Office Use Only

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	:	**UA1415**

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

PROJECT DURATION

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

Project Start date	1st July 2015					
Project End date	30th June 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

Title:	**First Name:**	**Surname:**
A/Prof	Jason	Able

Organisation:
The University of Adelaide

Mailing address:

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:**
Ms	Chelsea	DuBois

Organisation:
The University of Adelaide

Mailing address:

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

- This project has resulted in the generation of new knowledge based on the durum heat tolerance trials conducted over two seasons (2015/2016).
- These trials were conducted using the AGT-SAGIT Heat Chamber at Roseworthy.
- Selected durum germplasm was screened through the heat chamber using an assay that AGT developed, which targets post-anthesis heat stress (plants stressed for three consecutive eight hour days, 10 days after the main tiller finishes anthesis at 36°C with 40 km hr^{-1} winds).
- Across two seasons of experiments, we have identified new sources of heat tolerant durum parents that will contribute towards future variety development.
- In the medium-long term, and with the release of improved heat tolerant durum varieties, this will deliver enhanced productivity to growers who sow durum.

Project Objectives

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

This project aimed to and achieved the following outcomes:

1. Screening of elite, selected entries (in addition to recently released varieties) from Durum Breeding Australia's (DBA) Southern Node Breeding Program against leading bread wheat varieties in heat stress trials conducted with the AGT-SAGIT Heat Chamber;
2. Comparing and evaluating the relative heat stress tolerance and the physiological responses of the selected durum entries to the benchmark bread wheat varieties;
3. Identifying elite heat stress tolerant durum parents for further exploitation through breeding by using these materials in future crossing blocks; and;
4. Dissemination of the heat chamber trial results by updating growers (and consultants/advisors) at forums in 2016 and 2017 and field day events in both 2015 and 2016 at the heat chamber site (Roseworthy).

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 project objectives were achieved on time and within budget. This was largely due to the excellent collaboration with AGT and its staff members (particularly Paul Telfer). Data was received in a timely fashion so that analysis could be completed on time. This has been a value-adding project to the southern DBA breeding program that operates out of the University and which is largely funded through the GRDC and San Remo.

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 selected elite durum entries, benchmarking against lead bread wheat varieties	Y (years 1 and 2)	
Validate chamber methodology in durum	Y (year 1)	
Publish trial results for the SADGA website	Y (years 1 and 2)	
Annual progress report submitted to SAGIT	Y (years 1 and 2)	
Final report to SAGIT	Y (this file)	

Technical Information (Not to exceed <u>**three**</u> pages)

Provide sufficient data and short clear statements of outcomes.

INTRODUCTION

For notes on the experimental design, the data measurements recorded and the analysis conducted, please refer to UA1415 Supplementary File.pdf.

GERMPLASM SCREENED & DESIGN

- 31 selected entries from Durum Breeding Australia's Southern Program (S4 and S3 – all advanced germplasm potentially ready for progression to pre-variety release trials).
- 7 durum varieties including the latest variety released – DBA-Aurora. Other varieties were Yawa, WID802, Tjilkuri, Tamaroi and the two released AGT varieties, Hyperno and Saintly.
- 2 bread wheat varieties as checks (Halberd – identified by AGT as heat tolerant, and Wyalkatchem – identified by AGT as heat intolerant).
- Split-plot design with adjoining split-plots aligned in adjoining ranges. Three replicates, replicated by block (see Figure 1 for example layout).

Figure 1: Example trial design layout for the heat stress experiments (2015-2016).

GENERALISED SUMMARY OF HEAT TRIAL RESULTS

- Recorded measurements for traits including grain number, grain weight, head weight, spikelet number and thousand grain weight (TGW) resulted in significant differences being identified.
- These differences were either identified between genotypes and/or between the treatments (control vs heat/wind stressed).
- The results from both years (2015 and 2016) resulted in the identification of some potential heat tolerant germplasm (as seen in representative image depicted as Figure 2).
- Results of several key indicators for heat tolerance (e.g. TGW and HHI) in varieties such as the recently released variety DBA-Aurora and advanced entries including UAD1151101 and UA1151125 look encouraging after the two seasons of experimentation.
- The research findings from this SAGIT sponsored project will play an important role in selecting suitable parental combinations for future crossing blocks, which may lead to the development of new heat tolerant durum varieties 8-10 years from now.

