Pilot Project Report – DroughtSpotter Platform	
Project Title:	**Drought Response in Low-Cyanogenic *Sorghum bicolor* Mutants**
Scientists:	Viviana Rosati, Dr. Cecilia Blomstedt, Dr. Trevor Garnett, Assoc/Prof Ros Gleadow
Organisation:	Monash University

General information about the project design

Sorghum bicolor stands as the fifth most important cereal grown worldwide; its high drought and heat tolerance cementing the crop's widespread utilisation for both grain and animal forage in semi-arid and arid, subtropical environments. Sorghum produces the cyanogenic glucoside dhurrin in all tissues except the mature grain, which upon tissue disruption is broken down to release hydrogen cyanide gas.

In sufficient concentrations this compound will be toxic to livestock, resulting in cyanide poisoning and potentially death. High dhurrin concentrations have previously been linked to the drought-tolerant staygreen phenotype; however, the potential osmoprotective properties of dhurrin needs further exploration.

To assess drought response and elucidate the role of dhurrin in drought tolerance, this pilot study used

- unique EMS-mutants with low adult dhurrin levels (adult cyanide deficient class 1, *acdc1*),
- mutant sibling lines (generated in parallel but lacking the mutation),
- and wildtype plants.

All lines were grown under three different watering regimes (15%, 30%, and 100% field capacity of water) using the Australian Plant Phenomics Facility's *DroughtSpotter* platform; a gravimetric system allowing for precise and reproducible water application, as well as analysis of the transpiration dynamics of each individual plant.

The experimental design utilised six replicates of each line at each watering level. Plants were germinated and grown for 10 days under fully watered conditions, with a baseline harvest being undertaken on the 10th day. Drought treatments were then implemented and physiological growth parameters measured via destructive harvests at three developmental time-points (1 week and 1 day; 2 weeks and 2 days; 3 weeks and 3 days; after the beginning of the drought treatments). Directly before harvesting plants were imaged using the Plant Eye.

During the harvests, tissue from each of the roots, leaves, and stem of each plant was divided in two, with one half being snap frozen in liquid nitrogen for analysis of gene expression, and the other half freeze-dried for metabolomic analyses to be undertaken for the final harvest time-point at a later date.

This study is important for the evaluation of using sorghum as forage in determining whether the *acdc1* line is advantageous in water-limited environments, and if decreasing the toxicity of the crop can be coupled with increased drought tolerance.

Aims of the experiment

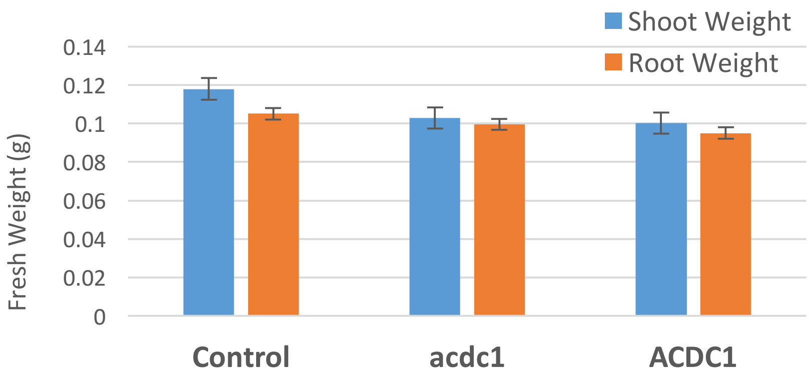
The aims of this experiment were:

- To examine the impact of altered dhurrin concentrations on drought tolerance at different stages of development in *Sorghum bicolor*.
- To quantify the impact of different levels of drought stress on growth in control and mutant plants.
- To examine the effect of drought on the regulation of dhurrin in the *acdc1 Sorghum bicolor* mutants.
- To evaluate the quality of the *DroughtSpotter* in providing differing watering regimes.

Key results and outputs

Growth of the three lines prior to the onset of drought stress.

a)



b)

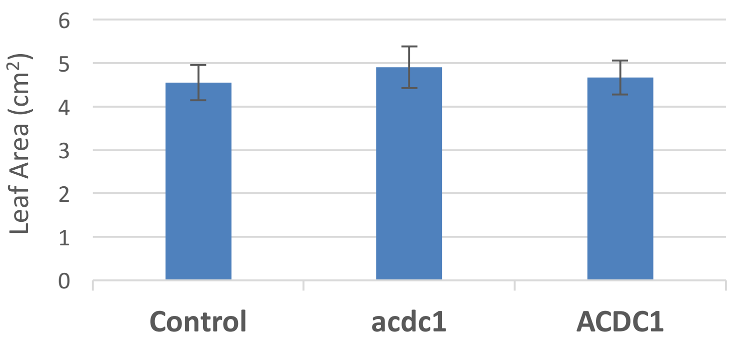


Figure 1: Harvest 1, baseline harvest at 10 days post germination of control, *acdc1* mutant, and mutant sibling lines (*ACDC1*) before drought treatments were initiated. **a)** Fresh weight of root and shoot tissues and **b)** leaf area (+/- SE).

