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**ANALYSIS OF THE RESPONSE OF A BROMELIAD, *VRIESEA GIGANTEA*, SUBJECTED TO DROUGHT**

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**ANALYSIS OF THE RESPONSE OF A BROMELIAD, *VRIESEA GIGANTEA*, SUBJECTED TO DROUGHT**

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## ABSTRACT

Drought can quickly alter physiological processes in plants not adapted to such conditions. Epiphytic plants, however, have been noted to have some tolerance to drought, which is evident in their water-holding tanks. Several physiological components were measured to determine if *Vriesea gigantea*, an epiphytic bromeliad, has drought tolerance. Water content, maximum quantum yield, protein concentration, and protein content were measured and compared between three control plants and three experimental plants, which were subjected to two weeks of no water. Water content showed a significant difference, indicating that the plants were being stressed compared to the control plants. The average maximum quantum yield was nearly identical (0.8), demonstrating the same likelihood of an electron being passed to photosynthesis and therefore photosynthetic processes are continuing at the same rate. Protein content resulted with a higher amount in the drought samples, as was also noticed in the darker bands of both Coomassie stained and Western blot gels compared to the control. The data obtained renders this species to be sufficiently drought tolerant at a two week, drought period.

## INTRODUCTION

Drought stress, depending on the severity and species involved, can alter physiological processes in plants. Depending on the physical adaptations to avoid stressors, many plants are adapted to slowing or stopping certain processes to promote survival. Growth reduction appears to occur first at a moderate stress level in order to continue other processes such as photosynthesis (English-Loeb et al., 1997). Physical injury may occur by losing leaves, or a time period of decreased rates in photosynthesis or oxidation while the plants adjust to low water availability (English-Loeb et al. 1997). A study completed by Loggini et al. (1999) compared two wheat cultivars, Adamello and Ofanto. They determined that the more drought-sensitive

Adamello reduced photosynthetic processes and built up biomass in response to a period of drought. Upon rehydration, Adamello returned to normal photosynthetic efficiency, proving its effective defense mechanism. The more tolerant Ofanto maintained normal growth activity as well as continued photosynthetic activity at the normal rate of the control, which was demonstrated by the lack of difference in pigment changes from control to drought samples and the small difference in PSII photon yield and net CO<sub>2</sub> assimilation (Loggini et al., 1999).

Drought may have a stronger negative effect on shallow-rooted epiphytes than on other plant types due of the lack of long-term, soil-root relationships in the former (Zotz et al., 2010). Epiphytes seem to be limited in distribution by access to water, which is supported by the expression of greater numbers located in areas with higher water availability (Zortz et al., 2010). Bromeliads tend to have very slow growth, allowing the species to survive in higher stress environments. This slow growth however, can also be a negative feature as it has led to declines in natural populations (Pickens et al., 2006). Several adaptations, such as tanks and crassulacean acid metabolism, have also helped these plants to avoid stressors like drought (Bader et al., 2009). Graham and Andrade (2004) noted that the lower drought tolerant plants were located at higher canopy heights, suggesting that more exposure may be correlated with more frequent water availability. The epiphytic characteristic of many bromeliads relates to this suggestion, allowing the plants access to more rainfall or dew the higher up the canopy they are located. Another notable adaptation of epiphytes is the ease which the tissue can rehydrate after a period of drought, resuming normal activities (Zotz and Andrade, 1998).

There are more than 3000 species and 50 genera of bromeliads that thrive in a wide variety of habitats (Pickens et al., 2006). The three subfamilies of bromeliads are *Tillandsioideae*, *Bromelioideae*, and *Pitcairnioideae*. Bromeliads express a wide variety of floral structures, colors, traits, seeds, and fruit types (Brown and Gilmartin, 1984). They generally are found in the New World Tropics and include mosses, cacti, and pineapple-producing plants (Miller, 2009). These plants have either epiphytic or lipophytic forms and generally use animals as pollinators (Schmid et al., 2010). Having small root bases, their varied leaf forms function as nutrient and moisture absorbers for the remainder of the plant (Krömer, 2006).

The bromeliad *Vriesea gigantea* is native to southern Brazil, and is found primarily in the Brazilian Atlantic Rainforest. It is an epiphytic, perennial species that plays an important role in the ecosystem due to its ability to hold liters of water in its tank-like structure (Palma-Silva et al., 2008). Populations of *V. gigantea* have bimodal pollination systems relying on bats, hummingbirds, and bees. Flowering occurs during the summer and seed dispersal occurs during the winter and spring. The seeds are small and usually dispersed by the wind (Paggi et al., 2010). *Vriesea* species have brightly colored flowers, require indirect light, and prefer a full central cup of water. Although they can survive cool temperatures, they prefer 55°F or higher. Because *Vriesea* species are naturally epiphytes, they require a rapidly draining soil mixture when potted.