Figure 2: Different durum germplasm showing clear significant spike differences in their response to the heat/wind treatment. The entry on the left shows significant desiccation/dis-colouration when compared to the entry on the right.

THOUSAND GRAIN WEIGHT (TGW) HIGHLIGHTS

- The latest variety, DBA-Aurora, which under non-limiting conditions (control) has exceptional TGW; recorded a 21% loss (in 2015 this was 22%, so consistency between the two experimental seasons is present).
- However, older varieties such as Tamaroi (when under non-limiting conditions also has very good TGW) recorded a 49% loss between the control (62.56 g) and heat/wind stress (31.69 g) treatments. In 2015, this loss was again very similar for this variety (at 46% loss).
- Several advanced breeding lines (listed below as examples) were identified that show lower reductions than DBA-Aurora or even small increases (not significant) when comparing treatments (control vs heat/wind stress). Comparisons to the heat tolerant bread wheat variety, Halberd, can also be made (13.07% loss between control vs heat/wind stress – 2016 data).
- The entries UAD1151101 (57.18 g control vs 58.43 g heat/wind stress in 2015; 56.51 g control vs 50.61 g heat/wind stress in 2016) and UAD1151112 (46.15 g control vs 47.40 g heat/wind stress in 2015; 53.19 g control vs 47.55 g heat/wind stress in 2016) are promising entries when using TGW as a heat stress indicator.
- In addition, the lines UAD1152020 showed only an 11% (2015) or 7% (2016) loss between the control and heat/wind stress treatments, while UAD1153177 showed only an 8% (2015) and 6% loss (2016).
- The results of these four advanced breeding lines above (UAD1151101, UAD1151112, UAD1152020 and UAD1153177) are all showing reduced loss when compared to the heat tolerant Halberd variety.
- Old varieties such as Tamaroi displayed very low TGW and were significantly impacted by heat stress (see Table 1).
- UAD1154192 which was entered into the 2016 and 2017 NVT trials displayed exceptionally large TGW (even superior to DBA-Aurora) with a value of 70.47 g in the control treatment (2016 data). However, under stress this line lost 41.17%, with a TGW of 41.46 g post heat/wind stress (2016). While this line may still be released as a variety in 2018, it certainly does not appear to be heat-tolerant, with Wyalkatchem (intolerant bread wheat check) only losing 29.11% of its TGW after stress (2016). Similar results can be seen from the 2015 summary data (see Table 1).

HEAD HARVEST INDEX (HHI) HIGHLIGHTS

- Significant differences between the control and heat/wind stress treatments were identified for HHI (grain weight divided by total weight of the intact head) across several entries.
- Compared with Halberd (3.7% gain) and Wyalkatchem (11.8% loss), several entries such as UAD1151108 (1.4% gain), UAD1151125 (2.2% gain) and UAD1153021 (4.7% gain) performed better than all other durum entries with an improved HHI when stressed.
- Varieties such as DBA-Aurora (9% loss) still performed better than Wyalkatchem but were not as superior as Halberd, while Saintly (22.6% loss) and Tamaroi (31.3% loss) were two poor performing varieties.
- However, the stand out entry again was UAD1151101. This line showed a small (2% in 2015; 0.5% in 2016) increase between the HHI obtained for the control vs heat/wind stress treatments, implying that grain weight and the other physical attributes of the spike (e.g. awns, glumes, etc.) were not affected by the heat/wind stress.
- Based on the results with the TGW (and other positive attributes) of the UAD1151101 line, it is an entry that will be further investigated as a parent (and potential variety in its own right during the 2017 season and beyond).

