Office Use Only	
Project Code	
Project Type	

FINAL REPORT 2020

Applicants must read the *SAGIT Project Funding Guidelines 2020* prior to completing this form. These guidelines can be downloaded from www.sagit.com.au

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

PROJECT CODE	UA717
PROJECT TITLE (10 words maximum)	
Enhanced N-use efficiency in durum through improved genetics	

PROJECT DURATION *These dates **must** be the same as those stated in the Funding Agreement.*	
Project start date	**1/07/2017**
Project end date	**30/06/2020**

PROJECT SUPERVISOR CONTACT DETAILS *(responsible for the overall project)*		
Title:	**First Name:**	**Surname:**
Prof	Jason	Able
Organisation:	University of Adelaide	
Mailing address:	PMB1, Waite Campus, School of Agriculture, Food & Wine, Glen Osmond, SA, 5064	

ADMINISTRATION CONTACT DETAILS *(responsible for all administrative matters relating to project)*		
Title:	**First Name:**	**Surname:**
Ms	Chelsea	DuBois
Organisation:	University of Adelaide	
Mailing address:	Research Branch, Level 4, Rundle Mall Plaza, 50 Rundle Mall, Adelaide SA 5000	

PROJECT REPORT: *Please provide a clear description for each 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.

In this project, we identified genetic variation in the sequence of the durum version of the nitrate transporter, NRT2.3, which is associated with higher NUE and grain yield in rice (Fan *et al*. 2016, *PNAS 113: 7118*). A subset of durum genotypes, comprising current leading durum varieties DBA Aurora and Saintly, together with ten lines expressing NRT2.3 variants, were evaluated in both controlled-environment and field conditions over two years, under two levels of N-input.

- Five durum genotypes expressed NRT2.3 variants containing an insertion or deletion, and additional lines contained single nucleotide polymorphisms (SNPs) and amino acid changes.
- Early-maturing varieties DBA Aurora, Saintly, AUS 20668 and 4WA 737 achieved the highest yields, TKW, and nitrogen harvest index. Later-maturing varieties were affected by frost and a dry finish to the season.
- NRT2.3 was expressed in developing spikes at GS85 in all genotypes surveyed. NRT2.3 expression in 2019 field trial spikes varied between genotypes and N-treatment, with no clear link with GPC (grain protein content).

Project objectives

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

This project aimed 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.

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.

Project objectives achieved

Project objectives were achieved. N-use efficiency of selected durum varieties - including leading varieties DBA Aurora and Saintly, selected advanced breeding lines and a sub-set of diverse germplasm – was evaluated through two years of field trials at Roseworthy, and one season under controlled growth conditions. We now have data on N-use efficiency of these durum lines, which can be used by durum breeding programs.

We also now have a better understanding of the underlying genetic background in the durum germplasm selected, by sequencing and analysing the gene product recently identified in rice which improved N-use efficiency by 40%. Five durum genotypes were identified that expressed NRT2.3 variants containing an insertion or deletion (INDEL). Unique single nucleotide polymorphisms (SNPs) and amino acid changes were detected in several durum lines.

List of personnel

Position	Names
Project leadership	Prof Jason Able, Prof Amanda Able and Dr Haipei Liu
Technical officer	Mr Sam Deed began the project, but left at the end of 2018. Dr Jacinda Rethus began in 2019.
Field trials	Mr Alistair Pearce
Nitrogen analysis	Ms Kathy Allder, Ms Sayeh Dehghanian

Difficulties encountered

There were some challenges in developing a robust quantitative PCR test for the detection of the NRT2.3 variants due to genetic complexity. Overall NRT2.3 expression levels were low in developing grains, with variants having extremely low abundance. It was not applicable to develop stringent, INDEL-specific qPCR primer sets for quantifying NRT variants in the grains. Thus, we quantified total NRT2.3 expression levels across the genotypes tested. Likewise, Fan *et al*. (2016, *PNAS 113: 7118*) reported qPCR results for total NRT2.3 expression.

Growing conditions in the field, namely, water availability and frost occurrences, impacted yields, particularly of later-maturing varieties and thus also affected grain protein content and NUE. With the limited grain stocks of foreign material, we were only able to run small-scale field and controlled-environment trials in year 1, and upscale to one full-size field trial in year 2. There was insufficient seed stock for multiple sites in year 2 field trials, which was unfortunate as a site such as Bordertown would have been ideal to evaluate in along with the Roseworthy site (which was selected).