The aim of this study was to determine if any physiological processes are altered during subjected drought stress in *Vriesea gigantea*, and how these alterations may affect other aspects of the plants. I measured protein content, maximum quantum yield, protein

concentration, and water content. I hypothesized that there would be a decrease in protein concentration as well as photosynthetic rates in plants subjected to drought. I also hypothesized that the protein content would remain the same in water-stressed plants, unless they produced additional distress proteins. The results of this study may lead to a better understanding of the mechanisms of drought tolerance in the epiphytic bromeliad, *Vr. gigantea*.

#### MATERIALS AND METHODS

*Plant Samples:* Plants were obtained from Michaels Bromeliads (<http://www.michaelsbromeliads.com/>). Six, young plants were used, each about 3-4 years old. Each plant was potted in moisture-control soil in a plastic pot with holes in the bottom to promote rapid draining. Plants were cultivated in a greenhouse under plant lights and they received fertilization, ventilation, and normal watering prior to the experimentation. The ambient temperature was kept at an average of 21°C during the day and 19°C at night. During the experiment, the control plants (n=3) received a cup of water each, applied to the soil and tanks, while the experimental plants (n=3) received no water for two weeks.

*Electron Transport During Light Reactions:* The three control plants and three experimental plants were placed in a dark room for 45 minutes prior to testing the photosynthetic processes in order to allow rest time prior to measurement. All testing was done in the dark, using a dim flash light for visibility. One plant was tested at a time. A whole leaf from the central cup was removed and placed with the top side facing the light, under the FlourPen apparatus. The program Light Curve was initiated and allowed to run in complete darkness. The maximum

quantum yield was recorded in order to measure the photosynthetic rates in control v. treatment plants.

*Water content:* Leaves retrieved for electron transport measurement were used to determine water content. The initial mass of each leaf was determined, recorded, and all leaves were placed in a drying oven at 50°C. After nine days, the mass of each leaf was recorded and used to determine the mass of water present in each leaf prior to drying.

*Protein concentration:* A leaf weighing approximately 0.8-1.0g was removed from each plant and placed on ice. Cold extraction buffer was prepared with the addition of PMSF (protease inhibitor) and DTT (reducing agent). Six cooled mortars and pestles were collected and labeled. Liquid nitrogen was poured over the leaves and the leaves were continuously ground until finely powdered. Approximately 1000µl of cold extraction buffer were added to each of the mortars and allowed to thaw prior to additional grinding. Once completely thawed and ground, another 1000µl of cold extraction buffer without PMSF and DTT was added, allowing the formation of enough supernatant for testing. Using a pipette, as much of the supernatant that could be removed from each mortar was collected and placed in microcentrifuge tubes. All six tubes were placed in a Centrifuge 5418 for five minutes at 14,000rpm. The supernatant was again removed and placed in a clean microcentrifuge tube. For each sample, two cuvetts were used to measure protein concentration. In each cuvette, 900µl of Coomassie Protein Assay, 100µl of water, and 2µl of the protein sample were added. This was done twice for each sample. The cuvetts were mixed using a Vortex Genie 2. Protein concentration was tested using absorbance measurements from DU730 Life Science UV/Vis

spectrophotometer after one and five minutes. These absorbance measurements were applied to a standard curve, allowing the calculation of protein content per  $\mu\text{g}$  for a denaturing Western Blot.

*Preparation of Gel for Western Blot and Coomassie Stain:* The samples were removed from the freezer and thawed by hand. SDS Page (1x) was used as the running buffer, while the loading buffer was prepared by adding DTT, glycerol, and salts to a protein buffer. The vertical Western Blot apparatus was set up with a 12% and a 4-20% gel. Once the samples had thawed, they were centrifugated for 60 seconds at 14000 rpm. In new microcentrifuge tubes, 80 $\mu\text{l}$  of the protein sample and 30 $\mu\text{l}$  of the loading buffer were added. The tubes were placed in the Isotemp at 75°C for 10 minutes. The 12% gel was loaded with each of the samples (1-6), two known protein ladders, and an additional sample of control one and drought one for further analysis. The 4-20% gel was loaded in a similar fashion, with lanes holding each sample (1-6) twice, two known protein ladders, and an additional sample of control one and drought one. The gel electrophoresis was conducted with 120Volts using a Hoefer PS300-B power source until the gel was complete. Lanes 1-7 of 12% gel were used for the Coomassie staining. Lanes 1-7 of 4-20% gel were not used. The remaining lanes of each gel were placed in separate containers with blot buffer and used for a Western Blot.