	TGW		HHI	
	2015	2016	2015	2016
DBA Aurora	-21.9 ↓	-20.9 ↓	-16.8 ↓	-9.4 ↓
HALBERD	-14.4 ↓	-13.1 ↓	-12.0 ↓	3.7 ↑
HYPERNO	-5.8 ↓	-22.4 ↓	-24.3 ↓	-6.4 ↓
SAINTLY	-20.0 ↓	-25.9 ↓	-26.2 ↓	-22.6 ↓
TAMAROI	-46.2 ↓	-49.3 ↓	-46.7 ↓	-31.3 ↓
TJILKURI	-21.7 ↓	-23.0 ↓	-28.6 ↓	-9.9 ↓
UAD1151024	-2.8 ↓	-10.9 ↓	-10.9 ↓	-3.1 ↓
UAD1151046	-8.5 ↓	0.3 ↑	-29.0 ↓	-8.9 ↓
UAD1151054	-18.5 ↓	-35.1 ↓	-52.4 ↓	-25.4 ↓
UAD1151056	-34.4 ↓	-19.8 ↓	-43.9 ↓	-19.0 ↓
UAD1151063	-39.2 ↓	-37.8 ↓	-63.1 ↓	-17.0 ↓
UAD1151064	-21.5 ↓	-24.9 ↓	-27.9 ↓	-7.2 ↓
UAD1151083	-15.2 ↓	-14.9 ↓	-34.9 ↓	-7.9 ↓
UAD1151096	-37.6 ↓	-33.7 ↓	-50.8 ↓	-31.8 ↓
UAD1151097	-21.6 ↓	-11.8 ↓	-39.1 ↓	-8.2 ↓
UAD1151101	2.2 ↑	-10.4 ↓	2.4 ↑	0.2 ↑
UAD1151104	-41.6 ↓	-38.6 ↓	-48.2 ↓	-18.1 ↓
UAD1151108	-39.8 ↓	-19.4 ↓	-23.7 ↓	1.5 ↑
UAD1151111	-22.1 ↓	-9.8 ↓	-37.8 ↓	-12.5 ↓
UAD1151112	2.7 ↑	-10.6 ↓	-23.5 ↓	-4.4 ↓
UAD1151114	-42.4 ↓	-33.0 ↓	-39.4 ↓	-14.7 ↓
UAD1151118	-34.7 ↓	-27.7 ↓	-27.4 ↓	-2.3 ↓
UAD1151120	-33.3 ↓	-28.8 ↓	-37.0 ↓	-12.6 ↓
UAD1151125	-17.2 ↓	1.8 ↑	-40.7 ↓	2.2 ↑
UAD1152020	-11.4 ↓	-7.2 ↓	-25.8 ↓	-10.9 ↓
UAD1152076	-27.8 ↓	-25.9 ↓	-28.1 ↓	-4.0 ↓
UAD1152081	-33.6 ↓	-28.4 ↓	-41.8 ↓	-11.5 ↓
UAD1152084	-28.4 ↓	-18.6 ↓	-38.7 ↓	-4.6 ↓
UAD1153021	-31.7 ↓	0.1 ↑	-33.1 ↓	4.7 ↑
UAD1153086	-27.5 ↓	-25.9 ↓	-26.7 ↓	-13.7 ↓
UAD1153177	-8.4 ↓	-5.9 ↓	-38.1 ↓	-14.7 ↓
UAD1153228	-33.3 ↓	-33.5 ↓	-45.5 ↓	-22.2 ↓
UAD1153324	-27.5 ↓	-15.8 ↓	-42.9 ↓	-21.0 ↓
UAD1154157	-10.9 ↓	-17.6 ↓	-22.6 ↓	-4.4 ↓
UAD1154187	-44.4 ↓	-29.0 ↓	-45.9 ↓	-26.4 ↓
UAD1154192	-45.1 ↓	-41.2 ↓	-55.1 ↓	-11.1 ↓
UAD1154220	-15.5 ↓	-38.7 ↓	-37.1 ↓	-7.8 ↓
WID802	-24.8 ↓	-20.3 ↓	-17.1 ↓	-8.7 ↓
WYALKATCHEM	-25.9 ↓	-29.1 ↓	-37.2 ↓	-11.8 ↓
Yawa	-14.2 ↓	-4.7 ↓	-39.2 ↓	-3.7 ↓

Table 1. Ranking loss for two important heat stress indicator traits (TGW and HHI) are shown across all germplasm evaluated over two seasons (2015, 2016). Green arrows indicate those lines that show significant promise as being a variety in their own right, or at a minimum being very useful as a parent in crossing blocks (for example, **UAD1151101**; which across two seasons has performed superior to the heat tolerant Halberd bread wheat variety). Those with red arrows indicate loss, with the % value indicating the severity. For example, with TGW and comparing DBA-Aurora with Tamaroi; while both varieties sustain high losses after heat stress, DBA-Aurora is < half the loss (averaging 21.5% over two years) when compared to Tamaroi (averaging 47.5% over two years). Factoring in that a variety like DBA-Aurora has superior TGW over other varieties (e.g. Yawa), the results for DBA-Aurora are still therefore superior to small TGW varieties.

