Pilot Project Report

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General information about the project design

Forage sorghum is an important pasture crop in Australia. It contains the cyanogenic glycoside dhurrin and when droughted can become lethal to stock animals. We have created 11 mutants with altered concentrations of dhurrin. The aim of this pilot project was to see if the phenotyping equipment at The Plant Accelerator® was able to assess growth rates of plants as a rapid through-put system that could be applied to the mutant progeny. In this pilot project we used a nitrogen treatment, rather than drought, as this was expected to result in the predictable differences in growth, chlorophyll and dhurrin concentration. Previous experiments have shown that dhurrin is highly dependent on N supply, plant phenology and ontogeny.

Two commercial varieties known to differ in dhurrin concentration (Line A with higher dhurrin content, Line B with lower dhurrin content) were grown for a total of six weeks in different nitrogen regimes.

During the growth period, plants were fertilised with nutrient solutions containing low (2 mM N) and high (10 mM N) levels of nitrogen. Six replicates for each line and nitrogen level were used (2 lines x 2 treatments x 6 replicates = 24 plants).

In addition, 3 replicates of each line were grown in a coco-peat potting mix with ready available nutrients and additional slow release fertilizer (Osmocote®). Those plants were used as germination and growth control, adding up to a total of 30 plants.

The plants were grown for two weeks before imaging and then a further four weeks, with three imaging runs per week. Given the small number of plants, the plants remained in a static greenhouse. They were transferred to the automated imaging system of The Plant Accelerator® for each imaging run and returned to the greenhouse afterwards.

At the end of the imaging period (after six weeks of growth), plants were destructively sampled. Leaf discs were taken from the third fully unfurled leaf for immediate analysis of dhurrin (fresh), or frozen in liquid nitrogen for later chlorophyll analysis. The lower part of the stem was also sampled and frozen in liquid nitrogen for later mRNA analysis at Monash University. Fresh weights were collected from all plant parts. Leaf area was measured using a leaf area meter. Roots were washed and weighed. All plant tissue was then dried overnight at 50°C in paper bags. Once back at Monash University, plant material was completely dried, weighed and then ground for total nitrogen, carbon/nitrogen ratio, nitrate, total dhurrin and chlorophyll determination. All parameters were measured in leaves, 'stem' (leaf sheath plus stem), and roots.

Key aims of the experiment

Our aim was to compare the growth and development of two commercial varieties of forage sorghum known to differ in dhurrin concentration. Our hypothesis was that changes in the defence pathways would also impact on the nitrogen use efficiency, growth rate, phenological development and dhurrin content. Whilst the NIR imaging system at The Plant Accelerator® is not suitable for non-destructive measurements of dhurrin concentrations within the leaves, it was hoped that both colour and fluorescence imaging would inform about chlorophyll and nitrate concentration of the plants. The results will be used to assess the efficacy of using this system to measure growth, health and composition of EMS mutants with altered cyanogenic status.

