SAGIT

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Project Code	
Project Type	

FINAL REPORT 2017

Applicants must read the *SAGIT Project Funding Guidelines 2017* prior to completing this form. These guidelines can be downloaded from www.sagit.com.au

Final reports must be emailed to admin@sagit.com.au as a Microsoft Word document in the format shown ***within 2 months*** after the completion of the Project Term.

PROJECT CODE :	AGT115

PROJECT TITLE (10 words maximum)
Genetic Characterisation and Exploitation of Heat Stress Tolerant Barley Germplasm

PROJECT DURATION

*These dates **must** be the same as those stated in the Funding Agreement*

Project Start date	01/07/2015					
Project End date	31/06/2017					
SAGIT Funding Request	2014/15	$	2015/16		2016/17	

PROJECT SUPERVISOR CONTACT DETAILS

The project supervisor is the person responsible for the overall project

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ADMINISTRATION CONTACT DETAILS

The Administration Contact is the person responsible for all administrative matters relating to the project

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PROJECT REPORT

Provide clear description of the following:

Executive Summary (200 words maximum)

A few paragraphs covering what was discovered, written in a manner that is easily understood and relevant to SA growers. A number of key dot points should be included which can be used in SAGIT communication programs

101 genotypes (including four wheat genotypes) were screened in the controlled environment assay in 2015 and 2016 at Roseworthy to screen for genotype diversity in adaptation to heat stress conditions. This process identified significant variation in grain yield determining traits for heat stress response. Providing scope to select for improved adaptation to heat stress conditions.

89 genotypes were grown in four environments in the 2016 growing season. The 2016 growing season was a year not characterized by heat stress conditions. This was shown in our study where only minimal variation in temperature conditions occurred, with the variation seen largely below heat stress thresholds. Temperature variation was still found to be important in determining grain yield and trait performance. However, due to the reduced stress conditions present, increased temperature was not always associated with decreased trait performance. Likely a consequence of the conditions present in the 2016 growing season, where increased temperature conditions below stress thresholds may have been associated with reduced waterlogging and lodging and therefore associated with increased performance.

In addition to SAGIT for funding this study, the authors would like to acknowledge efforts of the AGT team at Roseworthy for providing technical support in managing greenhouse and field trials, particularly Cassandra Bell who looked after much of the data collection. Additional thanks and acknowledgment goes to the farmer cooperators who hosted trials on their properties.

Project Objectives

A concise statement of the aims of the project in outcome terms should be provided.

The key aims and research outcomes from this project are as follows:

- Increased knowledge of varietal differences in heat stress tolerance in barley varieties.
- Benchmarking of barley heat stress tolerance compared to tolerance of wheat.
- Identification of heat stress tolerant parents suitable for targeting in a breeding program.

- Validation of the SAGIT-AGT heat chamber bioassay for use in screening barley.
- Validation of the heat chamber screening under field conditions at multiple AGT field sites. This also aids in the further dissection of heat stress interactions with genotype by environment interactions.

Overall Performance

A concise statement indicating the extent to which the Project objectives were achieved, a list of personnel who participated in the Research Project including co-operators, and any difficulties encountered and the reasons for these difficulties.

All components of the project were completed as planned. The only components of the project that were not fully conducive to the research outcomes of the project were beyond control of the project management and related to growing conditions in the field. Where low levels of heat stress conditions were evident across the environments sampled.

These reduced levels of heat stress in the field did impact on the ability to fully dissect the response to heat stress conditions that frequent flowering and grain fill periods in southern Australia.

Key Performance Indicators (KPI)

Please indicate whether KPI's were achieved. The KPI's ***must*** *be the same as those stated in the Application for Funding and a brief explanation provided as to how they were achieved or why they were not achieved.*

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
Screen barley germplasm set through heat chamber and in field	Y	
Validate chamber methodology in barley	Y	
Screen barley germplasm set through heat chamber and in field with methodology adjustments – if necessary	Y	
Screen barley germplasm set in multi-location experiments to field validate chamber results	Y	Material was screened across multiple field environments in 2016. Unfortunately for this project, there were minimal levels of heat stress conditions present in season that represented near ideal growing conditions.
Benchmark barley against wheat for heat stress adaptation	Y/N	Preliminarily benchmarking was conducted. Due to field conditions available further work is required.
Final Report	Y	

Technical Information (Not to exceed **three** pages)

Provide sufficient data and short clear statements of outcomes.

Following is a brief summary of the outcomes of this project. More detail is available in the supplementary technical report attached.

A set of 101 genotypes (including 4 wheat genotypes), were screened in a controlled environment assay in 2015 and 2016. Significant impacts on trait performance were identified as a result of heat stress for a number or traits (Table 1). A number of the traits measured were found to have a significant treatment by genotype interaction (Table 1). This indicates that the genotypes included in the study showed a differential response to the heat stress conditions imposed, and indicates potentially different levels in heat stress tolerance. The response of a subset of lines included in the study is shown in Figure 1, including total grain weight per head and harvest index of the primary tiller. These figures demonstrate that a range of response for each of the traits, a promising result indicating that potential differences in heat stress tolerance may be present within locally adapted and international germplasm. Further, this indicates that it may be possible to select for improved adaptation to heat stress conditions and potently combine different tolerance mechanisms.

Table 1. The traits measured in the controlled environment study along with the significance of the heat stress treatment, heat stress treatment by genotype interaction and the mean of each treatment if significant.