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

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

CONCLUSIONS

Based on the results from two years of trials (see Table 1 for summary), there are three lines that will be used heavily as donors in upcoming crossing blocks. These three lines include UAD1151101, UAD1151125 and DBA-Aurora and takes into consideration other factors such as plant type and performance in the field trial program that is run as part of Durum Breeding Australia.

The pedigree information (in confidence for the two UAD entries) for these three lines mentioned (in order of highest potential to lowest potential benefit) are:

UAD1151101
RH920318/Kalka///R622S/2*Kalka//Tamaroi////Worrakatta/2*Tamaroi//2*Kalka/////Senator Capelli/3*Kalka//Tamaroi

UAD1151125
ZenatiBouteille/Kalka//2*Tamaroi///Kalka////Kkna/////Worrakatta/2*Tamaroi//2*Kalka//////Senator Capelli/3*Kalka//Tamaroi

DBA-Aurora
Tamaroi*2/Kalka//RH920318/Kalka///Kalka*2/Tamaroi

In summary, while there are distinct differences in the parentage of these lines above, there are also some clear overlapping commonalities between the lines. For example, these three better performing lines above all have a reasonable percentage of Kalka in their parentage (much more so than Tamaroi, although that is present in all three as well). Kalka was not used as a germplasm entry in the trials in its own right (simply due to not having a confirmed pure source of seed – this variety early on in its existence was unfortunately significantly contaminated with bread wheat). Nonetheless, and since the completion of this project, pure Kalka seed has since been obtained from the 2016 harvest season and multiplied in sufficient quantities in order to be able to validate its individual performance in the heat chamber (see possible future work).

Intellectual Property

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

No new IP generated.

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.

- The generation of new knowledge based on the heat tolerance trial results has assisted in identifying heat tolerant durum parents that will contribute towards future variety development.
- In the medium-long term, and with the release of improved heat tolerant durum varieties, this will deliver enhanced productivity to growers who sow durum.
- The heat chamber trial results from both the 2015 and 2016 season are available on the SADGA website (http://durumgrowerssa.org.au/category/industry-news/).

- During Spring 2015 and 2016, the Durum Grower's Association visited the Heat Chamber Trial as part of their annual 'crop walks' tour. On both occasions there was excellent attendance at the field event with >50 growers and sponsors (2015) and >30 growers and sponsors (2016) attending. See below (Figure 3) for example photo where the heat chamber methodology and trial design was explained.
- There are no barriers to adoption if a suitable new variety can be generated. The parental materials that will be subsequently used in crossing blocks is owned by the breeding program.

Figure 3: Field day at AGT-SAGIT heat chamber (Roseworthy). Paul Telfer and Jason Able explain the design and importance of the research to growers and consultants attending.

POSSIBLE FUTURE WORK

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

We will take the information we have learnt from this screening evaluation and incorporate several of the lines as parents in the crossing blocks ahead. We will also work with AGT in moving forward and evaluating another set of durum germplasm in 2018 or 2019 (including pure Kalka seed source). This will be largely dependent on the funds received for the breeding program through the GRDC in renewal of our program. Having a rolling cycle (or near rolling cycle) of germplasm that has been evaluated through the heat chamber at Roseworthy over two seasons has significant merit and will be undertaken if the budget allows it.

AUTHORISATION
Name: A/Professor Jason Able
Position: Head, Department of Agricultural Science , School of Agriculture, Food & Wine
Signature:
Date: 23/08/17

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.

SAGIT UA1415 Heat Stress Durum Experiment Information (Trials in 2015 & 2016)

Experimental Design

- **Entries** – 38 Durum lines with additional wheat checks (Wyalkatchem-susceptible and Halberd-Tolerant)
- **Replicates** – 3 replicate, replicated by block
- **Pots/Experimental units** – 252 (12 ranges, 21 rows)
- **Treatments** – 2 treatments, an unstressed control and a heat stressed treatment (36°C with 40 kph wind speeds) with treatments randomised across split-plots
- **Secondary tillers** – If present, when the plant was due to be stressed (10 days after the primary tiller finished anthesis) a secondary tiller that was undergoing currently undergoing anthesis, was identified and tagged for further lab measurements
- **Design** – Split-plot design – Adjoining split-plots aligned in adjoining ranges

Experimental Methodology

Using the experimental design detailed above, single seeds were planted in 10cm x 10cm x 18 cm black olive pots. Pots were placed on an irrigation mat with computer controlled irrigation, supplying water on a daily basis. This process was managed such that after irrigation the mat was saturated without runoff, with irrigation occurring again before too much dryness was evident on the mat. Due to differences in plant growth, plant water requirements and greenhouse temperatures this is not a constant irrigation rate throughout the growing season.