*Coomassie Stain:* Lanes 1-7 of the 12% gel were removed and placed in a separate container with Coomassie stain. This gel was kept in the refrigerator overnight and then placed in destain with a chemwipe on a rocker until bars were visible. A photograph was taken and used for analysis.

*Western Blot:* Normal protocol for a Western Blot was used on lanes 8-12 of a 12% gel and lanes 8-16 of a 4-20% gel. The blot conditions were 150milliamps for about 2 hours. Upon completion, the nitrocellulose gel was removed and soaked in milk solution for 30-60 minutes. The membrane was washed once in TBS+T and then incubated overnight in the primary antibody wash, one with antibodies against photosynthetic proteins (PS1) and the other against core particles (CP), with a 1:1000 dilution. Using these antibodies, proteins specific to PS1 were identified and proteins that bind to chlorophyll were also identified on the CP treated membrane. After washing the membrane five times with TBS+T and being rocked 3-4 minutes each, the secondary antibody with a 1:5000 dilution was applied for 45 minutes. Again, the membrane was washed five times under the same conditions listed above, except only TBS was used. The membranes were marked for identification purposes. They were then washed with AP buffer and simultaneously washed with BCIP/NBT color developer for about two minutes. When bands appeared, the membranes were removed and placed in water to avoid over exposure.

*Statistical analysis:* One-tailed, paired t-tests ( $n=3$ ) with  $\alpha=0.05$  were used to test for differences in water content, protein content, and maximum quantum yield between control and treatment plants.

## RESULTS

*Maximum Quantum Yield:* The average maximum quantum yield ( $Q_{y_{max}}$ ) for the control group was 0.80, compared to the average  $Q_{y_{max}}$  of the drought group 0.81 with a standard

deviation of  $\pm 0.015$  for the control and  $\pm 0.010$  for the drought (Figure 1). The t-test determined no significant difference ( $p=0.21$ ).

*Water Content:* Water content varied slightly between the control and the drought groups. The average for control specimens was 87.2% and the average for the experimental group was 82.4% with a standard deviation of  $\pm 1.65$  for the control and  $\pm 1.07$  for the drought samples (Figure 2). The t-test determined a significant difference between groups ( $p=0.045$ ).

*Protein Content:* Average protein content of the control group was  $5.96\mu\text{g/g}$  of fresh weight, whereas the average protein content for the drought group was  $6.90\mu\text{g/g}$  of fresh weight with a standard deviation of  $\pm 0.52$  for the control and  $\pm 2.4$  for the experimental (Figure 3). The t-test determined no significant difference between groups ( $p=0.24$ ).

*Western Blot:* For the CP treated membrane, bars were visible in all lanes, but were much darker in the drought lanes (Figure 4). The PS1 treated membranes also showed bars in all lanes. Labeled drought lanes are visibly darker than control lanes. The protein ladder was also identifiable (Figure 5).

*Coomassie Stain:* The 12% gel showed similar protein abundance and expression in both control and drought samples (Figure 6). The identical protein bars are visible in all specimens.

## DISCUSSION

This experiment demonstrated that *Vriesea gigantea* has several drought resistant qualities. Average water content in the control group was significantly higher than that in the experimental group, indicating that the plants in the treatment group had significantly less

water after 2 weeks of drought conditions as hypothesized. However, the maximum quantum yield and protein concentration showed no significant difference in the data. Plants in the treatment (drought) group produced darker lines on the Western blots demonstrating that there was a slightly higher concentration of proteins in the treatment plants, which was unexpected. Coomassie staining indicated that an almost identical protein fingerprint occurred between all 6 samples. The drought samples appear to be slightly darker, indicating a higher concentration of proteins, which contradicts my hypothesis, but this finding must be investigated further. The drought samples had higher protein content per gram of fresh weight than control samples, but the results were not significantly different.

When subjected to drought, *Vr. gigantea* retains the ability to perform photosynthetic processes as well as synthesize similar amounts of protein as the species would under normal conditions. With significant difference in water stress, these plants appear to be physiologically unaffected after two weeks. Therefore, this species seems to be able to tolerate drought effectively which is expected since they are seasonal, lowland epiphytes (Zotz et al., 2010).