KEY PERFORMANCE INDICATORS (KPI) *Please indicate whether KPIs were achieved. The KPIs **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**	**If not achieved, please state reason.**
Amplify, clone, sequence and analyse the N-use efficiency gene in 100 durum lines.	Yes ☒ No ☐	
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}).	Yes ☐ No ☒	*Year 1 completed December 2018 (with 60 kg ha^{-1} and 120 kg ha^{-1}). Due to the limiting effects of tub use on yield, Year 2 tub trial was removed. Instead, a field trial in Year 2 was conducted in larger scale, with three replicates of each genotype and N-level in full-sized plots.*
Plan, conduct, harvest and analyse at least two field trials each year (for two years) in the district areas listed (with N-management rates as per tub-based trials).	Yes ☒ No ☐	
Publish trial results for relevant Farming Systems Groups, and the SADGA website/Twitter feeds.	Yes ☒ No ☐	
Annual progress reports submitted to SAGIT.	Yes ☒ No ☐	
Final report submitted to SAGIT.	Yes ☒ No ☐	

TECHNICAL INFORMATION (Not to exceed **three** pages)

Provide sufficient data and short clear statements of outcomes.

The N-use efficiency gene (NRT2.3) in 104 durum genotypes was amplified and screened for the presence of the high N-use efficiency allele (NRT2.3b). Deletions were identified in four durum genotypes and an insertion mutation was identified in one genotype (Figure 1). Unique single nucleotide polymorphisms (SNPs) and amino acid substitutions were identified in many other durum genotypes. Full-length sequences of the NRT2.3 gene in durum wheat were obtained for DBA Aurora, Saintly, and selected insertion/deletion lines.

Twelve durum genotypes were selected for glasshouse and field trials. These include commercial varieties DBA Aurora and Saintly as benchmarks, the five durum lines shown in Figure 1, and five durum genotypes with unique SNPs and amino acid substitutions.

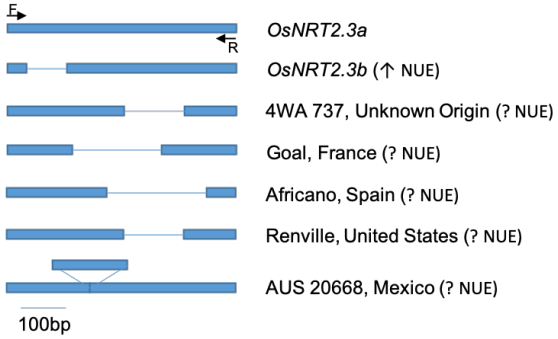

Figure 1. Schematic representation of a 509 bp region of the rice NRT2.3a and NRT2.3b gene showing the high N-use efficiency allele alongside unique alleles in five example durum genotypes that have been sequenced. *OsNRT2.3a* and *OsNRT2.3b* as reported by Fan *et. al.* (2016) *PNAS 113: 7118*. The NUE of the listed durum genotypes has not been studied to date.

The first year of glasshouse and field trial data revealed genotypic differences in physiological and morphological traits, grain protein content and yield components. In the Year 1 field trial, most genotypes achieved DR1 (13% protein) at both N-treatments (75 and 150 kg Ha^{-1} applied N, for low and high N treatments, respectively) with similar yield, indicating sufficient nitrogen under low N application. Environmental conditions reduced yields of later-maturing durum genotypes in the field. In contrast, there was a nitrogen response in the glasshouse tub trial, with high N treatment resulting in higher grain yield than the low N treatment. Both N treatments in the glasshouse trial yielded below the field trial, and grain protein content was below DR1, signifying that N was the limiting factor and that the use of tubs could be limiting the yield. Even so, the results of the glasshouse trial indicate that most of the genotypes have yield potential equal to, or higher than, the leading Australian varieties, DBA Aurora and Saintly, under ideal growing conditions.

Expression of NRT2.3 was confirmed in developing grains, with highest expression observed at GS85 (Figure 2). NRT2.3 expression was detected in spike samples from the Year 1 field trial, however limited conclusions could be made as not all genotypes were represented.

Figure 2. NRT2.3 expression in developing durum grains. Photos and the RNA material of developing grains were contributed by Dr Liu's ARC DE180100784 project (Liu et al. 2019 *Sci. Rep.*). Materials provided were collected from DBA Aurora at 5, 15, 25, 35 and 45 days post-anthesis (DPA) under controlled glasshouse conditions. 35 DPA is equivalent to GS85. Expression of NRT2.3 (RQ, relative quantity) was determined by quantitative PCR, and measured relative to the house-keeping gene, GAPDH.

