Office Use Only

Project Code	
Project Type	

FINAL REPORT 2016

Applicants must read the *SAGIT Project Funding Guidelines 2016* 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 : AGT031

PROJECT TITLE (10 words maximum)

Genetic Characterisation and Exploitation of Heat Stress Tolerant Wheat Germplasm

PROJECT DURATION

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

Project Start date	1/7/2012					
Project End date	30/6/2016 (revised)					

PROJECT SUPERVISOR CONTACT DETAILS

The project supervisor is the person responsible for the overall project

Title:	**First Name:**	**Surname:**
Dr	Haydn	Kuchel

Organisation:

Australian Grain Technologies Pty Ltd

Mailing address:

Roseworthy Campus, Roseworthy SA

Telephone:	**Facsimile:**	**Mobile:**	**Email:**

ADMINISTRATION CONTACT DETAILS

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

Title:	**First Name:**	**Surname:**
Mr	Andrew	Cecil

Organisation:

Australian Grain Technologies Pty Ltd

Mailing address:

PMB 1, Glen Osmond SA 5064

Telephone:	**Facsimile:**	**Mobile:**	**Email:**

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

Heat stress has large implications on cereal production in southern Australia with stressful temperatures during spring having large negative impacts on the grain yields achieved by producers. An increased understanding of the role of heat stress in adaptation and the physiology involved in order to develop breeding tools for selection of improved adaptation to heat stress conditions was a key intention of this project.

Increased understanding of the interaction of physiological processes interacting with heat stress conditions and further interactions with timing of stress conditions relative to plant development. This included identifying QTL for heat stress interactions in both controlled environment conditions and in the field. The value of these QTL for breeding purposes continues to be evaluated.

Key points

- Heat stress is a key limiting factor of cereal production in southern Australia.
- How varieties respond to heat stress conditions is very dependent on the timing of heat stress conditions. Sensitivity to both flowering stress and grain filling stress varies in Australian varieties.
- QTL for heat stress tolerance have been identified, their value as breeding tools and selection protocol continue to be evaluated as a component of Paul Telfer's PhD.

Project Objectives

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

The purpose of this project was to ensure that the knowledge generated in the previous AGT/SAGIT heat project(s) is exploited by breeders. The previous research found some very interesting results, however at the conclusion of that project there was still a significant research gap that needed to be filled before the results can be used by breeders (and therefore accessible by growers). The aim of the current project was to take the previous research outcomes that included developing a representative bio-assay to understand the extent of variation in heat stress tolerance, identify varieties with improved tolerance to improve our understanding of heat stress tolerance, and progress them to outcomes that are able to be used by breeders. In the proposed project we would aim to.

- Develop populations segregating for heat stress tolerance, that can then be used to identify the chromosome locations of genes conferring heat stress tolerance
- Develop molecular markers useful for breeding heat stress tolerance;
- Validate the environmental stability of heat stress tolerance over seasons and sites to better value the identified heat stress tolerance;
- Begin combining and introgressing levels of heat stress tolerance potentially greater than currently available in Australian germplasm from wild/unadapted lines into backgrounds ready for use in breeding.

The overall aim is to provide breeders the tools required to develop varieties with improved heat stress tolerance for South Australian growers. This project would act as the link between basic research (the current project) and project delivery (varieties).

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.

The project objectives were achieved. However, field validation of the genetic regions identified in this project and analysis of the results is ongoing as a component of Paul Telfer's PhD. The results will continue to be communicated, primarily in the form of published scientific articles.

Personnel who contributed to the project

- Paul Telfer – Project Manager
- Haydn Kuchel – Project Supervisor
- Cassandra Bell – Technical Assistant
- James Edwards - Project Supervisor
- Dion Bennett - Project Supervisor
- Andrew Cecil - Administration
- AGT Roseworthy team
- AGT collaborators who host AGT field trials

There were some issues encountered through the course of the project. This included the timing of delivery of the doubled haploid mapping populations. This caused a delay in the bulk up of seed delaying full scale field trials for a year. An extension to the project was negotiated with SAGIT, allowing a further year to the project allowing the field work planned to be conducted at the scale originally intended. All issues such as the one discussed were managed as to not adversely affect the project and its intended outcome.

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.***

Development of doubled haploid genetic mapping populations (pre main project)	Y	
Assessment and identification of variation for HST in populations in the heat chamber	Y	
Field assessment of exotic by adapted populations	Y	
Further assessment of populations in the heat chamber	Y	
Genetic mapping of heat stress tolerance	Y	Analysis and evaluation is continuing
Field value of key genetic regions (identified in mapping) established	Y – continuing	Analysis and evaluation is continuing as a component of Paul Telfer's PhD project to fully evaluate the genetic regions identified
Assess heat tolerance of exotic by adapted populations	Y	
Submit final report to SAGIT	Y	

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

Provide sufficient data and short clear statements of outcomes.

A brief summary of results is provided in this section, a more detailed technical report is attached.

The potential of heat stress to adversely affect grain production has been discussed widely across the grains industry in recent years, in part to the high frequency of heat stress conditions during flowering and grain filling producing visible effects on farmer's crops. The field work conducted in this project confirmed the negative impacts of heat stress on grain yield. As is shown in Table 1, the effects of heat stress occurring during flowering and grain filling can produce very large negative impacts in grain yield, with a larger magnitude of effect if heat stress occurs during flowering. This study involving a set of 24 Australian genotypes with different levels of heat stress tolerance grown at 13 locations in South Australia and in the Wimmera, Victoria in the 2013 and 2014 the growing seasons, where the climatic conditions were calculated for every plot in each experiment during both flowering and grain filling.

In addition to understanding the effects of heat stress in the data set, it also provided the opportunity to understand differences in genotype response to the stress conditions experienced. As shown in Figure 1, genotypes showed different responses to the heat stresses experienced across the range of environments, with some genotypes loosing yield at a lesser rate in the presence of heat stress than other genotypes. However, it was also shown that some genotypes expressing promising levels of heat stress tolerance when stress occurred during grain filling showed contrasting responses when stress occurred during flowering. Confirming that the interactions of heat stress with different physiological processes is complex. It does also indicate that pyramiding tolerance mechanisms together may improve adaptation to heat stress conditions in the southern Australian environment.

Table 1 Regression analysis results of field trials from 2013 and 2014 across seven locations and thirteen experiments in South Australia and the Wimmera, Victoria. For each climatic parameter, the significance of its correlation with site average yield is shown, along with the effect on grain yield for every one unit change in each climatic parameter.

Site	Sowing date	Grain yield (kg/ha)	May-Oct Rainfall (mm)	Av. temperature °C	Av. maximum temperature °C	No. of days > 30°C	Av. temperature °C	Av. maximum temperature °C	No. of days > 30°C	No. of days > 35°C
	2013			**flowering**	**flowering**	**flowering**	**grain fill**	**grain fill**	**grain fill**	**grain fill**
Angas Valley	**23 May**	1789	165.4	15.2	26.0	6.1	16.1	27.7	8.3	1.9
Booleroo	**17 May**	3119	292.6	15.2	24.7	2.4	16.1	26.3	7.2	2.0
Minnipa	**15 May**	2295	196.6	14.4	23.2	4.7	17.0	27.3	9.5	2.4
Pinnaroo	**28 May**	2318	223.7	14.1	24.1	3.1	16.5	27.4	9.9	3.2
Roseworthy	**17 May**	3489	302.2	14.2	21.9	2.0	15.9	26.3	8.7	2.5
Winulta	**10 May**	5222	388.2	13.1	20.2	1.0	15.3	24.8	5.4	0.0
	2014									
Angas Valley	**16 May**	3273	138.8	12.5	23.6	0.1	16.8	28.5	12.4	5.8
Booleroo	**19 May**	2969	187.8	14.5	25.9	4.7	19.3	31.5	17.3	8.2
Kaniva	**21 May**	3180	170.2	13.2	24.9	4.5	17.5	29.6	13.8	5.5
Minnipa	**7 May**	3434	227.4	12.5	21.7	0.5	17.0	26.7	6.0	0.8
Pinnaroo	**12 May**	2383	103.8	13.0	23.5	1.7	17.6	29.3	13.5	4.0
Roseworthy	**13 May**	4014	231.6	12.6	23.4	2.4	16.9	28.8	11.5	5.3
Winulta	**14 May**	3957	192.6	12.6	22.0	0.3	16.9	27.2	11.1	2.4
Significance (Pvalue)			*0.0003*	*0.0317*	*0.0054*	*0.0370*	*0.0675*	*0.0012*	*0.0256*	*0.0365*
% variance accounted for			83	37	54	24	22	40	33	34
Grain Yield Effect (kg/ha)			13	-773	-388	-302	-694	-442	-161	-182