Drought tolerance appears to be a shared characteristic in many bromeliads, especially among epiphytes living in habitats with diminished water resources. Slow growth in many species is a common way to survive through unfavorable conditions, since fast growth (active metabolism) is not advantageous during stressful times (Bader et al., 2009). If they had an active metabolism, too much energy and resources would be needed in order to survive. *Vriesea gigantea*, being a tank bromeliad, has the ability to store water, which is another beneficial trait to avoid drought stress (Bader et al., 2009).

With regard to maximum quantum yield, the average did not show a significant difference between groups (Figure 1). For reference, Nogues and Baker (2000) showed  $Q_{y_{max}}$  for rosemary, olive, and lavender leaves. The most dramatic difference over a two week drought period was shown in the rosemary leaves, with a drop in the control of about 0.2. Even the olive leaves in this study showed a 0.1 drop in the drought samples compared to the control (Nogues and Baker, 2000). *Vriesea gigantea* had no difference in  $Q_{y_{max}}$  between the drought and control plants, and therefore show some level of drought tolerance. For this species, it seems that the photosynthetic parameters were not affected as much as they are in other species. It is possible that at varying light intensities the total saturation of light at different periods of drought stress could alter the value. This should be studied further.

Due to limitations, we were unable to analyze the plants at different intervals of drought. Future studies could subject the plants to drought for four weeks or longer. Additional analysis may be conducted such as testing for glutathione concentration, peroxidase activity, and amount of oxidized proteins in both treatments. Additional stressor may also be tested, such as heat or over-exposure of light, to further mimic drought conditions.