The second year of field trials was completed in December 2019, with full-sized plots. Soil testing was conducted prior to sowing the Year 2 field trial to determine base N levels, from which applied N levels were calculated, for a final high N treatment of 150 kg Ha^{-1} and a low N treatment of 75 kg Ha^{-1}. Harvest data revealed that plot yields for both N treatments were similar (Figure 3). This is not surprising, as N topdressing was applied between stem elongation and anthesis. Early-maturing varieties gave the highest yields, with frost events and rising temperatures impacting yields of later-maturing durum genotypes. Rainfall was lower than average across both years of field trials. There were some seasonal differences, with 4WA 737 and Gerardo yielding comparably to the highest-yielding varieties in 2018, but with lower yields in 2019.

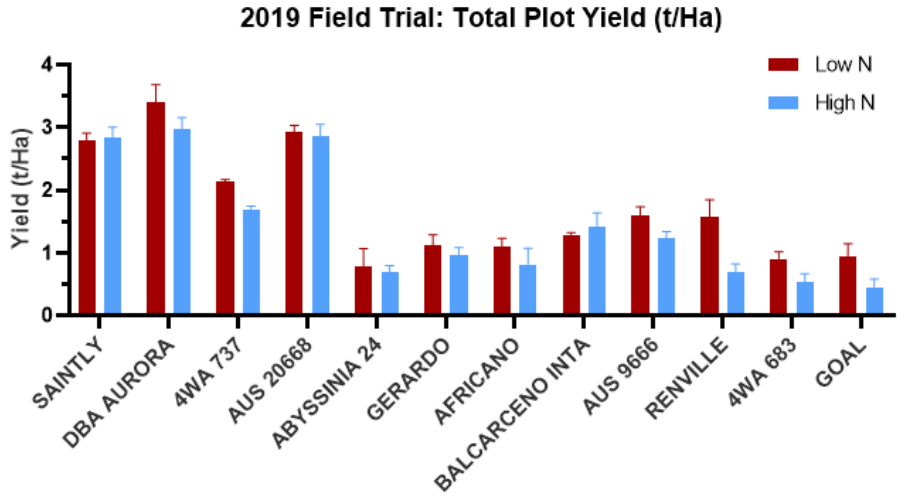

Figure 3. Year 2 field trial plot yields. Yields are reported in tonnes per hectare (t Ha^{-1}) equivalent. Plot size was 3.8 m x 1.3 m. Durum lines are listed in order of early-maturity to late-maturity.

Biomass cuts were taken from each plot (2 x 50cm rows) for more detailed measurements. Plant height, biomass, plant numbers, spike numbers, spikelet length, and the number of grains were comparable across N-treatments, but differing by genotype. Grain protein content was responsive to N application. The high N treatment lifted grain protein levels to above DR1 (13%) in the early-maturing varieties (Figure 4). Of these, 4WA 737 and AUS 20668 were close to achieving DR1 under low N input. Later-maturing varieties achieved DR1 under low N treatment. However, yields were reduced and often thousand-kernel-weight (TKW) was also lower (Figure 2, Table 1). Nitrogen harvest index (NHI) was greatest for the four early-maturing varieties (Table 1).

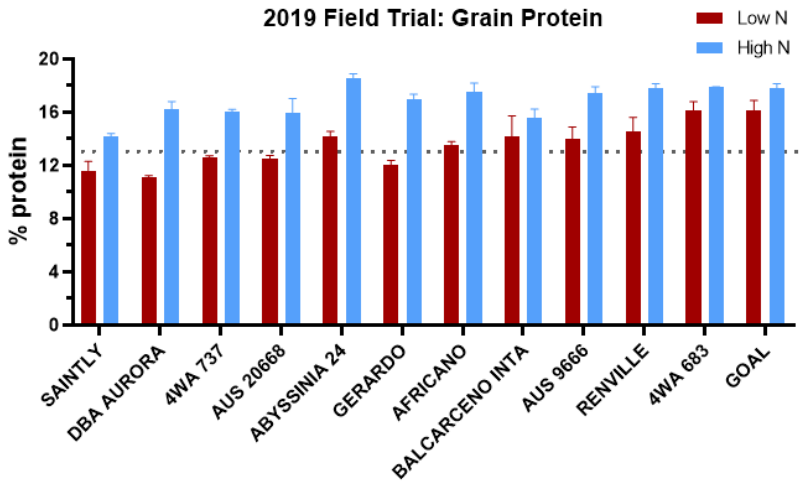

Figure 4. Year 2 field trial grain protein content. Grain N content was measured by the Dumas method, and converted to protein content using a correction factor of 5.7 for wheat, and adjusted for 11% moisture basis.