Trait	Treatment (P-value)	Treatment by genotype Interaction	Control Mean	Heat Stressed Mean
Fertility	<0.001	n.s.	1.76	1.80
TGW	<0.001	n.s.	55.26	49.37
Leaf2	<0.001	0.002	1.27	1.79
Leaf3	<0.001	0.024	1.81	2.49
Grain Weight (per head)	<0.001	0.012	1.47	1.30
Tiller Weight (Primary Tiller)	<0.001	n.s.	3.64	3.46
Head Weight (Primary Tiller)	<0.001	0.017	1.75	1.56
Grain Number (Per Head)	n.s.	n.s.	25.34	25.70
Harvest Index	<0.001	0.013	0.39	0.36

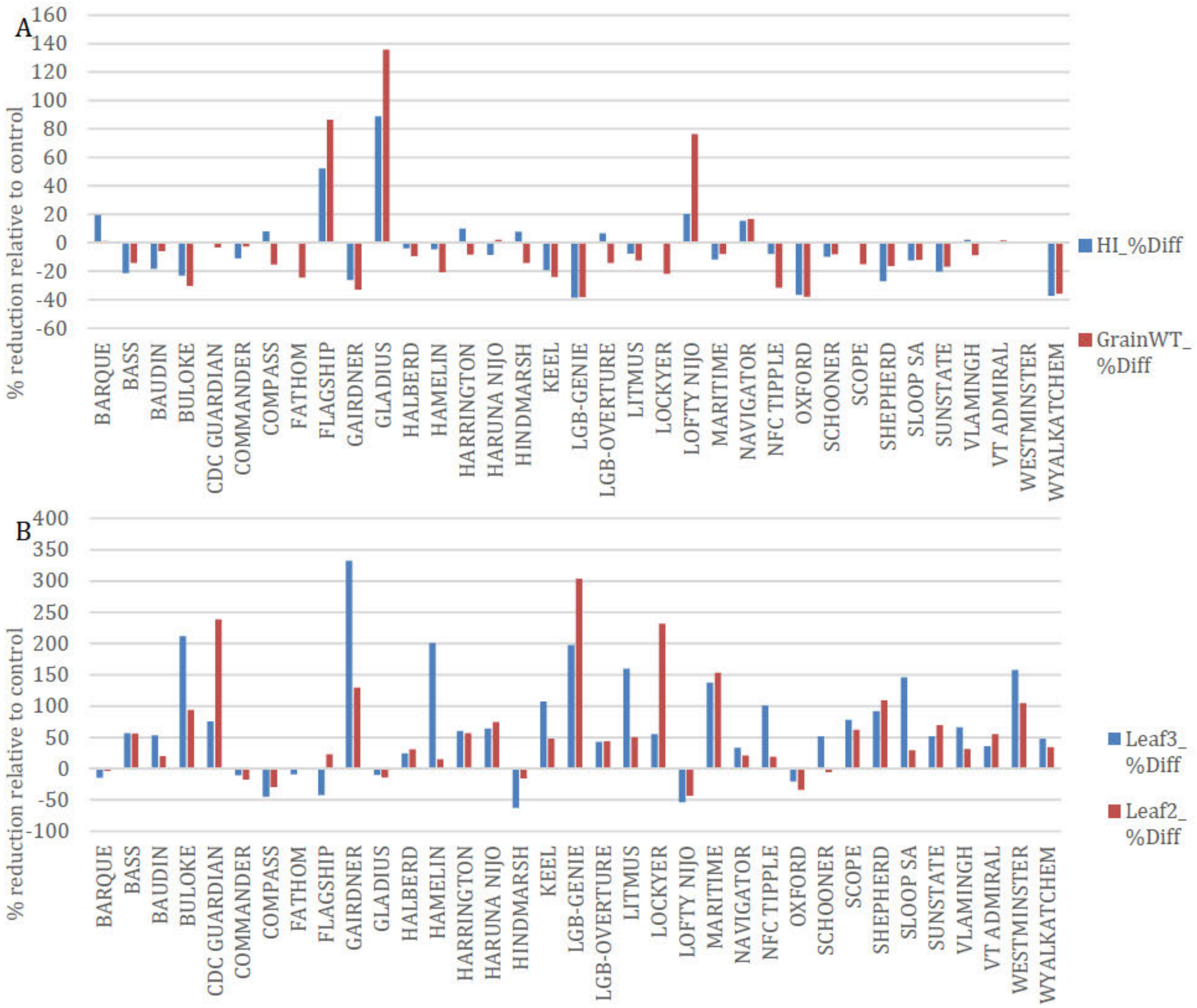

Figure 1. The percentage difference of the heat stress treatment relative to the unstressed control for (A) total grain weight and harvest index, and (B) Leaf2 (leaf senescence score at the end of heat stressing) and Leaf3 (leaf senesces score 7 days after the end of the heat stress treatment).

The same genotypes evaluated in the controlled environment assay (excluding a few genotypes found to be extremely un-adapted) were grown in field experiments in the 2016 growing season. The growing conditions present in 2016 were not conducive to the evaluation of heat stress tolerance, with relatively low heat stress conditions present. However, interactions with variable temperatures were still found to be significant for grain yield, along with screenings and thousand grain weight (TGW) (Table 2). Interestingly these were not always found to be negative relationships as found in previous AGT-SAGIT heat stress related research (Project AGT031). The temperature ranges experience in the field within the environments sampled were generally less than has been experienced in past research, and likely largely below stress impact thresholds. As a result positive relationships were identified for some interactions between climatic co-variates and genotypes for some traits. Possibly a result of increased temperature providing conditions conducive to reduced levels of lodging and waterlogging, and therefore potentially increased grain yield.