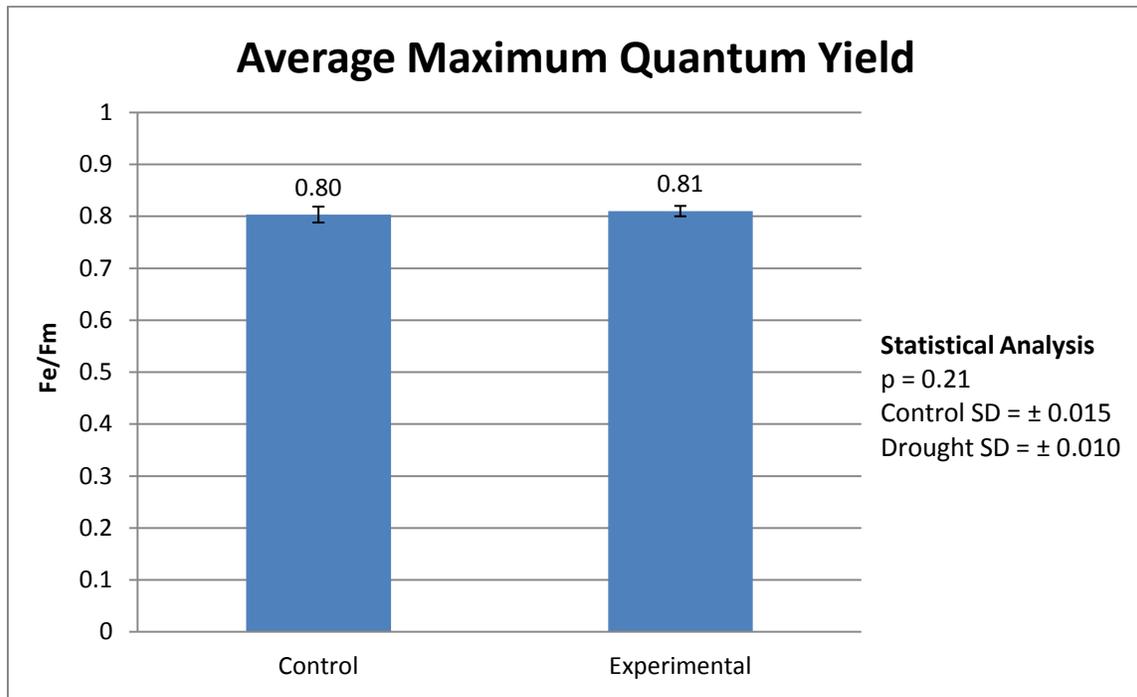
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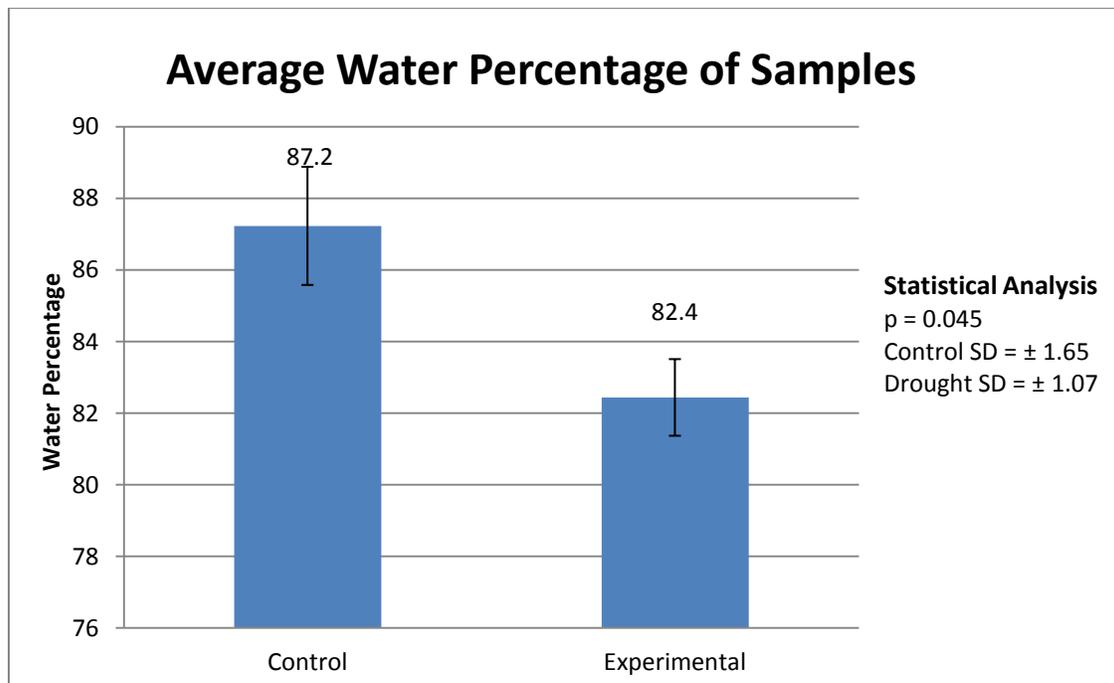
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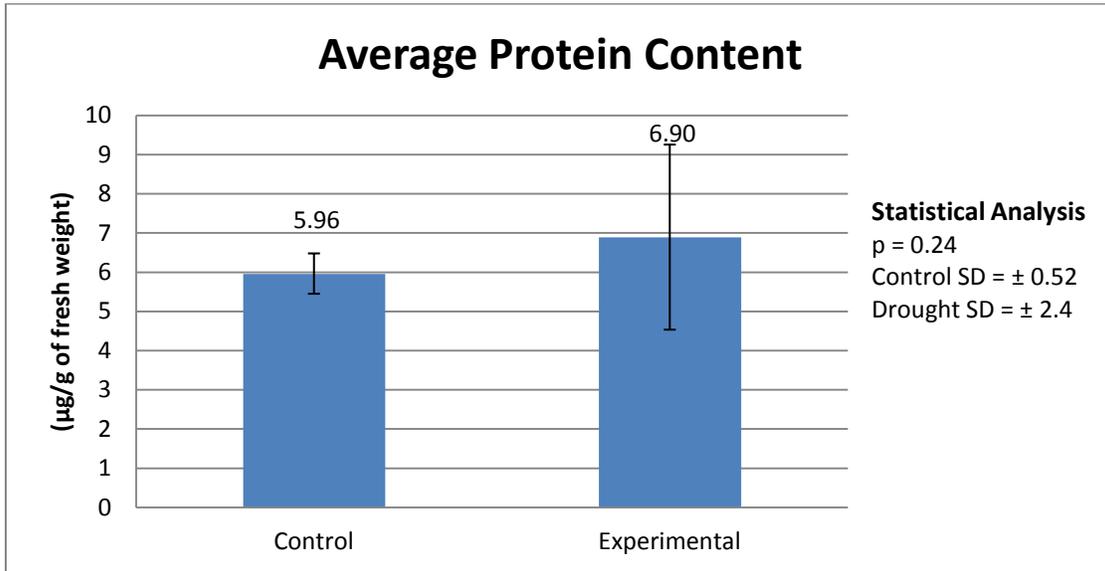
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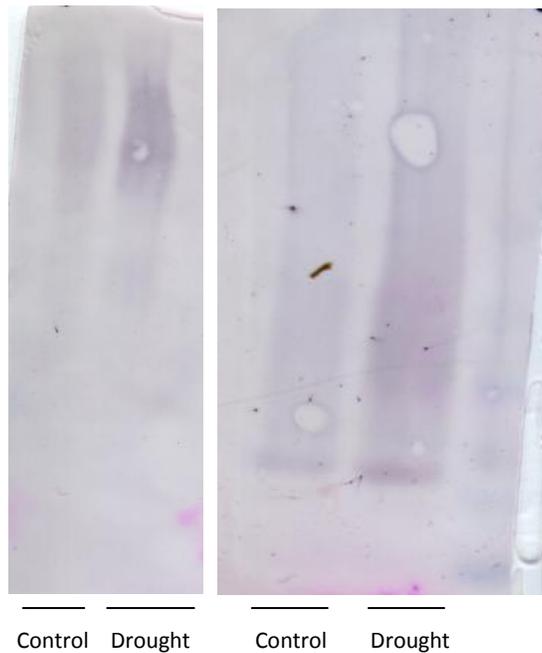
**Figure 1:** Average maximum quantum yield of control and drought groups.