No significant differences were found in the first, baseline harvest 10 days post germination (dpg) between the three lines for leaf area or root and shoot fresh weight.

Growth parameters of the three lines at the final harvest.

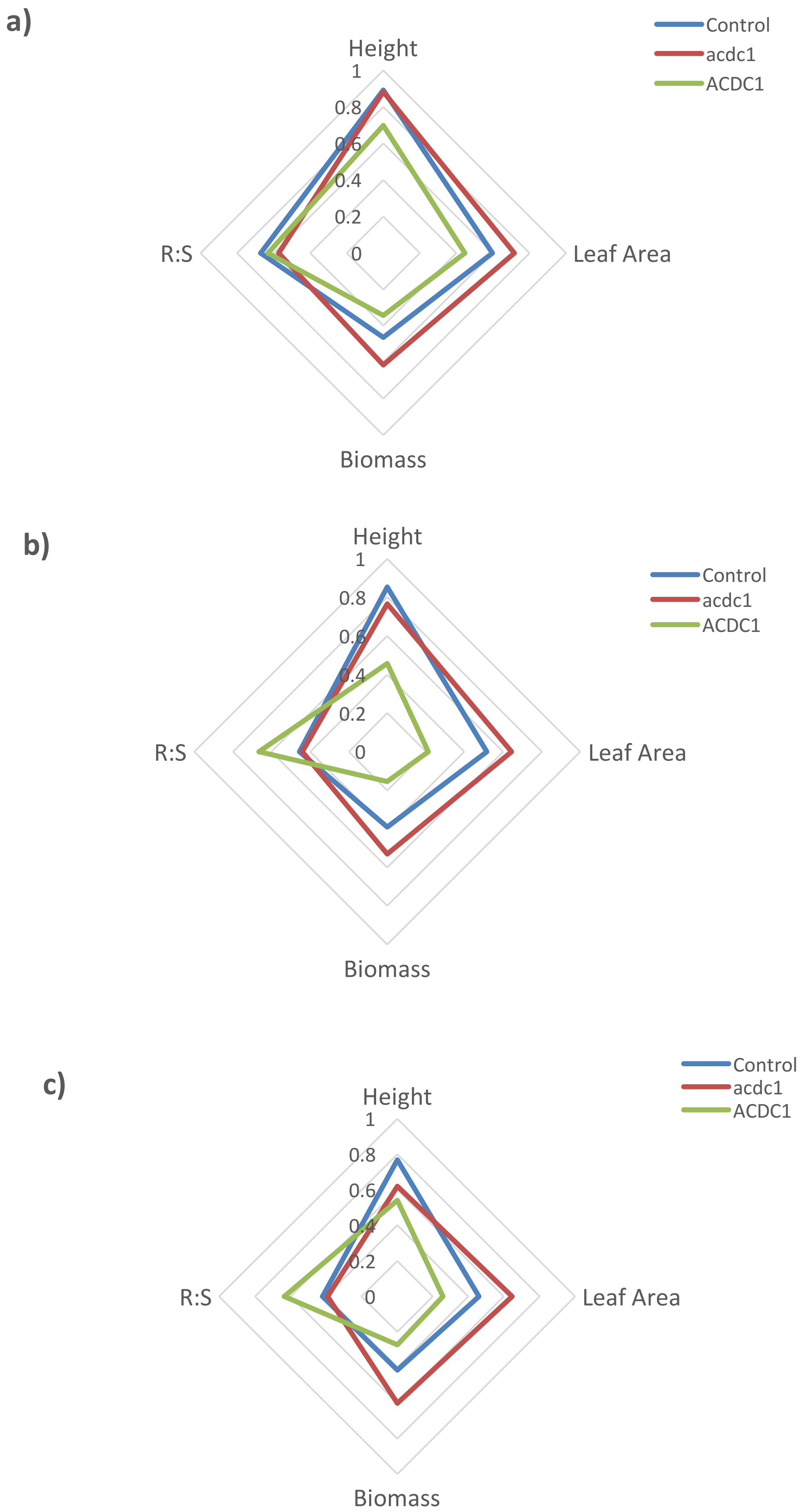


Figure 2: Growth parameters for Harvest 4 expressed as the average ratios for plants grown under three different watering levels **a)** 100% field capacity, **b)** 30% field capacity, and **c)** 15% field capacity.