Table 2. Summary of mixed linear model analysis of genotype by climatic covariate interactions across the environments included in the study. Significance (P value) of individual climatic co-variates (CV) and significance of genotype by climatic co-variate interaction (NAME.CV) is shown for grain yield, screenings and TGW.

	Grain Yield		Screenings % (2 mm)		TGW	
Climatic co-variate	**CV**	**NAME.CV**	**CV**	**NAME.CV**	**CV**	**NAME.CV**
Growing season rainfall	<0.001	<0.001	ns.	<0.001	<0.001	0.003
Anthsis average maximum temperature	<0.001	ns	<0.001	<0.001	<0.001	ns.
Anthesis days >30°C	<0.001	0.06	<0.001	<0.001	ns	<0.001
Anthesis days >35°C	ns.	ns.	ns.	ns.	ns	ns
Grain fill average maximum temperature	0.019	ns.	<0.001	ns.	<0.001	ns.
Grain fill days >30°C	<0.001	0.005	<0.001	<0.001	<0.001	<0.001
Grain fill days >35°C	<0.001	<0.001	0.02	<0.001	<0.001	<0.001

ns. = not significant

This non-linear relationship between increasing temperature and trait performance was confirmed with a factor analytic analysis of genotype by environment interaction. This analysis provides the opportunity to further dissect the drivers of trait performance across the environments included in the study. Environment loadings from the analysis when correlated with experiment mean climatic co-variates to give an indication of the influence on trait performance. Increasing temperature during both anthesis and grain filling were found to be important for the largest factor for grain yield determination, with large negative correlations produced. However, the relationship was less clear for the second factor of grain yield and for screenings % and TGW. Confirming that although temperature was important in trait performance determination, but not necessarily consistent with conditions experienced in previous studies for bread wheat where heat stress conditions were prevalent and found to be very important in trait performance with heat stress generally having large negative impacts.

Table 3. Correlations of environmental loadings with each factor produced from a factor analytic analysis of genotype by environment interaction analysis of grain yield, screenings % and TGW. Also shown is the % variance explained by each factor for each trait across all experiments included in the study.

Correlation	Grain Yield		Screenings %			TGW		
	FA1	**FA2**	**FA1**	**FA2**	**FA3**	**FA1**	**FA2**	**FA3**
% Variance explained by each FA	68.2	16.3	50.1	38.2	10.6	84.7	6	4.4
Growing season rainfall	0.30	-0.93	0.12	0.18	-0.30	-0.90	0.76	-0.19
Anthsis average maximum temperature	-0.80	-0.53	0.70	0.13	0.77	-0.60	0.05	0.72
Anthesis days >30°C	-0.72	0.66	-0.04	-0.54	0.82	0.33	-0.94	0.33
Grain fill average maximum temperature	-0.81	-0.04	0.21	-0.46	0.92	-0.42	-0.52	0.42
Grain fill days >30°C	-0.76	0.10	0.07	-0.59	0.89	-0.33	-0.64	0.31
Grain fill days >35°C	-0.42	0.08	-0.30	-0.83	0.62	-0.46	-0.59	-0.10

Conclusions Reached &/or Discoveries Made (Not to exceed one page)

Please provide concise statement of any conclusions reached &/or discoveries made.

Variation for heat stress adaption was found in the controlled environment assay which screens for performance under heat stress conditions during grain filling. This is a promising result, providing scope to select for improved tolerance to heat stress conditions in future barley varieties.

Field validation of controlled environment assay results is normally considered important to provide validation results in real world conditions, and to put any variation identified into context. Field validation was an important component if this project. However, only one season was able to be sampled within the scope of this project. The season was a record crop year with very low incidence of heat stress conditions experienced. This made it difficult to validate the results found in the controlled environment assay. The relevance of the controlled environment assay has been discussed and confirmed in previous work relating to heat stress adaptation of wheat (Project AGT031).

Despite the low incidence of heat stress conditions within the field environments sampled, interactions of temperature were found with differential genotype response also identified. Interestingly, compared to previous studies in wheat (Project AGT031) which showed predominantly negative impacts of heat stress, both negative and positive impacts of increased temperature were found in this study. This is likely an interaction of increasing temperature conditions below stress inducing thresholds and potently associated with near ideal growing conditions and potentially reduced lodging and water logging conditions.

Although not the results that was hoped for within the context of this study, this does confirm the complex relationship with temperature that crops have within our growing environment. That temperature is an important driver of grain yield and production, but when stress thresholds are exceeded negative impacts on grain yield and production are evident.

Intellectual Property

Please provide concise statement of any intellectual property generated and potential for commercialisation.

No IP with potential for commercialisation was generated in this project

Application / Communication of Results

A concise statement describing activities undertaken to communicate the results of the project to the grains industry. This should include:

- *Main findings of the project in a dot point form suitable for use in communications to farmers;*
- *A statement of potential industry impact*
- *Publications and extension articles delivered as part of the project; and,*
- *Suggested path to market for the results including barriers to adoption.*

Note that SAGIT may directly extend information from Final reports to growers. If applicable, attach a list of published material.

POSSIBLE FUTURE WORK

Provide possible future directions for the research arising from the project including potential for further work and partnerships.

To get a full picture of adaption to heat stress conditions in southern Australia, further field evaluation will be required. The levels of heat stress evident in the 2016 growing season were relatively low, resulting in inconclusive field evaluation in heat stress conditions.

