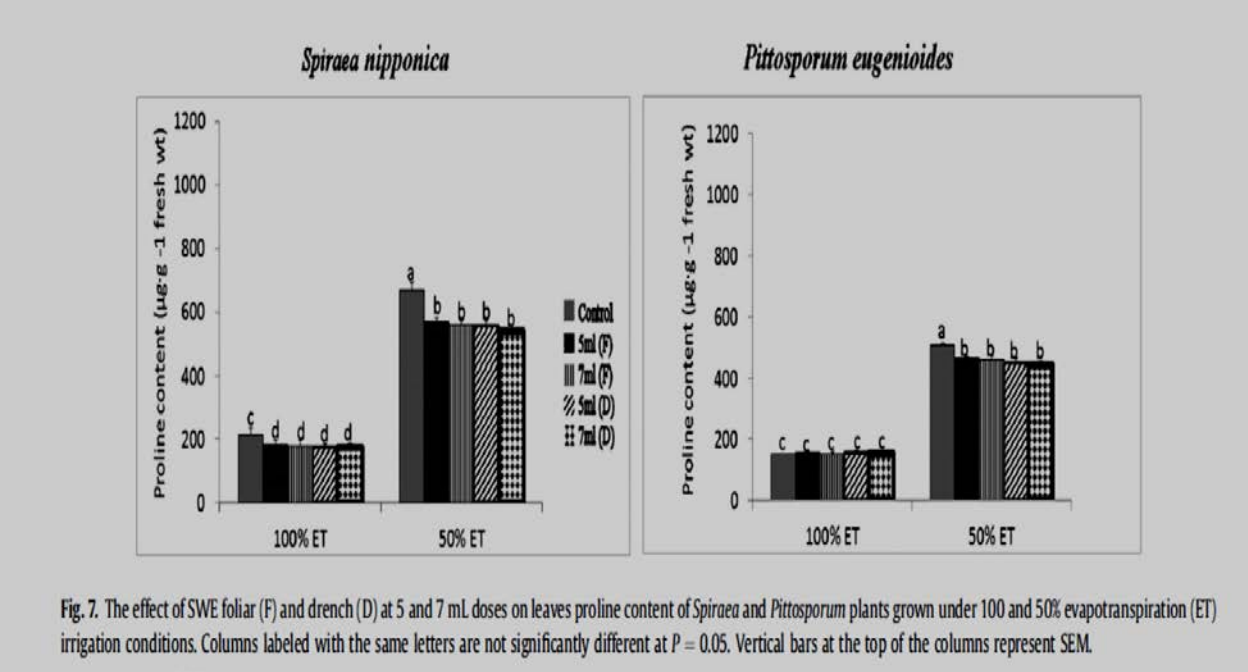

Fig. 7. The effect of SWE foliar (F) and drench (D) at 5 and 7 mL doses on leaves proline content of *Spiraea* and *Pittosporum* plants grown under 100 and 50% evapotranspiration (ET) irrigation conditions. Columns labeled with the same letters are not significantly different at $P = 0.05$. Vertical bars at the top of the columns represent SEM.

Figure 3: Graphs for proline content measurement using Acadian Seaweed extract (Elansary et al. 2016).

The work done on drought stress using one of our competitor's seaweed extract also showed that proline content is reduced under drought stress when applied by foliar and soil drench methods (Please refer to the graph –above -from the publication). The proline content measured in this report using drought stressed tomato plants treated with Seasol Commercial Seaweed Concentrate are consistent with the research published using seaweed extract from Acadian.

Conclusion:

This study concludes that application of Seasol Commercial Seaweed Concentrate improved tolerance to plant drought stress.

This conclusion is based on the findings that drought stressed tomato plants treated with Seasol Commercial Seaweed Concentrate had reduced levels of proline and display reduced wilting symptoms under drought stress.

These results support the hypothesis and purchasing the spectrometer used in this experiment.

References:

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39 (1):205-207. doi:10.1007/bf00018060

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Elansary HO, Skalicka-Wozniak K, King IW (2016) Enhancing stress growth traits as well as phytochemical and antioxidant contents of Spiraea and Pittosporum under seaweed extract treatments. Plant Physiol Biochem 105:310-320. doi:10.1016/j.plaphy.2016.05.024

Hayat S,Hayat Q, Alyemeni M,Wani A, Pichtel J,Ahmad A (2012) Role of proline under changing environments – A review.Plant Signaling &Behaviour 7:11, 1456-1466.

Shabnam N, Tripathi I, Sharmila P, Pardha-Saradhi P (2016) A rapid, ideal, and eco-friendlier protocol for quantifying proline. Protoplasma 253 (6):1577-1582. doi:10.1007/s00709-015-0910-6

Appendix:

Column A vs Column B	Water vs Seasol 1:400
Unpaired t test	
P value	0.0255
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.396 df=22
How big is the difference?	
Mean ± SEM of column A	404 ± 57.89, n=12
Mean ± SEM of column B	251.7 ± 26.23, n=12
Difference between means	152.3 ± 63.56
95% confidence interval	20.48 to 284.1
R squared (eta squared)	0.207
F test to compare variances	
F, DFn, Dfd	4.871, 11, 11
P value	0.0142
P value summary	*
Significantly different (P < 0.05)?	Yes