Genotype	NHI (LN)	NHI (HN)	TKW (Low N) ± SD	TKW (High N) ± SD
DBA AURORA	0.68	0.60	53.18 ± 11.6	51.24 ± 2.5
SAINTLY	0.70	0.66	43.62 ± 2.4	42.63 ± 1.2
4WA 737	0.50	0.55	39.78 ± 1.2	35.92 ± 1.0
AUS 20668	0.67	0.62	43.45 ± 14.7	46.49 ± 6.1
ABYSSINIA 24	0.31	0.22	31.80 ± 0.4	29.50 ± 1.4
GERARDO	0.41	0.29	41.55 ± 1.4	33.95 ± 5.5
AFRICANO	0.35	0.21	32.62 ± 4.1	27.62 ± 5.5
BALCARCENO INTA	0.38	0.39	39.94 ± 3.6	38.55 ± 2.6
AUS 9666	0.46	0.26	38.21 ± 1.0	35.13 ± 1.9
RENVILLE	0.42	0.19	37.45 ± 13.0	28.24 ± 1.4
4WA 683	0.25	0.10	30.69 ± 0.2	33.40 ± 0.7
GOAL	0.22	0.06	30.37 ± 3.4	29.49 ± 0.3

Table 1. 2019 Field trial nitrogen harvest index (NHI) and thousand-kernel-weight (TKW). NHI was calculated by (G_N x Gw) / ((G_N x Gw) + (B_N x Bw)), where G_N = grain N%, Gw = grain weight (g), B_N = biomass N% and Bw = biomass weight (g).

NRT2.3 gene expression was examined in spike tissue collected at GS85 (Figure 5). Durum genotype 4WA 737 was excluded from the analysis due to poor-quality data. NRT2.3 expression was down-regulated in the high N treatment for some varieties, including DBA Aurora. For others, NRT2.3 expression was up-regulated in the High N treatment. There was no correlation between grain protein content and NRT2.3 expression between the two N treatments. Whether this is also true for the abundance of NRT2.3 transcript

variants remains to be determined. Transcript levels of the NRT2.3 variants were not quantified due to very low expression value, and the challenges in designing a robust qPCR detection system.

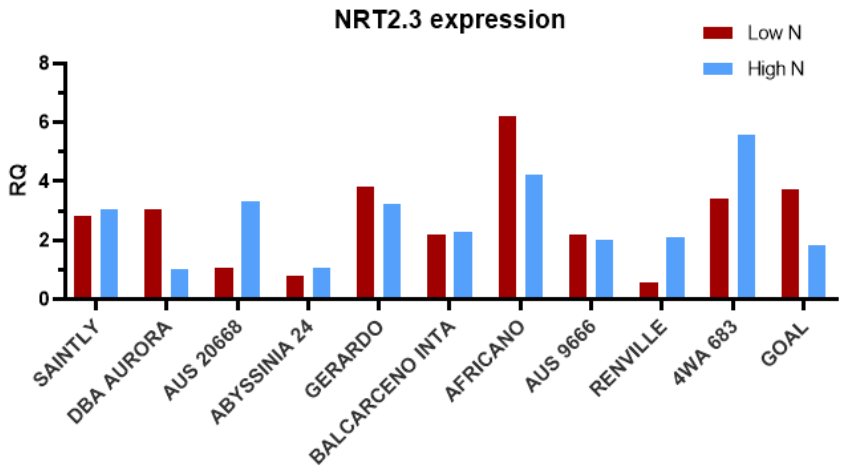

Figure 5. NRT2.3 expression in spikes at GS85. Spikes were collected from the 2019 field plots at GS85, followed by RNA extraction and cDNA synthesis. NRT2.3 transcripts were quantified by qPCR. Gene expression was shown as RQ (relative quantity), normalised to the reference gene, GAPDH.