AUTHORISATION
Name: Dr Haydn Kuchel
Position: CEO & Head of Breeding
Signature:
Date: 06/09/2017

Submit report via email to admin@sagit.com.au as a Microsoft Word document in the format shown ***within 2 months*** after the completion of the Project Term.

Genetic Characterisation and Exploitation of Heat Stress Tolerant Barley Germplasm

SAGIT – AGT115 Project Technical Report

Prepared by Paul Telfer, James Edwards and Haydn Kuchel

1.1 Introduction

The 2016 growing season was relatively free from heat stress conditions. However, heat stress conditions are common to many areas of Australia and particularly southern Australia. Southern Australia, which as a Mediterranean climate with spring typified by increasing high temperatures as the season proceeds. This period also aligns with important developmental stages in cereal crops such as wheat. Flowering is known to be sensitive to high temperatures with pollen viability adversely affected and seed set can subsequently be reduced if high temperatures are experienced. High temperatures during grain filling can also have adverse effects on grain filling duration, accelerated plant maturation and leaf area senescence potentially reducing grain size.

As a starting point for previous AGT-SAGIT research into the impacts of heat stress in the southern environment, a desktop study was undertaken to understand the magnitude of heat stress in the southern environment. The data set used in this study used average trial grain yield and a number of climatic variables from over 600 trial by year combinations from the NVT across southern Australia from 2005 to 2010. From this, significant negative impacts were found on grain yield with increasing heat stress conditions during both flowering and grain filling periods (Table 1). The magnitudes of damage found if stress occurred during flowering was higher than grain filling.

Table 1 Effect of various climatic variables on grain yield across over 600 bread wheat NVT field trials in southern Australia, 2005-2010. Average grain yield across all trials was 2530 kg/ha.

Growth Stage	Climatic variable	Unit	Effect (kg/ha)
Flowering	Rainfall	mm	22
	Avg daily min	°C	-161
	Avg daily max	°C	-371
	Days >30 °C	number	-379
	Days >35 °C	number	-837
	Avg Temp	°C	-490
Grain filling	Rainfall	mm	23
	Avg daily min	°C	-125
	Avg daily max	°C	-225
	Days >30 °C	Number	-130
	Days >35 °C	number	-179
	Avg Temp	°C	-244

1.2 Research questions

There were a number of research objectives within this project. In brief they are encompassed as follows:

- Increased knowledge of varietal differences in heat stress tolerance in barley varieties.
- Benchmarking of barley heat stress tolerance compared to tolerance of wheat.
- Identification of heat stress tolerant parents suitable for targeting in a breeding program.
- Validation of the SAGIT-AGT heat chamber bioassay for use in screening barley.
- Validation of the heat chamber screening under field conditions at multiple AGT field sites. This also aids in the further dissection of genotype by environment interactions explained by heat stress.

2. Understanding the role and impacts of heat stress in the southern Environment – methodologies used

The full methodology used in this project have previously been described (SAGIT project final report - AGT031).

In brief the controlled environment assay consisted of comparing an un-stress control treatment for each genotype with a heat stress treatment. The heat stress treatment consisted of exposing plants to three consecutive eight hour days to 36°C with 40 km hr^{-1} winds. Traits described in Table 2 were measured on all plants in the study.

Table 2. The traits measured in the controlled environment assay and the stage at which they are measured.

Trait	Trait description	Stage observed	Units
Leaf2	Visual leaf damage score (1-9)	End of heat treatment	1-9 score
Leaf3	Visual leaf damage score (1-9)	10 days post the start of heat treatment	1-9 score
Spikelet number per head	Number of spikelets per head	Physiological maturity	Spikelets $head^{-1}$
Grain number per head	Total number of grains in the mature head	Physiological maturity	Grains $head^{-1}$
Head weight	Total weight of head	Physiological maturity	g
Grain weight	Total weight of grain produced per head	Physiological maturity	g
Primary tiller weight	Total weight of primary tiller	Physiological maturity	g
TGW	Thousand Grain Weight (TGW)	Physiological maturity	g
Spikelet Fertility	Average grain number per spikelet	Physiological maturity	g
Harvest Index (HI)	Proportion of primary tiller mass present as grain	Physiological maturity	Grains $Spikelet^{-1}$
Head Fertility Index	Harvest index of head only	Physiological maturity	

A lot can be learnt in controlled environment studies, with many benefits from an experimental and statistical point of view. Despite this, understanding heat stress physiology in the field, the environment that farmers grow their crops, is important to confirm physiological trends identified in controlled environment conditions and to validate any tools developed for the real world. In this study four field environments were used in the 2016 growing season (Angas Valley, Booleroo, Roseworthy and Winulta). At each location climatic co-covariates (Table 3) were measured to enable evaluation of the impact of heat stress across environments and to evaluate individual genotype response to changing temperature conditions.

Table 2 The climatic variables calculated for each growing window in the field experiments to understand the seasonal temperature conditions.

