PROGRESS STATEMENT 2017

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Progress Reports must be submitted via email to admin@sagit.com.au as a Microsoft Word document

Project No: UA717	**Project Title:** Enhanced N-use efficiency in durum through improved genetics	
Previous Project(s) (If this project is on a similar theme to a previous funded project please provide code , title, years and investment details) N/A		
Organisation: The University of Adelaide		
ACN/ABN: 61 249 878 937		
Start Date: (This date must be same as in the Funding Agreement) 1st July 2017	**Completion Date:** (This date must be same as in the Funding Agreement) 30th June 2020	
Address: Research Branch, Level 4, Rundle Mall Plaza, 50 Rundle Mall, Adelaide SA 5000		
Principal Investigator: A/Professor Jason Able		**10% Time**
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Other Research Staff: *Professor Amanda Able (Investigator: 0.1 FTE), Dr Haipei Liu (Investigator: 0.1 FTE), Mr Samuel Deed (Technical Officer: 0.8 FTE)*		**100% Time (Samuel at 0.8 FTE)**
Administrative Contact: Ms Chelsea DuBois		
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1. BUDGET

Please include below a brief description of the main items required within each category for the current application year.

BUDGET			
Category	**$**	**$**	**$**
	Year 1	**Year 2**	**Year 3**
Salaries	71,773	57,371	61,300
Travel			
Operating		4,320	4,320
Capital			
TOTAL SAGIT CONTRIBUTION	71,773	61,691	65,620
Host organisation cash contribution	8,000*	8,000	
Host organisation in-kind contribution*	94,343	98,172	101,786
TOTAL HOST ORGANISATION CONTRIBUTION	102,343	106,172	101,786
Other funding bodies contribution			
Other third parties contribution*			
TOTAL NON-SAGIT CONTRIBUTION	102,343	106,172	101,786

*If it is not possible to specify amounts, then a description of the nature of the contribution should be given.

EXPLANATORY NOTES ON BUDGET ITEMS

Including budget variations

Mr Samuel Deed commenced duties on the 7th August 2017, approximately 6 weeks after the proposed start date of the project. As such, the first milestone has been delayed by approximately this amount of time.

*A detailed budget of operating costs is available upon request. In brief, costs have mostly included laboratory consumables and sequencing to date. We have spent in excess of $8000 on components for this project to date (~$10.5K). These costs have been host organisation contributions.

2. PROGRESS STATEMENT

Provide clear description of the following:

Project aims

This project aims to:

1. Benchmark N-use efficiency for the current commercially-grown and leading durum varieties (e.g. DBA-Aurora, Saintly), selected advanced breeding lines and a sub-set of diverse germplasm;
2. Understand the importance of the underlying background genetics in the durum germplasm selected, by amplifying, cloning, sequencing and analysing the gene product recently identified in rice to improve N-use efficiency by 40%. Through identifying gene variants (alleles) in the durum germplasm, new parents will be selected for creating novel breeding materials which will be integrated into the breeding program in the medium term; and;
3. Establish, analyse and validate a series of coordinated tub- and field-based trials in years 2 and 3 of the project to evaluate N-use efficiency in the selected durum germplasm with varying rates of N management. Tub-based assays will be conducted at the Waite Campus in controlled environment conditions, while field-based trials will be completed in areas with varying constraints including the Lower Mid-North (e.g. Roseworthy) and the Upper South East (e.g. Bordertown).

The outcomes of this project will have a direct impact on grower's profitability longer term through improved durum breeding strategies that target specific genetic backgrounds; and in this case, N-use efficiency.

Progress against the key performance indicators of the project

No.	KPI	Date to be completed
1	Amplify, clone, sequence and analyse the N-use efficiency gene in 100 durum lines ***Completion target by March 2018***	31/12/2017, 2018
2	Plan, conduct, harvest and analyse tub-based trials for two years at the Waite Campus in controlled environment conditions with varying rates of N-management (nil, 70 kg ha^{-1}, 120 kg ha^{-1}) ***To be commenced in 2018***	31/12/2018, 2019
3	Plan, conduct, harvest and analyse at least two trials each year (for two years) in the district areas listed (with N-management rates as per tub-based trials) ***To be commenced in 2018***	31/12/2018, 2019
4	Publish trial results for relevant Farming Systems Groups, and the SADGA website/Twitter feeds ***Report prepared and disseminated to SADGA***	31/03/2018, 2019, 2020
5	Annual progress reports submitted to SAGIT ***First report submitted January 2018***	31/01/2018, 2019, 2020
6	Final report submitted to SAGIT ***To be undertaken at end of project***	30/06/2020

KPI 1 is yet to be completely achieved. This is in part due to the late commencement of Mr Samuel Deed being employed on the project (7th August 2017). We have collected different tissues from 115 genotypes with diverse genetic backgrounds and maximum geographical spread. A complete cDNA collection has been generated from these materials, with the N-use efficiency gene amplified and cloned in 73 genotypes. We expect to have completed cloning of the gene in the remaining entries for which cDNA has been made and complete the sequencing analysis within the next 6 weeks.