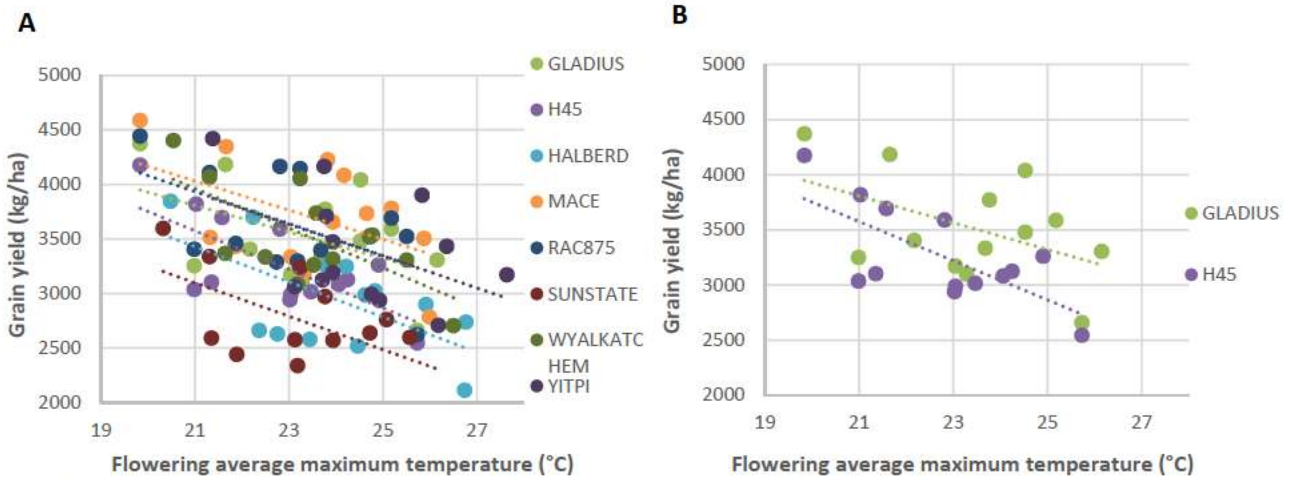

Figure 1 (A) The response for a subset of varieties to increasing average maximum temperature during flowering and (B) examples of contrasting responses by Gladius and H45 to increasing average maximum temperature during flowering.

Identifying the level of heat stress tolerance in Australian germplasm and potentially novel sources of tolerance in unadapted lines or landraces was a key component and outcome of this project. This was principally done using a controlled environment assay where extraneous and confounding factors could be minimized. Stress conditions involved plants being stressed for three consecutive eight hour days, 10 days after the end of anthesis at 36°C and 40 km hr^{-1} winds. This process confirmed that Australian varieties and breeding germplasm have some heat stress tolerance and also identified exotic material with potentially novel sources of tolerance. Lines identified through this process were used to create and identify doubled haploid mapping populations that can be used to identify genetic regions associated with heat stress tolerance. The mapping populations were screened through the controlled environment assay and in the field at multiple locations. The grain yield QTL identified at all field locations for each population is summarised in Table 2. The significance of the linear regressions of the magnitude of effect of each QTL with the climatic co-variates are also included in table 2. Significant interactions of a number of grain yield QTL were found with the heat stress conditions seen in the study. These results are promising and analysis and evaluation of QTL identified in the controlled environment assay and in the field continues unfunded to fully understand their interactions with heat stress related performance and their potential as breeding tools. Analysis is continuing to fully understand the interactions of grain yield and interactions of grain size under heat stress conditions and parallels between the field and the controlled environment assay.

Table 2 Summary of the grain yield QTL found to be significant at all three field locations in 2015, the significance of linear regression of the effects across all three locations with significant climatic co-variates. Also included are postulations of the identity of the QTL.

QTL Name	Population	Chromosome	Region (cM)		Climatic Variable				
						Grain filling			
					May-Oct rainfall	Days >30°C	Days >35°C	Sum of C°d >30°C	Sum of C°d >35°C
HGSMB151-HX46-3B	SCOUT/ MACE	3B	131	P	0.094	ns	0.095	0.058	ns
				% Var	0.980	ns	0.98	0.99	ns
HGSMB151-HX46-5B	SCOUT/ MACE	5B	0	P	ns	0.031	0.294	ns	0.074
				% Var	0.970	1	0.8	0.95	0.99
HGSMB151-HX47-2B	SCOUT/ GLADIUS	2B	50	P	ns	ns	ns	ns	ns
				% Var	ns	ns	ns	ns	ns
HGSMB151-HX47-2A	SCOUT/ GLADIUS	2A	25	P	ns	ns	0.003	ns	ns
				% Var	ns	ns	1	ns	ns
HGSMB151-HX47-2D	SCOUT/ GLADIUS	2D	42	P	ns	ns	ns	ns	ns
				% Var	ns	ns	ns	ns	ns
HGSMB151-HX47-7A	SCOUT/ GLADIUS	7A	66	P	ns	0.011	ns	ns	ns
				% Var	ns	1	ns	ns	ns
HX32151-7B	GLADIUS/ AUS17840	7B	42	P	0.045	0.066	ns	ns	0.008
				% Var	0.990	0.99	ns	ns	1
HX42151-2B	GLADIUS/ AUS17750	2B	56	P	ns	0.021	ns	ns	ns
				% Var	ns	1	ns	ns	ns

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

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

Heat stress and its interactions with the growing environment in the southern environment are extensive and complex. Studies in the field to understand the variation in heat stress tolerance in existing germplasm and implications of heat stress conditions found that not only is heat stress a potentially significant grain limiting factor, but also that genotype interactions with heat stress can vary greatly depending on when the stress occurs relative to developmental stage. Heat stress reduces both grain number (fertility) and grain size (TGW) as a consequence of different mechanisms within the plant being affected. Reductions in grain number due to pollen sensitivity during pollen formation and leading up until seed set, and grain size impacts a result of stress during grain filling increasing leaf senescence, reducing photosynthetic capacity and reduced grain fill duration.

The multiple effects of heat stress on genotype performance make categorizing relative tolerance as a whole more difficult, but from a breeding perspective provide promise that pyramiding tolerance mechanisms has the potential to improve the heat stress tolerance of future varieties for growers.

Seven doubled haploid populations were either made or sourced for this project to understand the underlying genetics of heat stress tolerance. These populations included parents that are exotic landraces with potential novel sources of heat stress tolerance. This material was screened under controlled environment conditions and in field condition in a number of environments in South Australia. This resulted in a number of QTL being identified for both trait performance and heat stress tolerance. Analysis and evaluation is continuing to understand the value of these genetic regions and potential to be utilized as selection tools in breeding. More data is currently being collected to confirm and further understand these relationships and to understand the effects of heat stress on important physical grain quality traits such as grain size and test weight.

Intellectual Property

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

NA

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.

Summary of the main findings and outcomes from the project

The interactions and implications of heat stress on wheat crops was studied, finding strong negative impacts of heat stress both in controlled conditions and in the field. In the field complex interactions between various physiological processes and heat stress at different developmental stages, confirming the complex interactions of heat stress in plants.

A large diverse set of bread wheat genotypes were screened to identify what heat stress tolerance is already present in Australian germplasm, to identify novel or alternative sources of heat stress tolerance and to understand the underlying genetics of heat stress tolerance. Reassuring levels of heat stress tolerance was found in current and historical varieties originating from southern Australia. QTL were found both in controlled environment conditions and the field. Work continues to understand the interactions of various tolerance mechanisms as well as genetic interactions with both grain yield and physical grain quality traits in the field and also the implications of genetic regions identified as interacting with heat stress adaptation and as potential breeding tools.

Potential industry impact

Heat stress has been shown to have considerable negative impacts on wheat production in southern Australia, Australia as whole and internationally. Producing a truly heat stress tolerant variety that not only maintains grain yield but also minimizes physical grain quality defects will be difficult to achieve, but incremental improvements through an increased understanding of heat stress interactions with physiology, adaptation and genetic tools will have very real and significant impacts on improved varieties into the future.

Potential path to market for project outcomes and findings

The results from this project are still evolving and will be published in peer reviewed journal articles allowing the knowledge gained through this project to be conveyed to the whole industry. Additionally, with close links to the AGT wheat breeding programs, this information and tools developed can be integrated into the routine breeding protocols and an improved knowledge of interactions with the environment and adaptation will accelerate the development and release of heat stress tolerant varieties.

Publications and Extension articles delivered as a part of the Project

- Presentation at the AGT open day in November 2012 to agronomists and other industry representatives.
- Presentations by Paul Telfer at the 2013 GRDC grower and adviser updates in Ballarat and Temora.
- Presentation by Paul Telfer to the South Australian Independent Agronomists group in 2013.
- An article published in a Kondinin Group publication in 2013.
- An article published in the GRDC groundcover 2013
- An article published in Eyre Peninsula Farming Systems Summary 2013.
- An article published in the 2013 Mallee Sustainable Farming R & D Compendium.
- Poster presentation made at the July 2013 International Drought Research Conference in Perth.
- Poster presentation made at the Wheat Breeding Assembly in Brisbane in 2013.
- Presentation and article presented at the 2014 Hart Field Day.
- Presentation made to the Crop Science Society (CSS) in 2014.