Developmental stage	**Degree day range relative to anthesis**
Anthesis	300°Cd before anthesis to 100°Cd post
Grain filling	100°Cd post anthesis to 600°Cd post

Climatic variable	**Explanation & units**
Growing season rainfall	May to October rainfall (mm)
Average maximum temperature	°C
Number of hot days	Number of days >30°C
Number of very hot days	Number of days >35°C
Curtesy of Chapman & Zheng	

3.1 Evaluation of barely material in the controlled environment assay

Experiments were conducted in the controlled environment assay in both 2015 and 2016 using the methodology previously reported (SAGIT project final report - AGT031). In 2015, 101 Genotypes were screened, this was reduced to 89 in 2016 to remove extremely un-adapted material. This experiment identified negative impacts on a range of physiological traits in response to the heat stress (Table 3). This included a range of traits that contribute directly to grain yield determination including TGW, showing an average 11% reduction of the heat stress treatment compared to the unstressed control. Additionally traits that may help explain how plants may respond to the stress conditions were also shown to be significant. An example of this is leaf senescence, measured by the Leaf2 and Leaf3 traits, where a reduction in viable leaf area (increased senescence) was observed both immediately after the end of the heat stress treatment (Leaf2) and seven days after the end of the heat stress treatment (Leaf3). Leaf2 and Leaf 3 showed a 41% and 37% increase in sensed leaf area of the heat stress treatment relative to the control treatment on average across all genotypes included in the study.

Table 3 The traits measured in the controlled environment study along with the significance of the heat stress treatment, heat stress treatment by genotype interaction and the mean of each treatment if significant.

Trait	Treatment (P-value)	Treatment by genotype Interaction	Control Mean	Heat Stressed Mean
Fertility	<0.001	n.s.	1.76	1.80
TGW	<0.001	n.s.	55.26	49.37
Leaf2	<0.001	0.002	1.27	1.79
Leaf3	<0.001	0.024	1.81	2.49
Grain Weight (per head)	<0.001	0.012	1.47	1.30
Tiller Weight (Primary Tiller)	<0.001	n.s.	3.64	3.46
Head Weight (Primary Tiller)	<0.001	0.017	1.75	1.56
Grain Number (Per Head)	n.s.	n.s.	25.34	25.70
Harvest Index	<0.001	0.013	0.39	0.36

A number of the traits measured were also found to have a significant treatment by genotype interaction (Table 3). This indicates that the genotypes included in the study showed a differential response to the heat stress conditions imposed, and indicating potential different levels in heat stress tolerance. The response of a subset of lines included in the study is show in Figure 1, including total grain weight per head and harvest index of the primary tiller. These figures demonstrate a range of responses for each of the traits shown, a promising result indicating that potential differences in heat stress tolerance may be present within locally adapted germplasm as well as international germplasm.