CONCLUSIONS REACHED &/OR DISCOVERIES MADE (Not to exceed one page)

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

- By screening 104 durum genotypes, the project revealed the genetic variation in the sequence of the durum version of the nitrate transporter, NRT2.3, In rice, the NRT2.3b variant is associated with higher NUE and grain yield (Fan *et al*. 2016, *PNAS 113: 7118*). Five durum genotypes were identified with NRT2.3 variants that have an insertion or deletion. Genotypes containing SNPs and amino acid changes were also identified.
- At GS85, NRT2.3 expression can be successfully profiled in all genotypes that were surveyed. NRT2.3 expression in spike samples taken from the 2019 field trial showed significant differences, subject to genotype and N-treatment.
- Early-maturing varieties gave the highest yields and nitrogen harvest index (NHI). This confirms Saintly and DBA Aurora as N-use efficient, leading varieties for commercial application.
- AUS 20668 and 4WA 737 have great potential in breeding programs, having high yields, high NHI, and GPC that was close to, or achieved DR1 (2019 and 2018, respectively) at low N input. They are early-maturing varieties, thus escaping adverse growing conditions that tend to occur later in the season in the Australian Mediterranean environment.
- Later-maturing varieties suffered yield penalties through frost events, a dry finish and increasing temperatures, thus masking full NUE potential.
- Measuring residual soil N and adjusting the amount of applied N was critical in observing N-treatment differences in the second year of field trials.

INTELLECTUAL PROPERTY

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

The project has generated new genetic information for germplasm which may have enhanced N-use efficiency. Such germplasm would subsequently be used as a parent to create enhanced N-use efficient durum lines for the breeding program. In the medium- to long-term this could translate to variety release.

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.

This project, UA717:

- Benchmarked 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.

- Identified sequence variation in the durum version of the nitrate transporter, NRT2.3, which in rice is associated with higher N-use efficiency and yield (Fan *et al*. 2016, *PNAS 113: 7118*).

- Confirmed the genotype-dependent expression of the nitrate transporter, NRT2.3 in developing spikes at GS85. In some lines, NRT2.3 was reduced under high N treatment, while for other varieties, NRT2.3 expression increased under high N levels.

- Identified early-maturing varieties AUS 20668 and 4WA 737 as potential parents in breeding programs to develop new durum lines with high N-use efficiency, high yield, GPC and NHI. These varieties express variants of the nitrate transporter, NRT2.3.

Nitrogen is one of the most expensive nutrients required by plants. Plants have been bred for traits that show improved yields in the presence of surplus nitrogen, however, this ignores a plants' ability to efficiently use nitrogen. The excessive use of nitrogen fertiliser hinders the profitability of growers and also has a negative impact on the environment. Approximately 50-70% of applied nitrogen is not absorbed by the plant, resulting in air and water pollution (Good *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.

The outcomes of this project identified germplasm to be used in breeding programs to specifically enhance NUE while maintaining high yield performance. With informed breeding decisions based on the new knowledge, the development of superior germplasm can be accelerated and will increase profitability for both growers and the industry when new varieties are released.

Southern Australia Durum Growers Association (SADGA) supported field days (5th September 2018, 11th September 2019) at the Roseworthy site, where we communicated with local growers (~50 stakeholders in attendance at each event) about this research and the findings to date as well as further experiments that were planned at that stage. During 2019, at the Wheat Breeding Assembly conference WBA2019 (20th-22nd August 2019), we promoted the research outcomes to industry, growers and researchers through a poster presentation and discussions. A peer-reviewed publication is in preparation.

POSSIBLE FUTURE WORK

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

Project UA717 can be further expanded to involve multiple field sites with ranging environments, carried out across multiple seasons. Project UA717 was successful in identifying genotypic variation in yield components, grain protein content and physiological traits within the selected durum genotypes. With increased grain stock of all genotypes, future work can be conducted at full-scale without limitation, to include multiple field conditions and gauge the genotype by environment effect across multiple years.

Alongside the multi-site field trials, testing the later-maturing genotypes under controlled-growth conditions would provide an opportunity to evaluate N-use in the absence of frost and drought so that these varieties can realise their full yield potential.

Additional factors to investigate further include:

- Timing of N application – test the most suitable developmental stage to add N earlier for yield boost as well as GPC boost, without adding the risks of haying-off.
- Examination of NRT2.3 expression in roots (known to be expressed at high levels in roots under very low nitrogen conditions, but not at normal/high N) – possible association with nitrogen uptake/transport, or in older leaves (post-anthesis, but before visible senescence) – possible association with photosynthetic nitrogen use efficiency.
- Examination of other nitrogen-related genes - involvement of transferring N from plant (straw, leaves) to grain during grain fill.

The durum genotypes identified in this project as having enhanced N-use efficiency could be used as parents in breeding programs to create enhanced N-use efficient lines. In the medium- to long-term this could translate to variety release. These varieties also provide benchmarks against which to screen other durum germplasm in breeding programs.