- Presentation made to the Mid-North High Rainfall Zone (HRZ) winter seminar in 2014.
- Presentation made to Peter Hooper's client group in 2014.
- Presentation made at the AGT open day in 2014.
- Presentation made to the GRDC low rainfall solutions network in Melbourne in 2014
- Presentation made to the GRDC heat stress review in Canberra 2014.
- Presentation and article presented at the 2015 WA Crop Updates in Perth.
- Presentation made to the Durum Growers Association at Roseworthy in September 2015.
- An article published in the Eyre Peninsula Farming Systems (EPFS) 2015 Summary.
- An article published in the Stock Journal, through a SAGIT Initiative discussing the project in 2015.
- A presentation was made to a heat stress research methodology review, a GRDC initiative, in Sydney 2016.
- Presentation to the Upper North Farming Systems group, in 2016

- Additionally, this project has formed the basis of the PhD project being conducted by Paul Telfer. There have been components of this that have involved communication to the University of Adelaide community and Academics.
    - Core component of the structured program involving the presentation of an introductory seminar, literature review and research proposal in 2014.
    - Major review presentation in 2015.
    - Peer reviewed published articles are currently being prepared forming both a part of Paul's PhD thesis but also communication of research results from this project to the scientific community.

POSSIBLE FUTURE WORK

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

Work continues to validate the results presented in this report and evaluate their potential as selection tools in a wheat breeding program. Field trials are occurring unfunded in the 2016 growing season at three locations for five of the doubled haploid mapping populations.

It was also found in this study that there are numerous physiological factors that interact to influence genotype adaption to heat stress. There are opportunities to further dissect the QTL identified and their role in adaptation to heat stress tolerance in the southern Australian environment. This would include a detailed study of a small number of lines that differ only for the QTL being studied in both the controlled environment with stress imposed at a range of growth stages, and also across a wide range of field environments to characterise and provide an accurate value for each QTL.

AUTHORISATION
Name: Dr Haydn Kuchel
Position: Chief Executive Officer & Head of Breeding
Signature:
Date:

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 Wheat Germplasm

SAGIT – AGT Project Technical Report

Prepared by Paul Telfer, James Edwards, Dion Bennett and Haydn Kuchel

1.1 Introduction

Heat stress has been a hot topic in the cereal producing sector in recent seasons with hot and dry conditions prevalent in many areas of Australia and particularly southern Australia. Southern Australia, has 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.

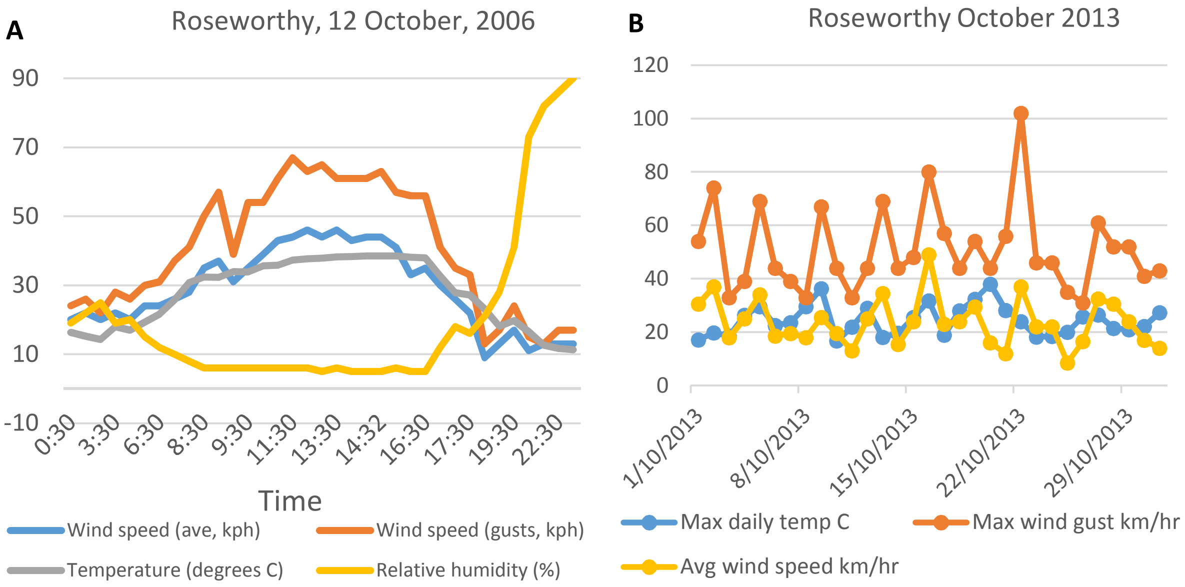

Figure 1 Climatic conditions reflective heat stress conditions in spring in Southern Australia. (A) shows typical daily conditions of a high temperature day indicating the daily cycle of temperature, wind, wind gusts and relative humidity. (B) show the 2-5 cycles of incrementally increasing temperatures, corresponding with increasing wind conditions, the proceeding day is then typified by much calmer and cooler conditions.

High temperature conditions during spring in southern Australia are often typified by 2-5 day cycles of incrementally increasing temperatures, often associated with increased wind speeds (Figure 1). Additionally, days exhibiting high temperature and high wind conditions are generally also very low in relative humidity.

Stressful high temperature conditions are generally of increasing frequency and severity as the season progresses, making stressful conditions during grain filling more common. This is the basis of the rational of focussing the SAGIT-AGT heat stress project around the investigation of heat stress tolerance during grain filling.

Although a lot remains unknown about future climate conditions. There is general agreeance that average temperatures will increase, increasing along with it the increased chance of heat stress conditions (Figure 2), possibly increasing the severity and frequency of the conditions that are already present in the southern environment.

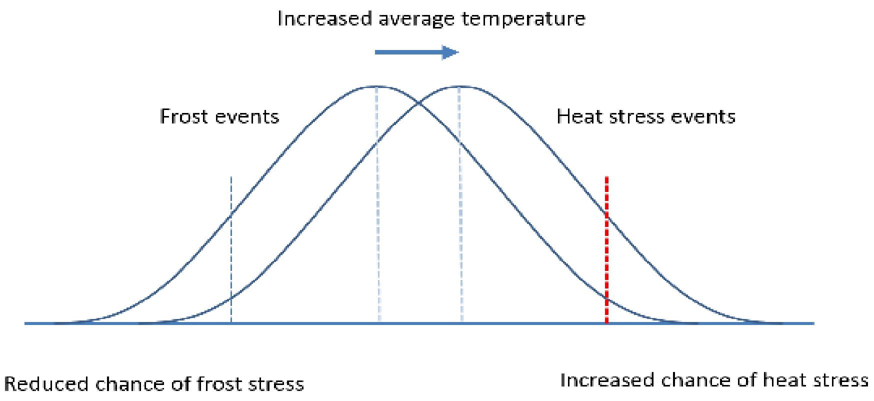

Figure 2 A diagram showing a bell curve of daily temperatures and the potential implications of increased average temperatures, and potential increased occurrence of heat stress conditions (Darren Ray, pers comms).

A desktop study was undertaken at the beginning of the project to establish an understanding of 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 occurring during flowering was higher than grain filling. However, this does not reflect the likely hood of stress occurring within each respective development stage and therefore, total economic impacts.

Table 1 Effect of various climatic variables on grain yield across over 600 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:

- Is there diversity for heat stress tolerance within Australian germplasm and Australian breeding germplasm?
- Is there other novel sources of heat stress tolerance available in exotic germplasm?

- Can a high throughput phenotyping system be developed and used for breeding selection purposes?
- Are there genetic tools that can be developed and used in a breeding program to aid in the selection of increased heat stress tolerance of wheat varieties for Australian and South Australian wheat producers?

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

2.1 The heat chamber, methodologies used and traits measured

Being able to accurately and reliably screen for heat stress tolerance during grain filling was core to the project. Field conditions are unreliable in producing consistent stressful conditions, further complicated by interactions with maturity and timing of stress events confounding interpretation of results. Conducting experiments within a controlled environment allows many of the confounding factors that would otherwise be adversely effecting experiment interpretation, to be controlled. Namely each genotype heat stressed can be stressed at the same growth stage under the same repeatable stressful conditions.

For this project experimental conditions were established to be representative of high temperature spring conditions in southern Australia. As such the targeted experimental conditions were 36°C with 40 km hr^{-1}. These conditions are achieved and maintained using a custom made heat chamber (Figure 3) using thermostatically controlled heating elements for 8 hours a day for three consecutive days. Additionally a fan in a wind tunnel connected to each end of the heat chamber creates a cycle of continuous wind through the chamber. At night the heat chamber is switched off and is allowed to return to the ambient temperature of the greenhouse that the heat chamber is housed in.

The developmental stage targeted in this project was early grain filling. This was based on local knowledge of the southern regions, where heat stress conditions are increasingly prevalent during spring and frequently align with this period. A specific target of 10 days after the end of anthesis was set as the target stress day, to be consistent with other research published including Esten Mason et al (2011). For the duration of the plants lifecycle plants are grown in a randomised split plot design in a greenhouse with plants for both heat stressing and an unstressed control. Plants are managed to optimise plant growth, with irrigation automated to ensure that plants are not drought stressed and aqueous nutrient solution applied routinely to ensure adequate plant nutrition. As plants approach heading and flowering twice weekly observations are taken to measure when anthesis occurs on the primary tiller. 10 days post the end of anthesis plants are removed from their original growing location and placed in the heat chamber for the prescribed heat stress treatment. After which plants are returned to their original location for the remainder of the maturation.

