

## Pilot Project Report

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<b>Project Id:</b>	#0030 Drought Tolerance of Parental Lines (10 barley lines, 2 watering regimes, 6 reps)

### Introduction

Drought is a complex stress which elicits a wide variety of plant responses. As such, genetic studies of drought are particularly difficult. Elucidation of the genetic basis of components contributing to drought tolerance is likely to be more tractable than that of overall drought tolerance. Certain of the traits which contribute to drought tolerance in plants and the high-throughput phenotyping techniques available to measure those traits are described in this paper. Given the dynamic nature of drought, plant development and the resulting stress response, the focus is on non-destructive imaging techniques which allow a temporal resolution and monitoring of the same plants throughout the experiment. Information on the physiological changes in response to drought over time is vital in order to identify and characterise different drought tolerance mechanisms. High-throughput imaging provides a valuable new tool which allows the dissection into a series of component traits of plant responses to drought.

### General information about the project design

Ten barley lines used as parents of mapping populations or for the generation of transgenic plants were compared for their behaviour under drought. We focused especially on their growth, transpiration, development and changes of these variables in response to soil water deficit.

The barley lines were grown under two stable watering regimes - well watered and -5 bar of soil water potential, as estimated by predawn leaf water potential of Golden Promise plants. All plants were imaged and watered every second day from day 30 to day 70 after sowing. Six replicates for each line and treatment were used.

Leaf area was determined from a calibration curve between imaging variables and true leaf area measured destructively on two wheat genotypes and a barley line.

Kinetics of leaf area were modelled with 3 parameters: final leaf area, relative growth rate (RGR), and time of inflexion point (time at which the growth rate starts to decrease, considered as a shift between vegetative stage and reproductive stage).



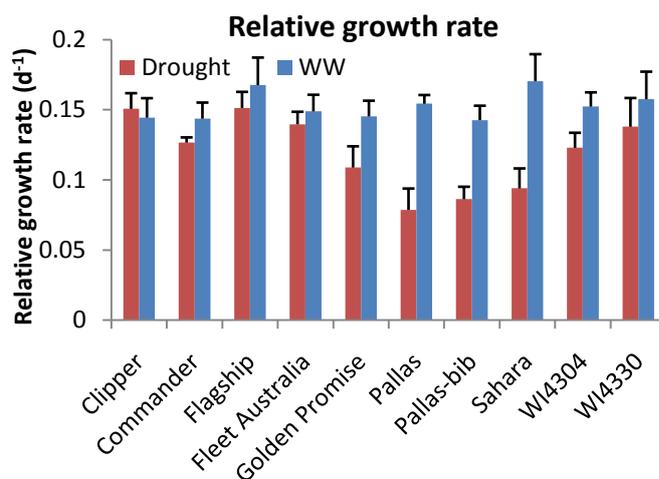
### Key aims of the experiment

- (i) Ranking genotypes for later genetic analyses of mapping populations
- (ii) Selecting three genotypes with different responses to drought for later microRNA analyses
- (iii) Testing and improving the framework previously developed for wheat under drought

### Key outputs

The genotypes showed very different behaviours under drought and were pooled into five groups according to their behaviour (Figure 1 and Table 1). Contrasting genotypes will be analysed in more detail in later experiments.

The differences between four South Australian adapted barley varieties (Commander, Flagship, Clipper and Keel) were small, decreasing the chance of finding interesting alleles in their progeny. In contrast, Sahara showed significantly different behaviour under drought to the adapted varieties, so the use of the Clipper by Sahara mapping population is likely to provide promising candidates for a forward genetics approach.



**Figure 1.** Relative growth rate for 10 barley cultivars in well-watered (WW) or drought conditions. Bars, average value of the 6 replicates. Errors bars, confidence interval at 0.95.

**Table 1.** Behaviour of ten barley genotypes in well-watered (WW) and drought conditions. Differences in developmental rate, relative growth rate (RGR), final leaf area and maximum growth rate were used to assign the genotypes to five distinct groups.

genotype	WW				Drought (compared to WW)		
	development	RGR (# wheat)	final area	Max growth rate	development	RGR	Max growth rate
Clipper	moderate	high	low	low	strong escape	no reduction	good maintenance
Flagship	moderate	high	low	low	escape	small reduction	good maintenance
Fleet Australia WI4304 WI4330							
Commander	moderate	high	low	low	strong escape	small reduction	low maintenance
Golden Promise	late	high	high	high	development time increases	reduction	good maintenance
Pallas Pallas-bib Sahara	moderate	high	high	high	development time increases	strong reduction	low maintenance

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

Drought tolerance is generally defined as the ability of crops to maintain yield under water-limited conditions. This trait is studied by the ACPFG drought focus group and field measurements have already provided interesting QTLs, currently in the process of backcrossing into an elite germplasm. However, this trait is subject to many Genotype x Environment (GxE) interactions, causing differences in particular QTLs in different field trials. It appears that the response of dynamic variables such as growth or transpiration to soil water deficit is more robust and less subjective to GxE interactions and can explain overall yield maintenance under drought.

In that context, phenotyping at The Plant Accelerator® allows us to (i) select parental lines differing in their response to drought, (ii) to analyse their progeny and (iii) to deliver stable QTLs involved in overall drought tolerance.

### Summary

Due to the non-destructive imaging possible at The Plant Accelerator®, we could collect enough data points to generate growth curves, measure relative growth rates and obtain the time of maximum growth. In addition, the automated watering and weighing enabled us to control the water regime and measure water use. All these parameters enable us to quantify traits contributing to drought tolerance and apply these measurements in a forward genetics approach with the aim of discovering the molecular genetic basis of traits that contribute to drought tolerance.