KPIs 2 and 3 are currently in the planning phase. We do not foresee any delays in achieving these as agreed.

Conclusions reached / discoveries made

This must include a dot point summary of progress to date, suitable for use in media articles. Provide more details which add to key findings (eg. tables, graphs) in an attachment of 1-2 pages.

- Different tissues of 115 durum lines have been collected and individual cDNA has been generated for all these materials.
- The N-use efficiency gene (NRT2.3) in 73 durum lines has been amplified and screened for the presence of the high N-use efficiency allele (NRT2.3b).
- Unique alleles have been identified in 12 durum lines and have been sequenced (see Figure 1).

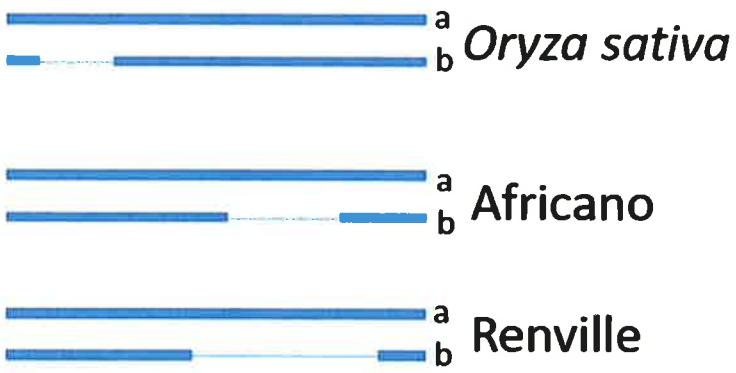

Figure 1: Schematic representation of a 510bp region of the NRT2.3 gene showing the 'a' & 'b' alleles in rice and unique alleles (NRT2.3b-1 and b-2) in two example durum genotypes that have been sequenced. *Oryza sativa* as reported by Fan et al. (2016) *PNAS 113: 7118*. Durum variety Africano is from Spain and Renville is from the United States.

- In conjunction with identifying the unique NRT2.3 alleles in as many durum lines as possible, we have also been screening amino acid substitutions that might affect protein function in the 510bp region.
- We have also undertaken the analysis of the complete NRT2.3 sequence. We have successfully designed primers that cover the whole coding region (~1700 bp). In time, this could lead to the identification of additional genetic variation which could contribute to better N-use efficiency.
- To date, with the sequencing data we have generated, several single nucleotide polymorphisms (SNPs) have also been detected. Whether these SNPs result in a different functional protein product is yet to be determined.
- We have found that the NRT2.3 gene is predominantly expressed in root tissue over coleoptile tissue.

Communication of results to farmers/industry

The results to date are ready for dissemination to growers and will be sent to SADGA for uploading to their website. In addition, and more meaningful for this project will be the presentation of these results to growers directly at the annual SADGA forums and other growers events (where the opportunity arises).

Nitrogen is one of the most expensive nutrients required by plants. The excessive use of nitrogen fertiliser hinders the profitability of growers and also has a negative impact on the environment. Plants have been bred for traits that show increased yield in the presence of excess nitrogen, however, this ignores a plants ability to efficiently use nitrogen. Approximately 50-70% of applied nitrogen is not absorbed by the plant and ends polluting the air and water (Good, A. G., *et al.* (2004) *Trends in Plant Science 9: 12*). In rice, a particular form of a nitrate transporter gene (NRT2.3b) has been shown to improve nitrogen use efficiency up to 40% (Fan *et al.* (2016) *PNAS 113: 7118*). A 40% increase in nitrogen use efficiency in durum wheat would significantly reduce the amount of nitrogen fertiliser required to achieve the 13% grain protein which is required for DR1 specification.

We currently have found unique NRT2.3 alleles in 12 durum lines. We are now in the process of screening for more unique alleles, uncovering the possible functional variance of different alleles, and getting the first full-length sequence of the NRT2.3 gene in durum. Durum lines with different alleles will be used in both tub- and field-based experiments to measure their performance under different N rates. We expect to see different N-use efficiency levels among these lines and based on these findings will be able to select for the most efficient NRT2.3 allele in durum.

Plans for the coming year

In the coming year, we will benchmark NUE efficiency in South Australia's commonly used durum varieties DBA-Aurora (and now to a lesser extent, Saintly). Following this, we will measure NUE of any variety which contains the NRT2.3b allele and/or any unique NRT2.3 allele (for example, and at a minimum – the lines Africano and Renville). Furthermore, we will also investigate any of the other 100+ varieties for which we will have data for, to determine whether any genetic variation that may/may not be present results in a different NUE response. These experiments will first be performed in tub-based glasshouse experiments to gain some preliminary data and then followed up with field trials.

3. AUTHORISATION OF THE PROJECT REPORT

Name: A/Professor Jason Able
Position: Head, Department of Agricultural Science , School of Agriculture, Food & Wine
Signature: 
Date: 18/01/18