A number of traits are measured both on the plants during their growing phase and on mature harvested plants with a focus on the primary tiller, additional data is collected on secondary tillers on an opportunity basis to gather information of heat stress response at other growth stages. A visual leaf senescence score and leaf chlorophyll levels (with a SPAD meter) are measured before heat stressing, immediately after and finally 10 days post the beginning of the heat stress treatment. This occurs on both the heat stressed plants and unstressed control plants, to quantify any differences in leaf senescence resulting from heat stress. After stressing, plants are allowed to mature before being harvested as whole plants and transferred to the lab for more measurements. Measurements taken in the lab include, number of spikelets per head, number of grain per head, weight of grain per head, weight of primary tiller, weight of intact head, flag leaf length, flag leaf width, peduncle length, thousand grain weight (derived from grain number and grain weight) and head fertility (derived from spikelet number per head and grain number per head) as a measure of grain number set. All measurements are taken on the primary tiller, just measurements involving the head are taken for secondary tillers. All results are analysed using the ASREML statistics package through R.

Figure 3 The heat chamber developed for the current project to produce relevant, controllable and repeatable conditions to screen for heat stress response in plants.

2.2 Basic methodology of field validation and field phenotyping

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.

However, as described earlier, there are a number of drawback of conducting field experiments to understand and identify heat stress tolerance in the field. One method used commonly internationally is to use delayed sowing, involving sowing experiments 1-2 months after optimal, such that plant maturation is occurring in hotter conditions. Although hotter conditions are achieved in such experimental conditions, these conditions are not representative of agronomically conducive conditions, and make interpretation of results difficult. The approach used for this project involved growing experiments with the same genetic material at multiple locations representative of the southern environment but inherently different for the climatic temperature conditions during flowering and into grain filling. Examples of this, and locations used in this project, include Winulta (Central York Peninsular) which has a maritime climate producing moderate temperatures during flowering and grain fill with relatively few frost and heat stress conditions. Contrasting is Angas Valley on the edge of the Murray/Mallee, which generally experiences larger diurnal temperature ranges during flowering and grain filling. Roseworthy generally sits between these two locations in temperatures experienced. Within each location temperature conditions experienced by the experiment are measured on 30 minute intervals. Using time of flowering information collected at Roseworthy and modelled to other locations using a degree day model, the temperature conditions experience by each genotype and each plot can be calculated. Across the environments included in the study, a range of stress conditions can be achieved providing an opportunity to study the impacts of heat stress on an environment level as well as on a genotype level. Alternatively, it provides a data set to understand the genotype by environment (GxE), specifically, the role of heat stress in genotype performance.

For this project, a number of climatic co-variables were calculated for both the flowering and grain filling developmental stages including those described in Table 2. A number of ways of describing the temperature conditions were used to encompass different ways in which the plants may experience heat stress. Although many of the climatic co-variates will be highly correlated, and this needs to be considered in the analysis and interpretation of results.

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

Developmental stage	**Degree day range relative to anthesis**	
Flowering	300°Cd before anthesis to 100°Cd post	
Grain filling	100°Cd post anthesis to 600°Cd post	
Climatic variable	**Abbreviation**	**Explanation**
Average temperature	avgt	
Average maximum temperature	avg_maxt	
Number of hot days	hot_days	Number days >30°C
Number of very hot days	very_hot_days	Number days >35°C

Co Chapman & Zheng

3. Heat stress probe study – understanding heat stress in the controlled environment and in the production environment

During the growing seasons of 2013 and 2014 a number of experiments were conducted with the aim of validating the assay as a tool for understanding heat stress adaptation in the production environment and to further understand the role of heat stress as a limiting factor in wheat production in the southern environment. To achieve this a set of 24 genotypes previously identified as possessing potentially different levels of heat stress tolerance, but still being representative of Australian varieties, were assessed in both the controlled environment assay and in the field at 13 locations.

3.1 Controlled environment results from the probe study

Experiments were conducted in the greenhouse and controlled environment assay as per the protocol previously described in this report. Unfortunately the controlled environment assay was not as informative as previously experienced in the project. Significant treatment effects were found as a result of heat stress in the heat chamber for some traits including a decline in thousand grain weight (TGW) (Table 3). However, few significant genotype by treatment interactions, those that indicate that genotypes are performing differently under the conditions experienced, were identified. There are a number of possible reasons for this. The number of lines selected for the probe study may not be large enough or have a range in response diverse enough to be identified as statistically significant for the heat stress performance traits targeted. Additionally, an improved experimental design was implemented from the 2013 growing season onwards, with a split-plot approach used rather than a blocking by treatment approach. There is a possibility that some of the previous statistical differences identified in the controlled assay experiments had been related to underlying differences associated with block location and associated changed environmental conditions rather than heat stress response *per se*.

Table 3 Significance of traits measured in the controlled environment probe study experiment

Trait	Genotype		Treatment		Genotype treatment interaction	
Fertility	<0.001	***	ns		ns	
TGW	<0.001	***	0.041	*	ns	
HI	<0.001	***	0.073	.	ns	
Flag leaf length	<0.001	***	ns		0.079	.
leaf2	<0.001	***	<0.001	***	0.003	**
spad2	<0.001	***	<0.001	***	0.002	**

3.2 Field results from the probe study conducted during the 2013 and 2014 growing seasons

Over the 2013 and 2014 growing season the same set of 24 genotypes screened in the controlled environment assay were grown in 13 experiments around South Australia. As previously described the climatic conditions were calculated for each plot in each experiment, the experiment mean climatic co-variates are shown in Table 4. Also shown as is the effect and significance of relationships with site mean grain yield across all 13 experiments in the study for a range of climatic co-variates. Linear regression analysis did show strong negative impacts of heat stress on grain yield. Strong year effects were found for some traits, as illustrated in Figure 4. This indicates that there are more complex interactions with environmental factors additional to heat stress *per se* as measured, complicating the interpretation of the impacts of heat stress in the southern Australian environment.

Table 4 Regression analysis results of field trials from 2013 and 2014 across seven locations and thirteen experiments in South Australia and the Wimmera, Victoria. For each climatic parameter, the significance of its correlation with site average yield is shown, along with the effect on grain yield for every one unit change in each climatic parameter.

Site	Sowing date	Grain yield (kg/ha)	May-Oct Rainfall (mm)	Av. temperature °C	Av. maximum temperature °C	No. of days > 30°C	Av. temperature °C	Av. maximum temperature °C	No. of days > 30°C	No. of days > 35°C
	2013			**flowering**	**flowering**	**flowering**	**grain fill**	**grain fill**	**grain fill**	**grain fill**
Angas Valley	**23 May**	1789	165.4	15.2	26.0	6.1	16.1	27.7	8.3	1.9
Booleroo	**17 May**	3119	292.6	15.2	24.7	2.4	16.1	26.3	7.2	2.0
Minnipa	**15 May**	2295	196.6	14.4	23.2	4.7	17.0	27.3	9.5	2.4
Pinnaroo	**28 May**	2318	223.7	14.1	24.1	3.1	16.5	27.4	9.9	3.2
Roseworthy	**17 May**	3489	302.2	14.2	21.9	2.0	15.9	26.3	8.7	2.5
Winulta	**10 May**	5222	388.2	13.1	20.2	1.0	15.3	24.8	5.4	0.0
	2014									
Angas Valley	**16 May**	3273	138.8	12.5	23.6	0.1	16.8	28.5	12.4	5.8
Booleroo	**19 May**	2969	187.8	14.5	25.9	4.7	19.3	31.5	17.3	8.2
Kaniva	**21 May**	3180	170.2	13.2	24.9	4.5	17.5	29.6	13.8	5.5
Minnipa	**7 May**	3434	227.4	12.5	21.7	0.5	17.0	26.7	6.0	0.8
Pinnaroo	**12 May**	2383	103.8	13.0	23.5	1.7	17.6	29.3	13.5	4.0
Roseworthy	**13 May**	4014	231.6	12.6	23.4	2.4	16.9	28.8	11.5	5.3
Winulta	**14 May**	3957	192.6	12.6	22.0	0.3	16.9	27.2	11.1	2.4
Significants (Pvalue)			*0.0003*	*0.0317*	*0.0054*	*0.0370*	*0.0675*	*0.0012*	*0.0256*	*0.0365*
% variance accounted for			83	37	54	24	22	40	33	34
Grain Yield Effect (kg/ha)			13	-773	-388	-302	-694	-442	-161	-182