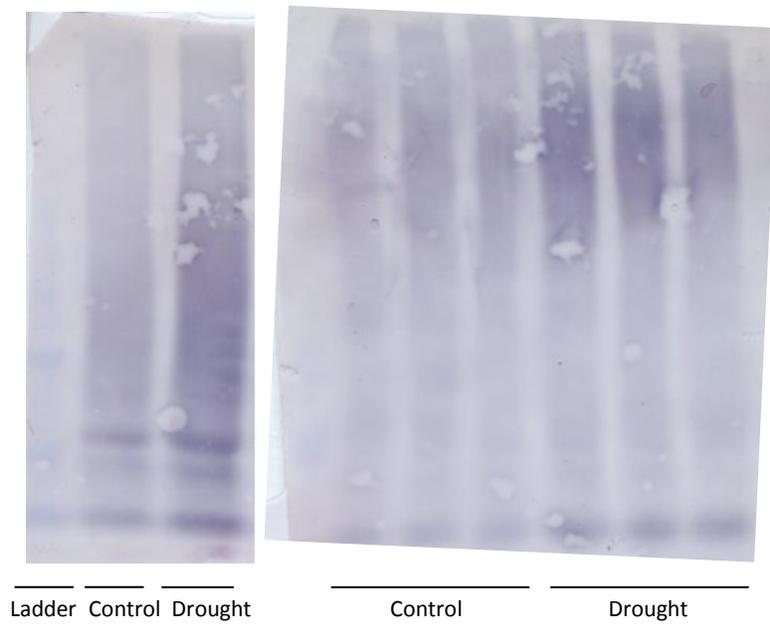
**Figure 2:** Average percentage of water in control and drought groups shown with standard deviations.



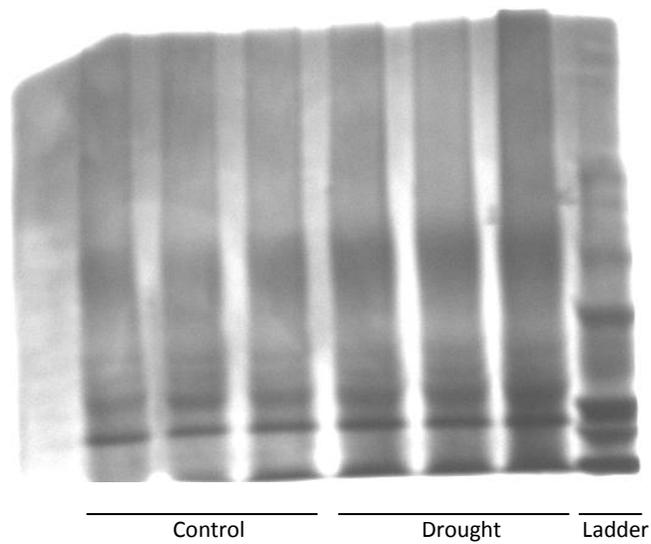
**Figure 3:** Average protein content for control and drought groups.



**Figure 4:** Western Blots of CP treated membranes (4-20% gel on left, 12% gel on right).



**Figure 5:** Western Blots of PS1 treated membranes (12% gel on left, 4-20% gel on right). A basic protein ladder is shown. Control and drought lanes are indicated.



**Figure 6:** Coomassie stain of 12% gel with three control samples, three drought samples, and a protein ladder.