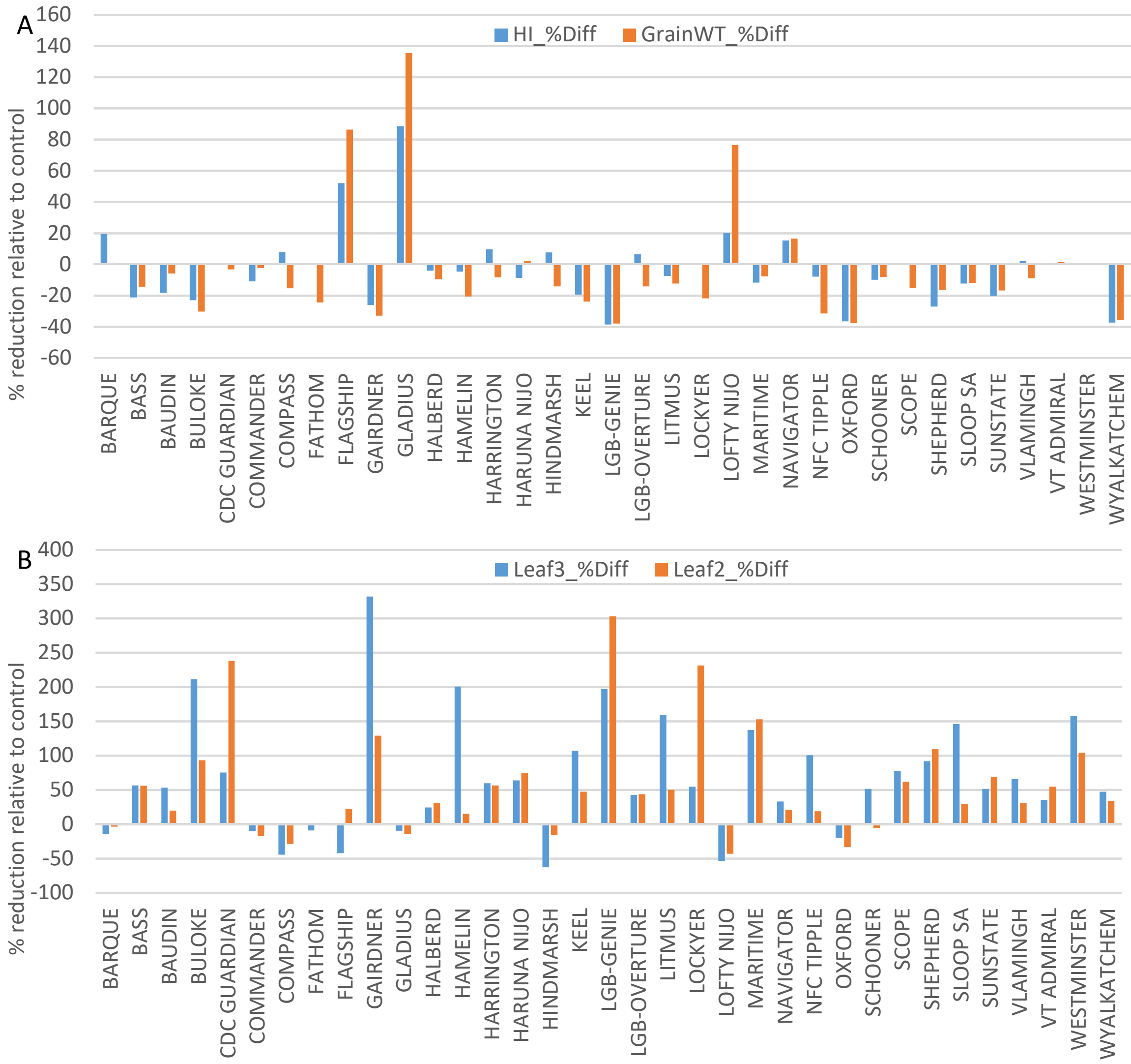

Figure 1. The percentage difference of the heat stress treatment relative to the unstressed control for (A) total grain weight and harvest index, and (B) Leaf2 (leaf senescence score at the end of heat stressing) and Leaf3 (leaf senesces score 7 days after the end of the heat stress treatment).

3.2 Results for field evaluation across multiple environments in the 2016 growing season.

Compared to the preceding few years the amount of heat stress present in the southern Australian growing environment was greatly reduced, with the 2016 growing season experiencing well above average rainfall conditions and generally low heat stress conditions (Table 4), achieving a record cereal crop for both South Australia and Australia as a whole. The results of this study (Table 5) showed that across the environments included in the study, the impact of heat stress on grain yield was less than has previously been reported (Table 1), and in multi-year and multi-environment studies evaluating the heat stress tolerance of wheat (SAGIT project final report - AGT031). However, in a year of low heat stress incidence and near ideal growing conditions, there was a significant relationship with increasing average maximum temperature during grain fill and reduced grain yield (-436.2 kg ha^{-1} per °C) and an increase in screenings percentage (0.21 % per °C). Demonstrating that even in low heat stress conditions, heat stress did play a role in grain yield determination across the four environments sampled.

Table 4. The mean grain yield, screenings % (2mm) and TGW for each experiment, along with the mean climatic co-variates experienced.

Experiment	Grain Yield (kg ha^{-1})	Screenings % (2 mm)	TGW	Growing season rainfall	Anthsis average maximum temperature	Anthesis days >30°	Anthesis days >35°C	Grain fill average maximum temperature	Grain fill days >30°C	Grain fill days >35°C
Angas Valley	4144	2.1	42.5	221	21.3	2.6	0.0	26.5	9.0	1.3
Booleroo	4659	0.6	48.6	340	20.9	2.3	0.0	27.2	11.0	2.8
Roseworthy	4174	2.6	43.3	480	22.6	0.9	0.0	27.1	9.4	1.8
Winutla	6003	0.9	46.1	381	19.7	0.0	0.0	23.3	3.2	0.4

Table 5. Results of a linear regression between experiment mean grain yield and the mean climatic covariates measured at each field location, P-value and effect on trait performance is shown if significant.

Trait	Grain Yield		
Cliamtic Co-variate	P value	% Var	Effect (kg ha^{1})
Growing season rainfall	ns.	ns.	ns.
Anthsis average maximum temperature	ns.	ns.	ns.
Anthesis days >30°C	ns.	ns.	ns.
Anthesis days >35°C	ns.	ns.	ns.
Grain fill average maximum temperature	0.085	0.84	-436.2
Grain fill days >30°C	ns.	ns.	ns.
Grain fill days >35°C	ns.	ns.	ns.

ns. = not significant

3.3 Evaluation of genotype response to heat stress conditions across multiple field environments

Grain yield and physical quality data for each genotype was collected from each experiment. This data was analysed using mixed linear models to determine the impact and interactions of heat stress on genotype performance. This is summarised in Table 6, and the response of a subset of genotypes for each of the climatic co-variates with significant genotype by climatic co-variate interactions are shown in Table 7. There were a wide range of responses in grain yield to increased temperature conditions, with some genotypes responding favourably to increased temperature conditions, while others responded negatively. These responses are interesting, and not what we have traditionally associated with increased temperature conditions. However, the 2016 growing season was an extremely wet season and it is possible that in such a year increased temperature conditions may be associated with reduced water logging, less lodging and potentially increased grain yield. Although not a usual response, in what was an unusual season, it is a plausible explanation. As such it is difficult to draw solid conclusions on relative tolerance of genotypes to heat stress conditions and further investigation across more seasons and environments would be required to accurately characterise heat stress tolerance.

The study was primarily based around assessing the relative tolerance of barley in heat stress conditions, to parallel previous work conducted in wheat (SAGIT project AGT031). Additionally, barley is understood to be incrementally more tolerant to other abiotic stress conditions, namely frost. Four wheat genotypes were included in this study to do some preliminary benchmarking of barley heat stress tolerance compared to wheat. Although there was minimal heat stress conditions present across the environments included in the study, the mean response to many of the climatic covariates of barley and wheat (Table 7) showed that wheat often produced a more negative response. Although difficult to draw conclusions, it does elude to a differential response to changing temperature conditions between the two species. Further investigation with a larger range of heat stress conditions would be required for further characterisation.

Table 6. Summary of mixed linear model analysis of genotype by climatic covariate interactions across the environments included in the study. Significance (P value) of individual climatic co-variates (CV) and genotype by climatic co-variate interaction (NAME.CV) is shown for grain yield, screenings and TGW.