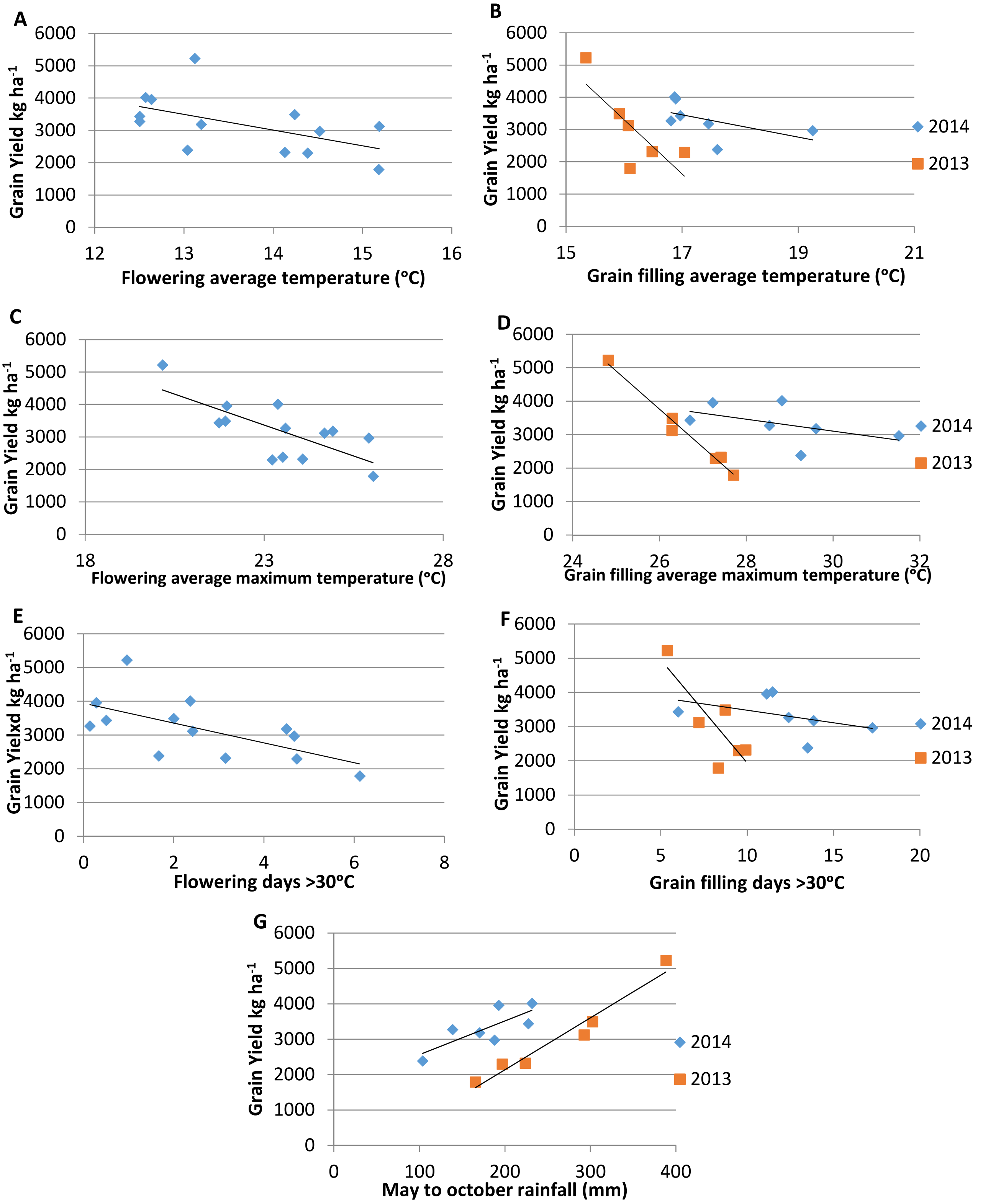

Figure 4 Linear regression relationships of site mean grain yield with (A) flowering average temperature, (B) grain filling average temperature, (C) flowering average maximum temperature, (D) grain filling average maximum temperature, (E) flowering days >30°C, (F) grain filling days >30°C and (G) May to October rainfall.

Analysis was conducted to determine if different genotypes were performing differently in response to the heat stress conditions for grain yield and other physiological traits (Table 5). There were a number of significant relationships found for both grain yield and the other traits measured except for screenings where no significant interactions were found. Some varies were shown to respond to the presence of heat stress at a different rate as shown in Figure 5. In this example Gladius was shown to maintain grain yield under the presence of increasing flowering average maximum temperatures during flowering better than H45. Adding further complexity, it was also shown that different varieties showed different relative performance advantages depending on the when stress occurred. Some varieties showed promising levels of tolerance when stress occurred during grain filling while showing contrasting responses if stress occurred during flowering. This confirmed that interactions of heat stress with different physiological mechanisms is complex, but also indicated the potential of pyramiding tolerance mechanisms together to improve the adaptation to heat stress conditions in the southern Australian environment.

Table 5 The significance of genotype interactions for grain yield and other physiological traits with the climatic co-variates measured in the study.

Climatic co-variate	Grain yield		HLW		Screenings	Fertility		TGW		Head HI	
May to October rainfall	<0.001	***	ns		ns	<0.001	***	0.034	*	0.017	*
Flowering average temperature	0.042	*	ns		ns	ns		ns		ns	
Flowering average maximum temperature	<0.001	***	0.001	**	ns	ns		ns		ns	
Flowering days >30°C	0.078	.	ns		ns	ns		ns		0.015	*
Flowering days >35°C	ns		0.052	.	ns	ns		ns		ns	
Grain filling average temperature	ns		ns		ns	ns		ns		ns	
Grain filling average maximum temperature	0.092	.	ns		ns	ns		ns		0.007	**
Grain filling days >30°C	0.085	.	ns		ns	0.012	*	ns		ns	
Grain filling days >35°C	0.100	.	ns		ns	ns		ns		0.043	*

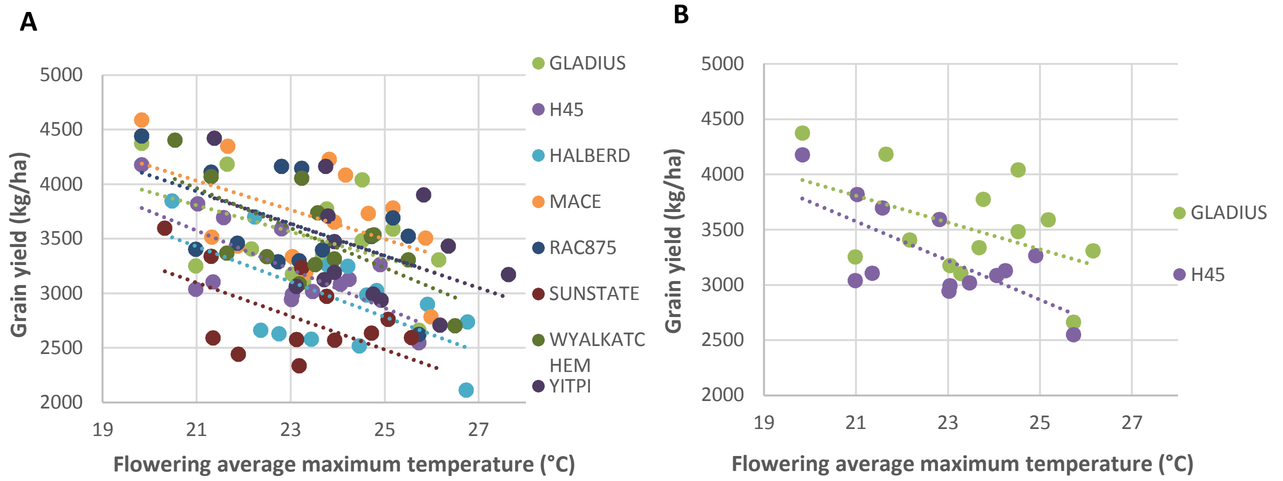

Figure 5 (A) The response for a subset of varieties to increasing average maximum temperature during flowering and (B) examples of contrasting responses by Gladius and H45 to increasing average maximum temperature during flowering.

4 Genetic diversity for heat stress tolerance

4.1 Identifying genetic diversity in heat stress tolerance – Australian germplasm and beyond

Over the course of this project 1944 genotypes were screened through the controlled environment assay (described in 1.3), equating to 7104 pots over 11 experiments. This material was made up of a diverse set of Australian varieties, both current and historical, international varieties of interest, AGT breeding lines, exotic material and genetic mapping populations.

The initial stages of the project were primarily targeted at characterising the level of heat stress tolerance in Australian and international varieties, to understand the range in genetic variation for heat stress tolerance and the level of adaptation to heat stress tolerance already present in Australian varieties. Promisingly it appears that Australian varieties both recent and historical, have useful levels of heat stress tolerance. As can be seen in Figure 7 the thousand grain weight and fertility for a selection of Australian varieties is shown. Halberd along with others, was shown to express promising levels of heat stress tolerance.

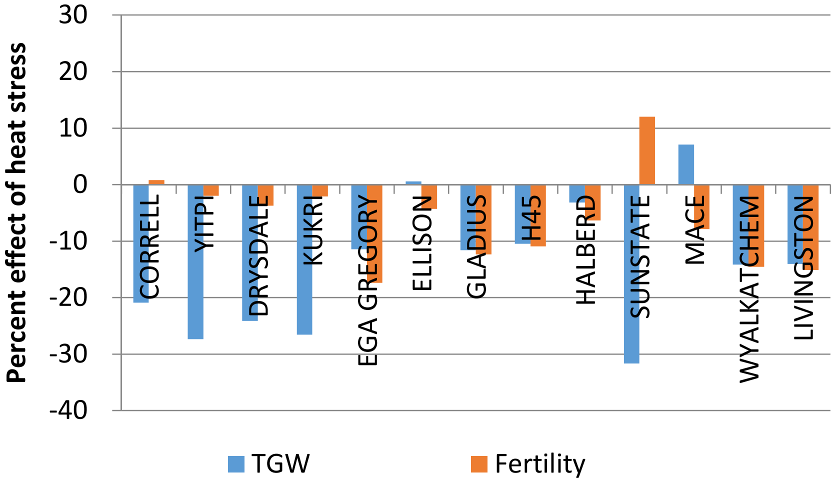

Figure 7 Fertility and thousand grain weight (TGW) response as a result of a heat stress treatment in the controlled environment assay for a selection of Australian varieties expressed as the percentage of the unstressed control.