Example figures

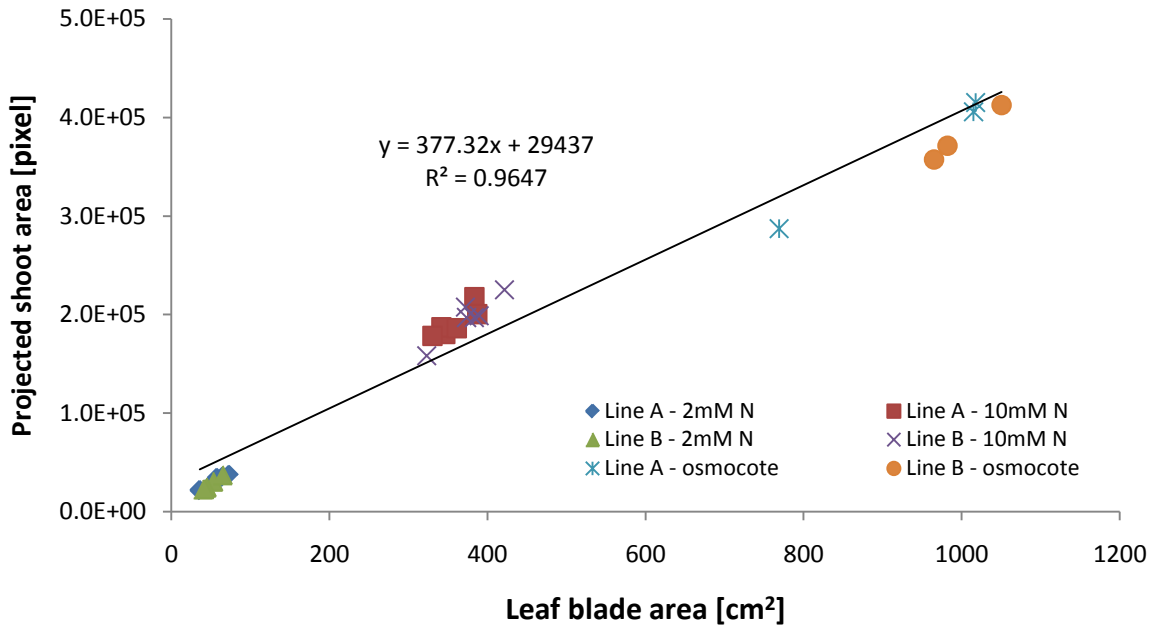

Figure 1: Comparison of leaf blade area and projected shoot area. Leaf blade area was measured destructively using a leaf area meter at the end of the experiment. The projected shoot area was determined from three colour images (two side view images, one top view image) taken at time of harvesting.

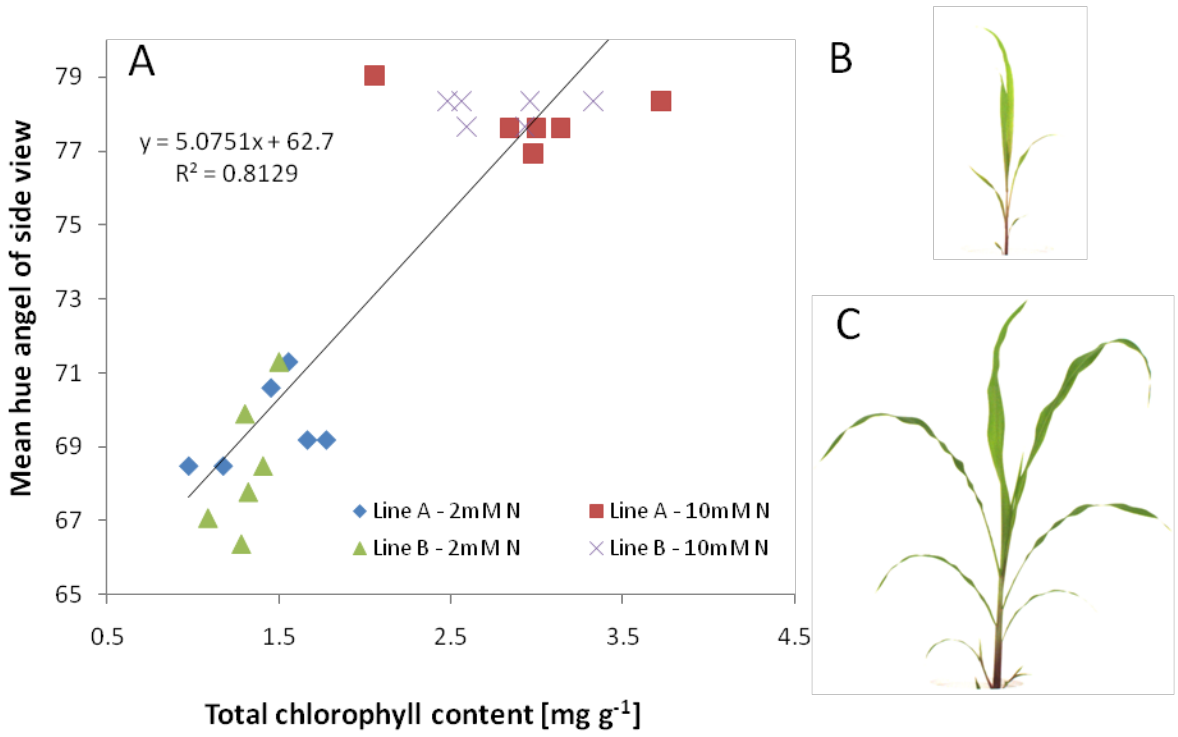

Figure 2: Comparison of chlorophyll content and hue of the side view images. (A) Total chlorophyll was measured from leaf discs (leaf three) harvested on the last day and compared to the mean hue extracted from the side view images. (B) Example images of a plant grown in 2 mM N and (C) 10 mM N showing differences in greenness.