	Grain Yield		**Screenings % (2 mm)**		**TGW**	
Climatic co-variate	**CV**	**NAME.CV**	**CV**	**NAME.CV**	**CV**	**NAME.CV**
Growing season rainfall	<0.001	<0.001	ns.	<0.001	<0.001	0.003
Anthsis average maximum temperature	<0.001	ns	<0.001	<0.001	<0.001	ns.
Anthesis days >30°C	<0.001	0.06	<0.001	<0.001	ns	<0.001
Anthesis days >35°C	ns.	ns.	ns.	ns.	ns	ns
Grain fill average maximum temperature	0.019	ns.	<0.001	ns.	<0.001	ns.
Grain fill days >30°C	<0.001	0.005	<0.001	<0.001	<0.001	<0.001
Grain fill days >35°C	<0.001	<0.001	0.02	<0.001	<0.001	<0.001

ns. = not significant

Table 7. The effect on grain yield and TGW for every unit change in each climatic co-variate as determined across all environments included in the study. Temperature co-variates with a significant genotype by climatic co-variate interaction are displayed. The mean trait effect for barley and wheat genotypes are also displayed.

	Grain Yield (kg ha^{-1})				TGW (g 1000 $grains^{-1}$)			
Genotype	**Anthesis average maximum (°C)**	**Anthesis days >30°C**	**Grain fill days >30°C**	**Grain fill days >35°C**	**Anthesis average maximum (°C)**	**Anthesis days >30°C**	**Grain fill days >30°C**	**Grain fill days >35°C**
BARQUE	-311.2	386.8	109.3	280.9	0.20	1.22	0.71	1.06
BASS	-304.0	48.8	44.2	-24.2	0.42	-0.36	0.33	0.31
BAUDIN	-315.5	-52.9	-22.3	-302.3	0.65	1.44	0.33	-0.95
BULOKE	-275.1	-305.2	24.5	137.4	0.21	0.51	0.39	0.62
CDC GUARDIAN	-275.2	-116.8	15.9	35.3	1.61	-0.76	0.14	0.01
COMMANDER	-310.3	205.3	27.3	-153.4	0.37	1.58	0.66	0.39
COMPASS	-330.1	9.7	61.5	-327.4	0.15	0.93	0.47	0.46
FATHOM	-241.4	-148.2	89.3	448.9	0.25	2.66	1.09	5.64
FLAGSHIP	-220.5	-20.1	9.5	-39.1	0.23	0.64	0.38	0.00
GAIRDNER	-264.5	-2.0	14.2	5.1	0.36	0.35	0.61	0.77
HAMELIN	-330.3	135.8	-6.6	-381.9	0.39	0.26	0.21	0.54
HARRINGTON	-296.5	122.1	-20.2	-108.0	0.72	-0.04	0.39	0.76
HARUNA NIJO	-473.6	19.4	-117.1	-68.7	0.51	-0.27	0.50	0.01
HINDMARSH	-213.6	-534.3	-35.0	-393.6	0.31	3.15	0.81	-0.21
KEEL	-331.6	932.4	172.6	-68.7	0.43	1.64	0.46	0.01
LGB-GENIE	-262.0	-105.5	70.1	100.2	0.34	-0.02	0.34	0.18
LGB-OVERTURE	-251.5	-206.7	162.2	426.2	0.21	-0.98	0.23	0.28
LITMUS	-357.8	19.4	145.5	-68.7	0.41	-0.27	1.02	0.01
LOCKYER	-217.5	-79.0	101.3	266.9	0.31	0.65	0.57	0.93
LOFTY NIJO	-300.0	284.3	100.1	505.8	0.26	-0.01	0.77	1.98
MARITIME	-264.2	-231.8	24.2	-44.0	0.23	-0.31	0.19	-0.25
NAVIGATOR	-290.6	246.4	81.1	407.1	0.33	0.49	0.65	0.66
NFC TIPPLE	-256.4	-96.6	87.0	248.8	0.09	0.62	0.38	0.45
OXFORD	-226.5	-29.2	130.5	262.2	0.09	-0.67	0.11	-0.98
SCHOONER	-301.1	-150.3	-10.7	-359.1	0.23	-0.33	0.46	1.80
SCOPE	-241.0	-117.6	15.2	-65.0	0.47	0.66	0.39	0.35
SHEPHERD	-257.2	124.5	126.0	557.7	0.17	-0.20	0.49	1.60
SLOOP SA	-266.8	-20.7	44.5	320.3	0.25	0.26	0.84	3.67
VLAMINGH	-271.0	-321.8	17.2	81.1	0.08	-0.29	0.27	-0.09
VT ADMIRAL	-416.6	19.4	118.4	-68.7	0.41	-0.27	0.05	0.01
WESTMINSTER	-214.7	-223.2	-27.1	-84.0	0.18	-0.70	0.14	0.05
GLADIUS	-307.0	-818.4	-184.4	-1332.8	0.34	-3.37	-0.71	-5.60
HALBERD	-176.4	-772.8	-73.8	-530.8	0.11	-2.52	-0.10	-3.21
SUNSTATE	-160.7	19.4	-185.8	-68.7	0.03	-0.27	-0.14	0.01
WYALKATCHEM	-263.6	-343.3	-130.8	-525.8	0.09	-1.45	-0.31	-2.60
BARLEY MEAN	**-281.2**	**-9.3**	**59.1**	**93.4**	**0.37**	**0.28**	**0.43**	**0.63**
WHEAT MEAN	**-226.9**	**-478.8**	**-143.7**	**-614.5**	**0.14**	**-1.90**	**-0.32**	**-2.85**