4.2 Exotic and potentially novel sources of heat stress tolerance

Potently novel sources of genetic tolerance to heat stress can be found in genetic sources that have not undergone selective breeding in the Australian environment where heat stress is a characteristic of our growing environment. This can be done through targeting varieties or landraces from areas in the world where the targeted stress is prevalent and natural selection or human selection may have developed alternative sources of tolerance. To identify such novel tolerance and genetics a Focused Identification of Germplasm Strategy (FIGS) was used (El Bouhssini et al. 2011). For this project a set of 330 exotic genotypes were selected from areas of the Middle East where late season heat stress similar to that experienced in southern Australia, is common. All of these lines were screened through the controlled environment assay to identify lines that expressed tolerance to the yield determining traits fertility and thousand grain weight. As shown in Figure 8 there were lines expressing various levels of tolerance to both fertility and thousand grain weight.

Lines identified as possessing potentially useful levels of heat stress tolerance were selected and crosses made to a selection of adapted Australian varieties for further investigation including creating doubled haploid mapping populations discussed later in this report.

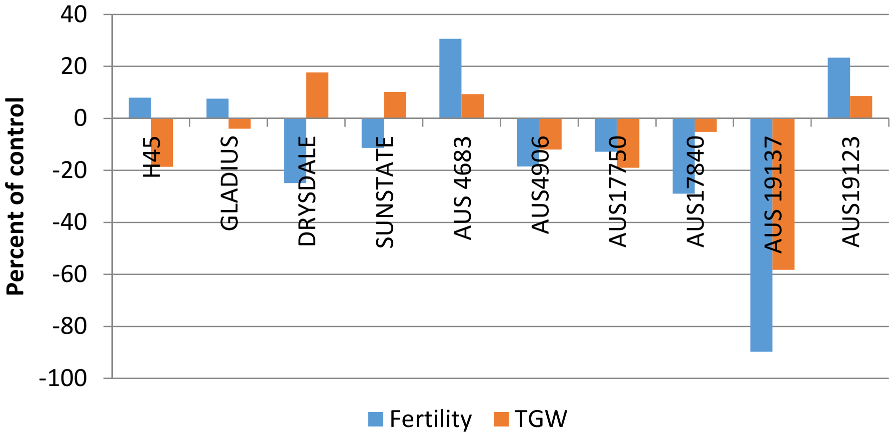

Figure 8 Fertility and thousand grain weight response as a result of a heat stress treatment in the controlled environment assay for a selection of FIGS lines expresses as a percentage of the unstressed control treatment.

5. Other findings of project

5.1 Growth stage interactions with heat stress responses

Principally the data gathered in the controlled environment experiments is targeting the primary tiller, ten days after the end of anthesis. Other tillers at different growth stages when put into the heat chamber offer an opportunity to understand phenotypic responses to heat stress at different developmental stages. Given the opportunity basis of secondary tillers, it is very difficult to get a balanced dataset representing a range of development stages to allow genotypic comparisons in performance at the various growth stages. However, using the dataset can be used in its entirety to understand the relative differences in performance at key developmental stages.

The traits principally targeted to evaluate tolerance in controlled environment experiments in this project have been fertility and thousand grain weight. These are not only key yield determine traits that carry relevance to field conditions, they also reflect physiological process in the plants that are known to be susceptible to heat stress and abiotic stress in general; the number of seed set and grain filling ability. The response in fertility and thousand grain weight at a range of growth stages relative to the unstressed control treatment is shown in Figure 9. At earlier growth stages fertility or grain number set was adversely affected with the magnitude of negative impacts on fertility declining until the end of flowering. The response in thousand grain weight is contrasting, with strong positive impacts on grain weight at earlier growth stages, when there are fewer grains to fill as a result of the reduction in fertility. This response is not surprising, with a number of studies showing that if heat stress occurs during pollen formation, the impacts on pollen viability and the final seed number set can be very significant. Strong negative impacts on gran weight were evident at later growth stages, particularly after the end of flowering and during grain filling.

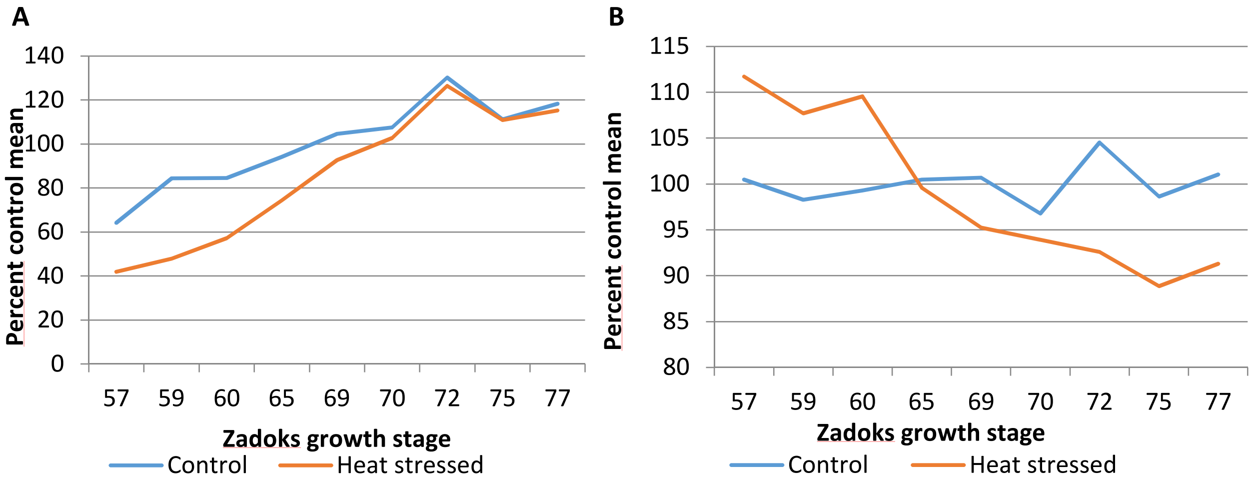

Figure 9 the response of (A) fertility and (B) thousand grain weight at a range of growth stages to heat stress compared to the unstressed control captured in controlled environment conditions.

To further understand this relationship an experiment was designed to target a range of known sensitive growth stages from booting to target pollen formation, through heading, flowering up until early grain filling the normal stage of stressing for this project. As is shown in Figure 10 the relationship for decreasing sensitivity to the heat stress conditions imposed on fertility or grain number set declined from booting through flowering to early grain filling. Unfortunately the heat stressing was so successful that grain number was reduced in this experiment that there was insufficient data available to analyse the response to timing of heat stress to thousand grain weight.

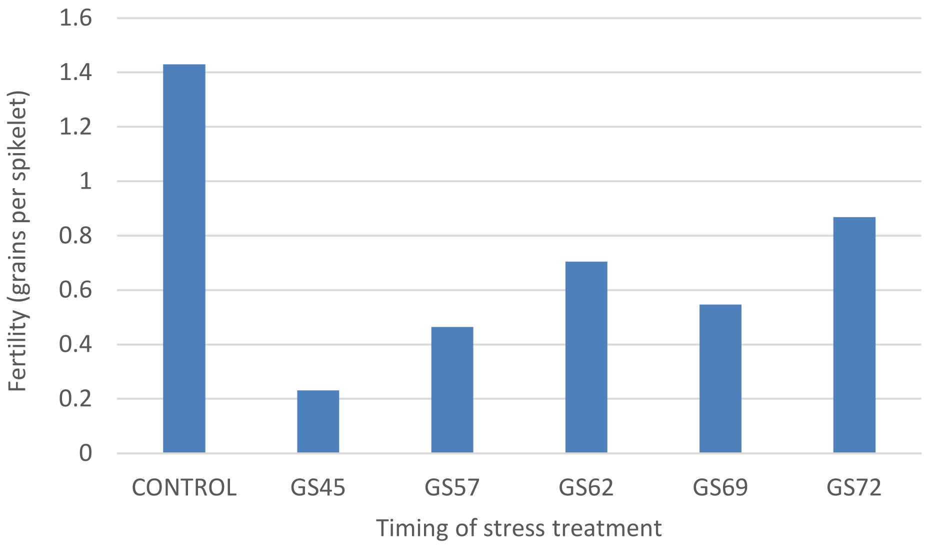

Figure 10 The fertility (grains set per spikelet) at a range of developmental stages ranging from booting to through to early grain filling.

6. Development of genetic mapping populations

The early stages of the SAGIT/AGT heat stress project involved screening a range of germplasm (Australian varies, AGT breeding lines, and exotic germplasm) to identify if there was variation in heat stress tolerance, and if there was potently novel sources of tolerance that could be introgressed into Australian germplasm to improve the heat stress tolerance of Australian varieties. Once variation was identified doubled haploid mapping populations were developed to understand the genetics responsible for the heat stress tolerance and to identify the locations of genes to allow the development of genetic tools to aid in breeding.