As plants were planted in a very low nutrient potting mix (coco peat), once the three leaf stage was reached, an aqueous complete nutrient solution was applied weekly.

As plants approached heading, twice weekly maturity observations were taken. This allowed for complete head emergence on the primary tiller to be observed. At this point a piece of tape was applied to the stem of the primary tiller to aid in identification. Heading was used as an indicator of imminent anthesis, with the recording of the date that anthesis is completed on the primary tiller.

Ten days after the end of anthesis; plants, both those designated as controls or for heat stressing, were removed from the irrigation mat. The primary tiller was tagged with a barcoded tag for later lab identification and the flag leaf of the primary tiller was given a visual damage score. This 1-9 score is roughly proportion to the area of leaf that is not viable (Score 1 – 0-10% of leaf area not viable, through to 9 – 90-100% of leaf area not viable). In addition, if a secondary tiller was present, it was also labelled with a barcoded tag for later lab identification.

Plants to be heat stressed were staked with bamboo, and tied loosely to this to ensure plant integrity during the heat stress treatment. Staked plants were then aligned in trays designed to hold the pots and trays were positioned in the heat chamber (Figure 1) aiming to have the heads and flag leaf region in the flow of hot air. Drip irrigation was applied to each pot individually, with irrigation occurring morning and evening. Control treatment plants were returned to the irrigation mat as originally located without stress applied.

At the completion of the three days of heat stress (normally the following morning), plants were removed from the heat chamber. A second leaf damage score was recorded, as it was for the control plants (stressed plants were then returned to the irrigation mats). A third leaf damage score was observed on both the control and heat stressed plants 10 days after the initial leaf score was observed.

Plants were then left to mature under the growing conditions described above. At maturity, irrigation ceased allowing full senescence to be managed evenly. Plants were harvested by cutting off at ground level. Tillers that have a barcoded tag were collected and separate plants individually bagged in to paper bags. Plants were dried in drying oven for 48 hours before processing in the laboratory.

Figure 1: The heat chamber developed for previous bread wheat projects with AGT and SAGIT, and which was used for the current project to produce relevant, controllable and repeatable conditions to screen for heat stress response in durum germplasm.

Data/Laboratory File Information

Data collected from the experiment included the following and analysis for some of these traits has been conducted (see progress report).

- **Matanthjul** – The day of year that the main tiller reached the end of anthesis (GS69)
- **tillernum –** For primary tillers this will be '10' indicating 10 days after anthesis, for secondary tillers this is the growth stage at the start of the heat stress treatment
- **batchno** – Batch number indicates the group and order that plants were stressed (that is, all plants listed as batch 1 were the first plants to be stressed and were all in the chamber at the same time)
- **stressjul** – The day of year the stress treatment started for a particular plant
- **leaf1** – 1-9 leaf damage score at the start of the treatment (before stressing) – 1 no damage, 9 - 90-100% unviable leaf area

- **leaf2** – 1-9 leaf damage score at the completion of the three days of stressing
- **leaf3** – 1-9 leaf damage score approximately 10 days after the initial leaf1 score
- **pedunclel** – Length of the peduncle
- **flagleafw** – With of the flag leaf at maximum width
- **flagleafl** – The length of the flag leaf
- **spikeletno** – The number of spikelets on the head
- **grainwt** – The weight of the grain in the head
- **tillerwt** – The weight of the whole intact primary tiller
- **headwt** – The weight of the whole intact head
- **grainno** – The number of grains contained in the head
- TGW (‘**thokernwt’**) – derived by (‘**grainwt’/’grainno’)*1000**
- **‘Headhi’** (harvest index of head) – derived by (‘**grainwt’/’headwt’)**
- Harvest index (‘**harindex’**) – derived (‘**tillerwt’/’grainno’)**
- **‘fertility’** (average number of grains per spike) – derived by (**‘grainno’/’spikletno’**)

Statistical Analysis

GenStat (Release 15.3) and R were used to analyse selected trait measurements from the recorded data.

As we are primarily interested in identifying germplasm that yields well under heat stress conditions, grain yield determining traits including fertility, TGW and Headhi have been given priority. However, other secondary traits such as grain number, grain weight, head weight, spikelet number and tiller number are also considered important.