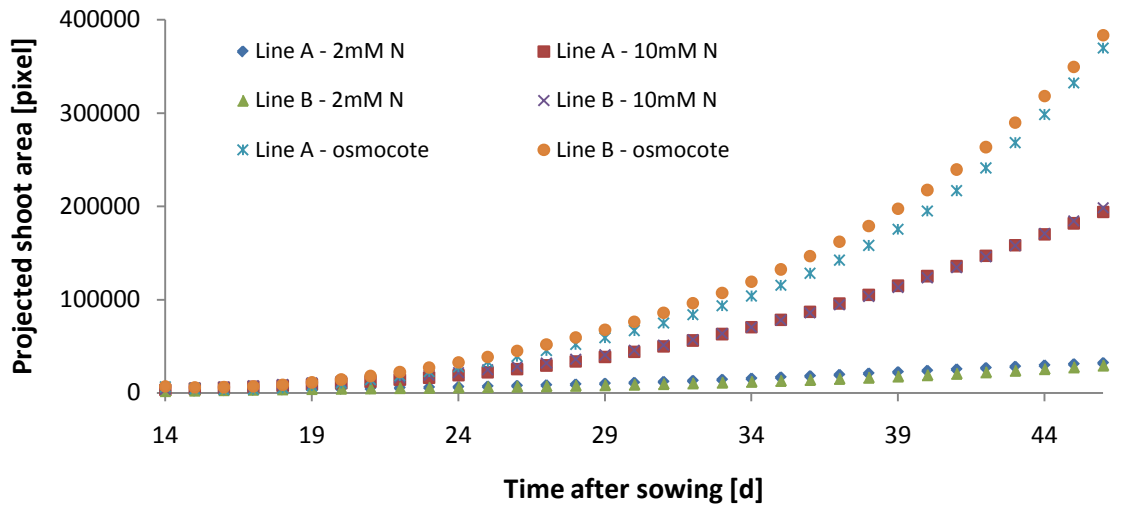

Figure 3: Growth of sorghum plants under different nitrogen regimes over time. A 4th order polynomial was fit using the projected shoot area extracted from the colour images. Both the 10 mM and 2 mM nitrogen treatment limited plant growth compared to the potting mix containing osmocote, possible due to an additional phosphate limitation in the two nitrogen treatments. There is no significant difference in size between the two lines, concordant with the final destructive harvest data.

Key outputs

- Preliminary data was been presented at a cyanogenesis workshop hosted by Monash.
- I am currently drafting a paper with the working title of "Fast throughput method for analysis of N allocation and nitrogen use efficiency in sorghum" and the target journal of New Phytologist. Bettina Berger is expected to be a co-author. Additional data analysis is required before this can be complete.
- Mark Crowe from the Australian Plant Phenomics Facility used our data in presentations promoting the use of The Plant Accelerator® for high-throughput plant phenotyping.

How data obtained from The Plant Accelerator® provided new insights into your research

- The size analysis showed that difference between the treatments arose early and were maintained. There was no difference in plant size between the two commercial lines, resolving that dispute.
- There were, however, differences in growth rate between the two commercial lines, and there was some interaction with nutrient treatment (data not shown). This needs further examination and research to be understood more fully. The growth data really provides something new and not able to be collected without cumbersome serial harvests.
- Subsamples for leaf chlorophyll from the third fully expanded leaf could be misleading about the overall chlorophyll (greenness) of the plants. The imaging gives an integrated measure, which is more indicative of productivity. Possible differences in the fluorescence imaging are currently being assessed.
- It was not possible to gain any insights into dhurrin content as the NIR imaging is not suitable for the analysis of chemical compounds. The NIR imaging will be tested for its use in water stress studies in upcoming projects.
- The Plant Accelerator® is, in my opinion, an excellent way to screen large numbers of sorghum individuals for growth and basic composition.
- In future, I think this system would work better using a soil mix with slow release fertilizer (e.g. Osmocote) and to use the automated watering system in the Smarthouse™ to impose water stress.