Following the screening of a large set of diverse germplasm six doubled haploid genetic mapping populations were created, with an additional existing populations sourced. These are as follows;

- Three bi-parental populations made from the three adapted parents, Gladius, Scout and Mace (Referred to as the GSM populations) – three parents showing different heat tolerance characteristics in the controlled environment assay and also represent different pedigree backgrounds from southern Australian plant breeding programs.
- RAC1548/GLADIUS – RAC1548, an AGT breeding line that showed promising results in the controlled environment assay.
- AUS17840/GLADIUS – AUS17840, an exotic introduction showing promising results in the controlled environment assay.
- AUS17750/GLADIUS – AUS17750, an exotic introduction showing promising results in the controlled environment assay.
- HALBERD/KENNEDY – Halberd was identified as a heat stress tolerance variety in this study as well as previous studies (Mason et al. 2010; Mason et al. 2011). This population was sourced from Dr Daryl Mares, University of Adelaide.

7. Heat chamber phenotyping of genetic mapping populations

7.1 GSM

No heat treatment by genotype QTL were identified in the greenhouse controlled environment assay, a disappointing result. However, QTL for traits believed to be important for tolerance to heat stress and are also important for grain yield determination were identified. QTL identified confer that a genetic region is associated with trait performance, and in this instance that the performance advantage of a particular allele was retained in the presence of heat stress. A number of these QTL were closely located to and likely are, known phenology genes. A number of the identified QTL for thousand grain weight appear to be associated with photoperiod genes, including Ppd-B1 on chromosome 2B. Additionally FT/Vrn3 loci appear to have been identified on chromosome 7A and 7D, although these genes are known to influence vernalisation requirements they also have a role in photoperiod requirements. Interactions of developmental genes and flowering times genes with many yield determining plant attributes is somewhat understood, with various developmental genes interacting to determine tillering number, spikelet number, grain number and also grain size, measured as thousand grain weight in this study. However, additionally interesting in this case is that with the assay used, and heat stress occurring at a fixed time relative to anthesis (ten days post the end of anthesis), the role of the identified QTL can be thought to be independent of heat stress influence. Something that in field conditions may be an important factor.

7.2 RAC1548/GLADIUS

Similarly to the GSM populations there was no heat by genotype tolerance tolerance QTL identified, main effect QTL were identified.

7.3 AUS17840/GLADIUS exotic population

In the controlled environment assay screen of the AUS17840/GLADIUS exotic population, genotype by heat stress treatment interactions were found to be significant for both traits of interest, fertility and thousand grain weight. This indicates that there are regions in the genome that confer different levels of performance under the different stress conditions and therefore potential to tolerate the stresses imposed to a better degree.

Analysis is still continuing in conjunction with statisticians to quantify the significance and magnitude of effect of putative QTL. Involved with this process is understanding the fit of putative QTL with those currently mentioned in literature and those known to be related to other traits. Preliminary results are shown in Table 6. This includes main effect QTL, those related to the overall performance of the trait regardless of the treatment imposed, and tolerance QTL, signalling a difference in trait performance in response to the stress imposed. Although this is an area of continued exploration, there are a number of interesting genetic regions for both trait performance but also for tolerance, including a number of QTL that are playing a role for both. Promisingly there were favourable QTL identified with the improved phenotype associated with the exotic source, AUS17840, not just the adapted parent Gladius.

Table 6 Summary of main effect and tolerance QTL identified in the AUS17840/GLADIUS (HX32) population for thousand grain weight and fertility in the controlled environment assay. Main effect and tolerance QTL for each trait that are potential collocated are aligned in the table.

Trait	Main Effect				Tolerance Interaction			
	Chromosome	**Favourable parent**	**Effect**	**Position cM**	**Chromosome**	**Favourable parent**	**Effect**	**Position cM**
TGW (grams per 1000 grains)	2A	AUS17840	2.5	0				
	2B	AUS17840	1.5	150				
					2D-1	AUS17840	0.3	50
					2D-2	GLADIUS	0.3	175
					3A	GLADIUS	0.3	100
	3B	GLADIUS	1	100	3B	GLADIUS	0.3	75
					4A	AUS17840	0.35	50
	4D-2	GLADIUS	1.5	100-150				
	5A-1	GLADIUS	1.5	50				
	5A-2	AUS17840	1.5	120				
					5B	GLADIUS	0.35	50-75
					5D	GLADIUS	0.35	25
	6A	GLADIUS	1.5	50				
	6B	GLADIUS	1.5	0				
	6D	GLADIUS	2.5	150	6D	GLADIUS	0.25	150
					7A-1	GLADIUS	0.25	25
					7A-2	AUS17840	0.25	100
	7B	GLADIUS	2	0				
					7B	AUS17840	0.3	50
					7D	GLADIUS	0.3	175
	Chromosome	**Favourable parent**	**Effect**	**position**	**Chromosome**	**Favourable parent**	**Effect**	**position**
FERTILITY (grains per spikelet)	1A	AUS17840	0.15	25	1A-1	AUS17840	0.0375	0
					1A-2	GLADIUS	0.03	75
	1D	GLADIUS	0.15	75				
	2A	GLADIUS	0.2	75	2A	GLADIUS	0.025	75
	2D-1	AUS17840	0.2	25				
	2D-2	AUS17840	0.275	150	2D	AUS17840	0.0275	150
	3B-1	GLADIUS	0.15	50	3B-1	GLADIUS	0.03	0-50
					3B-2	GLADIUS	0.03	150
	3B-2	AUS17840	0.175	75-100				
					5B	GLADIUS	0.03	0
	5B	GLADIUS	0.175	150				
					5D	GLADIUS	0.03	0
	5D	GLADIUS	0.175	200				
	6A	AUS17840	0.2	50-75				
	6B	AUS17840	0.175	50-100	6B	AUS17840	0.03	50
					6D	AUS17840	0.0275	75
	7A	GLADIUS	0.2	25	7A	GLADIUS	0.0275	25-75
					7B	AUS17840	0.03	0-25
	7D	AUS17840	0.2	200	7D	GLADIUS	0.0425	125-175

8. Field phenotyping of genetic mapping populations

The set of doubled haploid mapping populations (with the expectation of the HALBERD/KENNEDY), were bulked up in the field in the 2014 growing season and grown at multiple field locations (Angus Valley, Roseworthy and Winulta) in the 2015 growing season using the protocol described in 2.2, totalling 5,880 yield plots across the three locations.

QTL analysis was conducted for each population at each location for grain yield. A number of QTL were identified within most populations, totalling 90 across all of the populations and experiments. Efforts were focused on QTL that were identified as significant within all environments for each population. As shown in Table 7, eight QTL were found to be significant at all three environments. Using the magnitude of effect of each of the QTL from each environment and regressing this against the site mean of each climatic co-variable, the effect of the grain yield performance QTL in the presence of changing stress conditions could be quantified. As is shown in Table 7 and Figure 11, a number of the QTL targeted for further investigation showed associations with some of the climatic co-variables, namely the number of days in excess of 30ºC and 35ºC and the accumulated thermal time on days that exceeded 30ºC and 35ºC. These results are very promising and further investigation is likely to add more value to these results. Additionally a set from each population is being grown in the same locations again in the 2016 growing season. This will give a total of six environments to evaluate the performance and value of the QTL.

Analysis of results of thousand grain weight and HLW from the 2015 yield trials as currently occurring. It is hoped that this will add value to the promising QTL for grain yield identified in the field and the thousand grain weight QTL found in the controlled environment assay. Grain yield stability under heat stress conditions is of utmost importance to the ability of plant to be adapted heat stress conditions. However, grain size stability is also incredibly important as small reduction in grain size as a result of heat stress can have significant impacts on the value of deliverable grain.

Promisingly it appears that a tolerance QTL identified in the AUS17840/GLADIUS population on chromosome 7B in the controlled environment assay potentially conferring thousand grain weight heat stress tolerance to heat stress, was also have been identified as a grain yield QTL in the field study conducted in 2015.

Further opportunities exist to use other QTL identified that were not found to be significant at all three locations, and to understand their association with the climatic co-variates measured in each environment. Evaluation of other traits of interest is currently underway, and a second year of field data will help validate these putative QTL.

Table 7 Summary of the grain yield QTL found to be significant at all three field locations in 2015, their effect on yield and the significance of linear regression of the effects across all three locations with significant climatic co-variates. Also included are postulations of the identity of the QTL.