By the final harvest (Harvest 4) the sibling lines had a greater root to shoot ratio than the control and *acdc1* lines at 30% field capacity of water (P=0.026 and P=0.042 respectively) and a greater root to shoot ratio than the controls at 15% field capacity of water (P=0.024), while the sibling line was significantly shorter than both other lines at 100% field capacity of water (P<0.001). The *acdc1* mutant had a greater leaf area than the sibling line at 100% and 30% field capacity of water (P=0.048), and also a higher biomass under fully watered conditions (P=0.002).

Total Water Use.

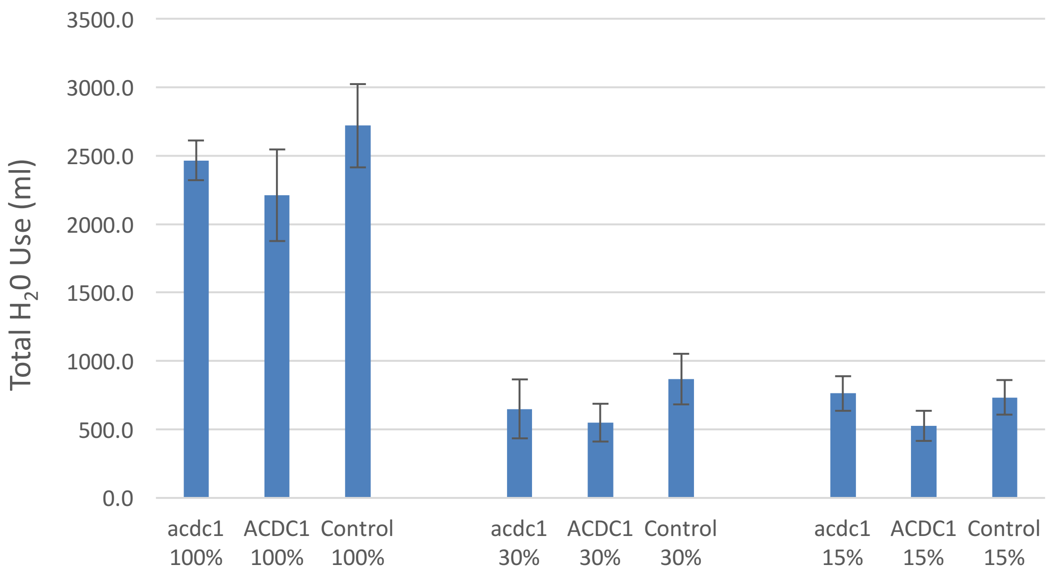


Figure 3: Total water use for the *acdc1* mutant, sibling line (ACDC1), and controls under 100%, 30%, and 15% field capacity of water (+/- SE).

No significant differences in water use between the three lines within treatments was observed.

While preliminary analysis of growth data has been completed, metabolomics and gene expression analysis is currently in the process of being undertaken for this experiment. Once completed it is envisaged that the data will be presented at the international Plant Biology 2017 conference, and ComBio 2017, while being drafted for publication.

Although plants were imaged with the PlantEye prior to harvesting, software issues were experienced during the pilot project testing phase, which impacted on the quality of image analysis. As a result, the software was upgraded and the issues experienced during this pilot study have now been addressed.

Statement on how data obtained from the DroughtSpotter provided new insights into your research

The baseline harvest (before the drought treatments were imposed) showed that there are no significant differences in growth in the early stages of development between the three lines. However, by the final harvest the *acdc1* mutants often maintained both a greater leaf area and biomass than the sibling lines at the same watering levels. This implies that the mutant line can equal or better the sibling line in leaf area and biomass, with matching water requirements. At this stage further analysis, particularly of the dhurrin concentrations between lines and treatments, is required. As drought is known to increase dhurrin concentrations in control plants it is necessary to understand whether this also occurs in the developmental *acdc1* mutants, and to what extent. As well as to analyse whether dhurrin concentrations are differing due to changes in expression of the biosynthetic genes. If dhurrin concentrations remain lower than the control and sibling lines then it appears that it is possible to lower the toxicity of sorghum, while maintaining drought tolerance.

The *DroughtSpotter* is an excellent platform to apply accurate, reproducible amounts of water to large numbers of individual plants for growth and compositional analysis under different levels of water limitation.