3.4 Heat stress conditions and their influence on genotype by environment interactions in the field as determined by a factor analytic analysis of genotype by environment interaction

A factor analytic analysis of genotype by environment interactions was conducted to further investigate the role of temperature conditions experienced across the study on grain and grain yield determining trait performance. This style of analysis allows for various factors that may be important for determining trait performance to be identified. Often it is used to identify which environments are performing similar or are being driven by similar environmental factors. However, it also provides an opportunity to further investigate the specific environmental drivers that are influencing trait performance, by correlating environmental loadings produced from the analysis with experiment mean climatic co-variates. In this study two factors were identified for grain yield, which together accounted for 84.5 % of the variance (Table 8). This process also showed that all of the climatic co-variates with variation across the environments included in the study were important for the largest factor (FA1) in determining grain yield, producing significant negative correlations with environmental loadings.

The story was not so clear for screenings % and TGW. Climatic co-variates produced significant interactions with experiment loadings for each of the factors for each trait. However, these were either negative or positive depending on the combination of trait, environmental loading and factor. As previously discussed, this is likely a result of the season, where increased temperature conditions, where perhaps not in excess of stress thresholds and may have conferred improved growing conditions; reduced water logging, reduced lodging and the like, making relationships with stress conditions less clear.

Table 8. Correlations of environmental loadings with each factor produced from a factor analytic analysis of genotype by environment interaction analysis for grain yield, screenings % and TGW. Also shown is the % variance explained by each factor for each trait across all experiments included in the study.

Correlation	Grain Yield		Screenings %			TGW		
	FA1	FA2	FA1	FA2	FA3	FA1	FA2	FA3
% Variance explained by each FA	68.2	16.3	50.1	38.2	10.6	84.7	6	4.4
Growing season rainfall	0.30	-0.93	0.12	0.18	-0.30	-0.90	0.76	-0.19
Anthsis average maximum temperature	-0.80	-0.53	0.70	0.13	0.77	-0.60	0.05	0.72
Anthesis days >30°C	-0.72	0.66	-0.04	-0.54	0.82	0.33	-0.94	0.33
Grain fill average maximum temperature	-0.81	-0.04	0.21	-0.46	0.92	-0.42	-0.52	0.42
Grain fill days >30°C	-0.76	0.10	0.07	-0.59	0.89	-0.33	-0.64	0.31
Grain fill days >35°C	-0.42	0.08	-0.30	-0.83	0.62	-0.46	-0.59	-0.10

3.5 Controlled environment assay data and its role in explaining field genotype by environment interactions

Genotype scores form the factor analytic analysis of genotype by environment interaction can be correlated with controlled environment data to identify links in genotype performance. This style of analysis has previously shown significant relationships between results collected in the controlled environment assay and the relevance of this data in determining genotype performance in the field (SAGIT project AGT031). As shown in Table 9, this was not the case in this study, with relatively low correlations with traits found to have a significant heat stress by genotype interaction in the controlled environment assay when correlated with genotype scores from the field. There was a relatively low incidence of heat stress across the field environments captured in this study in the 2016 growing season. The low correlations produced confirm that the environment produced in the controlled environment assay were not representative of what was experienced in the field in the 2016 growing season. Previous experience in wheat has shown that the controlled environment assay does relate to performance in the field under heat stress conditions.

Table 9. Correlations of controlled environment performance data with genotype scores from a factor analytic model of genotype by environment interaction analysis of grain yield, screenings % and TGW. Controlled environment data is the genotype performance under the heat stress treatment (Heat) and the % effect relative to the control treatment (Heat Effect).

Correlation	Grain Yield		Screenings			TGW		
	FA1	FA2	FA1	FA2	FA3	FA1	FA2	FA3
Grainwt_Heat	-0.04	0.04	0.22	-0.05	-0.05	-0.02	-0.14	-0.26
Grainwt_Heat Effect	-0.10	-0.13	0.17	-0.04	0.06	0.09	0.04	-0.25
HI_Heat	-0.01	0.27	-0.13	-0.01	0.00	0.11	-0.16	0.14
HI_Heat Effect	-0.07	-0.07	-0.16	-0.01	0.03	0.26	-0.12	0.16
leaf2_Heat	-0.05	-0.20	0.23	0.03	0.08	-0.20	-0.02	0.00
leaf2_Heat Effect	0.07	-0.15	0.07	-0.18	0.15	-0.15	0.08	-0.10
Leaf3_Heat	-0.14	-0.14	0.29	0.07	0.07	-0.26	-0.03	0.10
Leaf3_Heat Effect	0.02	-0.03	0.11	-0.06	0.05	-0.22	0.01	0.04

3.6 Concluding remarks

The 2016 growing season was not a good season for studying the impacts of heat stress on grain yield and adaptation of barley to heat stress conditions. It was however, generally much more positive for cereal growers. Comparative to previous seasons low levels of heat stress conditions were seen in the growing environments studied, making it difficult to evaluate differing genotype responses to heat stress conditions. Temperature was found to be important in driving grain yield and trait performance, however, this was not found to be the normal relationship of increasing heat stress conditions having a negative effect. With increasing temperatures below stress thresholds impacting positively on trait performance under some circumstances, likely as a result of other adaptation and agronomic factors of reduced lodging and potentially reduced waterlogging.

The controlled environment assay did identify differing levels of response to heat stress conditions for a number of grain yield determining traits. Confirming that genetic variance for heat stress tolerance does exist and therefore breeding opportunities do exist to further improve adaptation.