QTL Name	Population	Chromosome	Region (cM)	EXPT	Angus Valley	Roseworthy	Winulta		Climatic Variable					Postulations
										Grain filling				
									May-Oct rainfall	Days >30°C	Days >35°C	Sum of C°d >30°C	Sum of C°d >35°C	
HGSMB151-HX46-3B	SCOUT/ MACE	3B	131	Effect kg/ha	-127.7	-202.2	-234.5	P	0.094	ns	0.095	0.058	ns	
				LOD	2.4	3.0	5.0	% Var	0.980	ns	0.98	0.99	ns	
HGSMB151-HX46-5B	SCOUT/ MACE	5B	0	Effect kg/ha	151.7	179.8	179.4	P	ns	0.031	ns	ns	0.074	
				LOD	3.9	2.7	3.1	% Var	0.970	1	ns	ns	0.99	
HGSMB151-HX47-2B	SCOUT/ GLADIUS	2B	50	Effect kg/ha	-181.2	-356.7	-219.6	P	ns	ns	ns	ns	ns	Ppd-B1
				LOD	30.6	61.2	16.6	% Var	ns	ns	ns	ns	ns	
HGSMB151-HX47-2A	SCOUT/ GLADIUS	2A	25	Effect kg/ha	-72.8	-102.6	-125.2	P	ns	ns	0.003	ns	ns	
				LOD	5.1	5.5	6.0	% Var	ns	ns	1	ns	ns	
HGSMB151-HX47-2D	SCOUT/ GLADIUS	2D	42	Effect kg/ha	172.5	444.5	271.9	P	ns	ns	ns	ns	ns	Ppd-D1
				LOD	27.6	91.4	26.0	% Var	ns	ns	ns	ns	ns	
HGSMB151-HX47-7A	SCOUT/ GLADIUS	7A	66	Effect kg/ha	-71.5	-128.4	-123.4	P	ns	0.011	ns	ns	ns	FT/Vrn3
				LOD	4.7	7.9	5.6	% Var	ns	1	ns	ns	ns	
HX32151-7B	GLADIUS/ AUS17840	7B	42	Effect kg/ha	-275.3	-341.4	-361.3	P	0.045	0.066	ns	ns	0.008	FT/Vrn3
				LOD	20.0	11.2	19.1	% Var	0.990	0.99	ns	ns	1	
HX42151-2B	GLADIUS/ AUS17750	2B	56	Effect kg/ha	-275.9	-478.9	-423.8	P	ns	0.021	ns	ns	ns	Ppd-B1
				LOD	25.9	46.9	35.9	% Var	ns	1	ns	ns	ns	

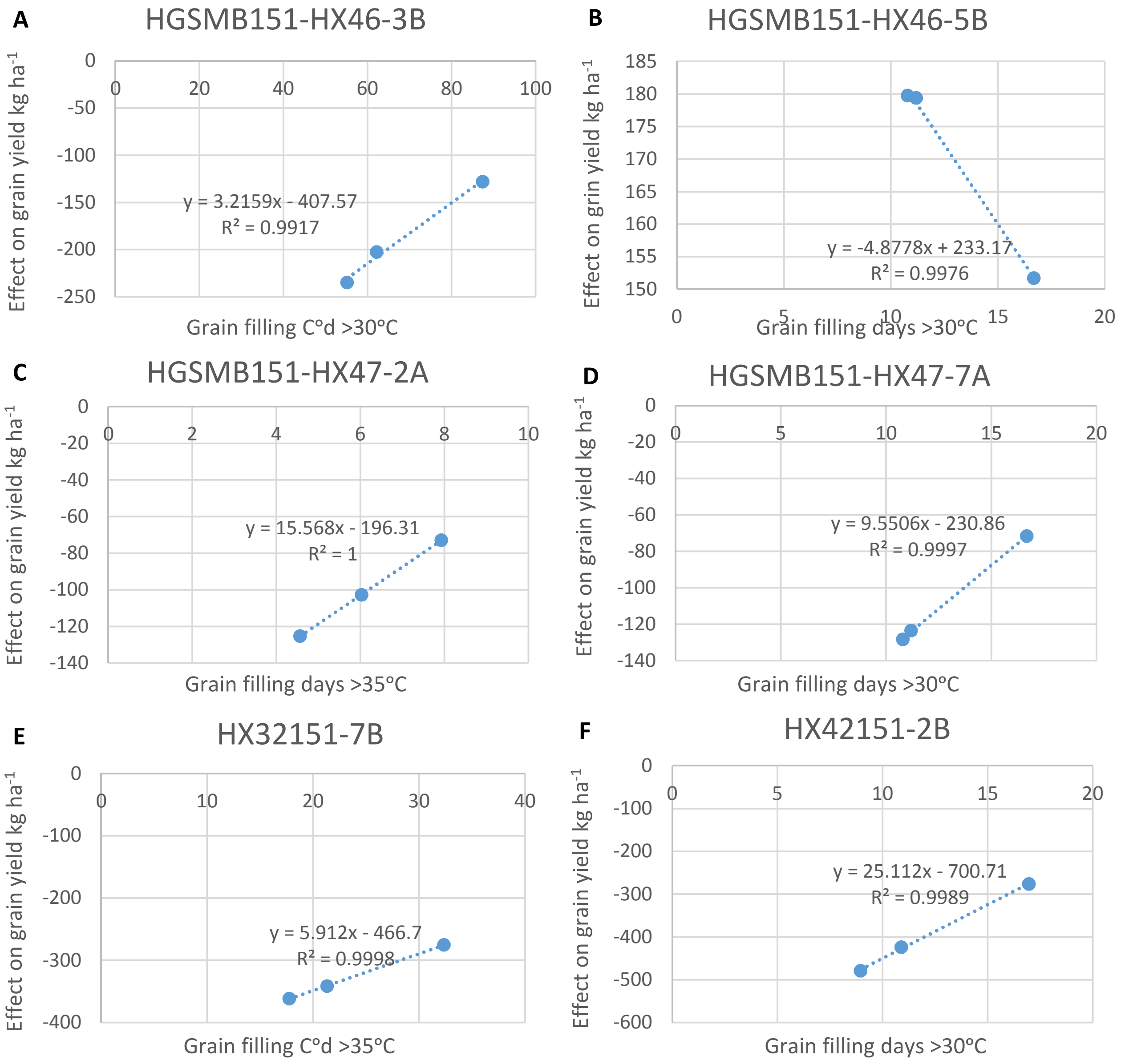

Figure 11 The linear regression relationships of QTL effects from each of the environments and site mean climatic co-variates for (A) HGSMB151-HX46-3B and accumulated thermal time >30°C, (B) HGSMB151-HX46-5B and grain filling days >30°C, (C) HGSMB151-HX47-2A and grain filling days >35°C, (D) HGSMB151-HX47-7A and grain filling days >30°C, (E) HX32151-7B and accumulated thermal time >30°C, and (F) HX42151-2B and grain filling days >30°C.

9. Value of field/genetic traits identified

Quantifying the value of genetic and phenotypic traits is difficult as there are often a number of confounding factors such as environmental interactions that make evaluation and interpretation difficult. However, there is also a lot of value in quantifying the value of traits as it helps researchers and breeders understand the importance and implications of a trait. In this project a number of QTL were found both in the controlled environment assay and in the field. Although analysis and evaluation of the germplasm and QTL already identified is continuing, with more data being added, we can start to gauge the effect of some of the QTL identified. For QTL identified solely in the controlled environment assay, the field value is difficult to confer to the field, as the conditions although intended to be representative of the field, still are a contrived environment and assessed on a single plant basis. The method used for the QTL analysis in the field experiments produces an effect of that QTL in each environment, giving it relevance to real growing environments. In this instance we were able to show that there were significant interactions with heat stress conditions across the environments with apparent significant interactions with grain yield. Although these interactions appear significant and could have lasting impacts going forward, it is prudent to wait for further data before commenting too heavily on the value of these traits.

As mentioned the analysis of thousand grain weight and HLW results from the 2015 yield trails is currently occurring. It is hoped that this will help tie the results seen in the controlled environment assay together with that seen in the field. The link between grain size and grain yield is not only important from a grain yield determining point of view but also from an economic grain value point of view. Previous data collected in this study has shown the promising ability of the controlled environment assay to explain variation in grain yield in field conditions.

10. Potential of genetic outputs/genetic tools

In this project a number of QTL were identified from both controlled environment testing and in the field as already described in this report. There is potential that breeding tools can be developed out of this project although further data currently being collected will confirm if this is the case. The populations of interest have been planted again funded by AGT in the 2016 growing season allowing another three environments to be added to the dataset.

In addition to the work which is still in progress there may be opportunities to learn further about the key QTL identified and how they interact with yield determination in plants and associated physiology. A set of lines selected for the presence and absence of the QTL of interest can be used for a targeted detailed study to facilitate in depth research into heat stress interactions both using controlled environment assays and in the field to understand interactions with stress adaption and grain yield over a wide range of environments. It was shown that different genotypes can show significantly different levels of adaption depending on when stresses were imposed, understanding the finer details of key QTL and physiological traits will further our understanding of these complex plant interactions to facilitate combining QTL for better adapted varieties for southern Australian environments.

11. References

El Bouhssini, M. Street, K. Amri, A. Mackay, M. Ogbonnaya, F. C. Omran, A. Abdalla, O. Baum, M. Dabbous, A. & Rihawi, F. (2011). Sources of resistance in bread wheat to Russian wheat aphid (Diuraphis noxia) in Syria identified using the Focused Identification of Germplasm Strategy (FIGS). *Plant Breeding* **130**(1), 96-97.

Mason, R. E. Mondal, S. Beecher, F. W. & Hays, D. B. (2011). Genetic loci linking improved heat tolerance in wheat (Triticum aestivum L.) to lower leaf and spike temperatures under controlled conditions. *Euphytica* **180**(2), 181-194.

Mason, R. E. Mondal, S. Beecher, F. W. Pacheco, A. Jampala, B. Ibrahim, A. M. H. & Hays, D. B. (2010). QTL associated with heat susceptibility index in wheat (Triticum aestivum L.) under short-term reproductive stage heat stress. *Euphytica* **174**(3), 423-436.