Office Use Only	
Project Code	
Project Type	

FINAL REPORT 2022

Applicants must read the *SAGIT Project Funding Guidelines* 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	UA419
PROJECT TITLE	(10 words maximum)
Saline evaluation of a wheat population identifying novel salinity tolerance	

PROJECT DURATION *These dates **must** be the same as those stated in the Funding Agreement.*			
Project start date	**1/07/2019**		
Project end date	**30/06/2022**		
SAGIT Funding	**2020**	**2021**	**2022**
			$0

PROJECT SUPERVISOR CONTACT DETAILS *(responsible for the overall project)*		
Title:	**First Name:**	**Surname:**
A/Prof	Stuart	Roy
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Telephone:		
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ADMINISTRATION CONTACT DETAILS *(responsible for all administrative matters relating to project)*		
Title:	**First Name:**	**Surname:**
Ms	Chelsea	DuBois
Organisation:	The University of Adelaide	
Mailing address:	Research Services, North Terrace Adelaide, SA, 5005	
Telephone:		

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 South Australia it has been estimated that approximately 50% of farms are at risk of showing signs of transient salinity. Mocho de Espiga Branca is a high sodium (Na^+) accumulating wheat landrace, offering opportunities to introduce novel salinity tolerance mechanisms into Australian wheat cultivars.

We had previously developed a Mocho de Espiga Branca × Gladius wheat recombinant inbred line (RIL) population to identify regions of DNA linked to novel salinity tolerance traits (SAGIT projects UA317, UA418). In this project we grew a selection of this material in a mild/moderate saline field site at Roseworthy to determine whether the incorporation of traits from Mocho de Espiga Branca could enhance yield in the field.

As Mocho de Espiga Branca is a wheat landrace and does not have agronomic traits which make it easily comparable to Australian cultivars, 64 Mocho de Espiga Branca × Gladius RILs were carefully selected from greenhouse trials. These RILs had a similar time to flowering and height as Australian cultivars but differed in their ability to accumulate salt. We identified 3 RILs which could accumulate >10× leaf Na^+ while showing a 5-25% increase in yield in the field when compared to their other parent, Gladius. A further 5 RILs accumulated >10× leaf Na^+ while exhibiting the same yield as Gladius.

Project objectives

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

1. Assess growth and yield of a novel bread wheat population in a saline field
2. Determine the genetic regions that translate between the glasshouse (SAGIT project UA418) and field experiments (this project) to identify the genetic regions (regions of the plant's DNA) underpinning the salinity tolerance of these lines
3. Determine which lines are carrying the genetic regions for improved salinity tolerance for breeding into Australian germplasm

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.

Personnel who participated in the Research Project
Dr Allison Pearson (Project lead until 31st December 2019), A/Prof Stuart Roy (Project lead from 1st January 2020, plant phenotyping and soil sampling), Laura Short (growth of plants, plant phenotyping, soil sampling and processing, harvest), Christine Trittermann (growth of plants, plant phenotyping, soil sampling and processing, harvest), Dr Abdel El Habti (soil sampling), Dr Julian Taylor (field site design and statistical analysis), Alistair Pearce (sowing and harvesting of plots).

The project successfully identified germplasm with enhanced salinity tolerance in the field. These plants accumulated significantly higher concentrations of shoot Na^+ and a greater yield.

As detailed below the project successfully met the first 5 KPIs and is working towards completing the 6th KPI.

Initially problems were encountered for KPI 1 "Preparation of bulked seed and statistical design of field trial". Mocho de Espiga Branca × Gladius recombinant inbred line (RIL) seed harvested from the summer nursery in 2019/2020 was of poor quality for sowing in field trials in 2020. This was due to a number of reasons, including differences in the timing to flowering of some of the lines – Mocho de Espiga Branca, in addition to having good salt tolerance, flowers over a month later than Australian cultivars and is >100cm tall. The delayed flowering phenotype and tall height (more biomass) of the plants, compromised their growth in the summer nursery. Rather than compromise 2020 field trials with poor seed, a variation to the project was requested. In this variation UA multiplied seed in the greenhouse over the 2020 winter season, ready to sowing in field trials in 2021. In addition to producing good quality grain, another growing season in the glasshouse allowed the identification of lines which accumulated high concentrations of Na^+ (which they got from Mocho de Espiga Branca) but had a flowering time and plant height similar to elite Australian germplasm (which they got from Gladius). This allowed us to sow RILs in 2021 which would all flower at the same time (thereby not adding an additional level of complexity in different growing season lengths).

Field site characterization and growth of the plants and analysis of the plants at the site was successfully completed (KPIs 2-5). Initially high saline field sites in Lower Light were selected for trials, however, it was decided to move the trial to a site with more moderate level of salinity. The plants at this site would accumulate enough Na^+ to compromise their growth (if they did not have good salinity tolerance mechanisms) but would not kill the plants – it is hard to get performance values of salt sensitive dead plants for comparison to salt tolerant living plants. A good variation in plant phenotype was observed. Some plants had the usual bread wheat salt tolerance (low accumulation of Na^+ and good yield), others had the novel salinity tolerance mechanism (high accumulation of Na^+ and good yield); while others had no salinity tolerance and poor yield. Three RILs which accumulate high Na^+ and had a 5-28% increase in yield over Gladius were identified in the field. An aliquot of seed from these three lines has been provided to Ag Consulting & Research (http://agconsultingco.squarespace.com) for a field trial on the Yorke Peninsula in 2022. This will allow independent verification of the performance of these lines and multiple seed for future trials.

KPI6 “QTL analysis” is still ongoing. 237 individual QTL for different salinity tolerance traits (yield, growth, ion accumulation etc) have so far been detected and in the greenhouse and RILs were selected for field trials based on QTL linked to growth and Na^+ accumulation. Further analysis of other QTL and their effect on yield in the field is ongoing.

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.
Preparation of bulked seed and statistical design of field site	Yes ☒ No ☐	
Site characterization before seeding with soil cores and EM38 mapping, followed by planting of plots	Yes ☒ No ☐	
Site characterization at anthesis, collecting soil cores, EM38 mapping and plant measurements	Yes ☒ No ☐	
Harvest	Yes ☒ No ☐	
Data analysis of measurements taken	Yes ☒ No ☐	
QTL analysis of data against sequence information	Yes ☐ No ☒	Partially completed. In SAGIT project UA418 237 genetic regions were identified linked to enhanced salinity tolerance in the greenhouse. Knowledge of these loci allowed the selection of material for field trials and was

		used in the initial analysis of field trials. While the effects of some of 237 salt tolerance QTL has been determined, others are still being investigated.
	Yes ☐ No ☐	
	Yes ☐ No ☐	

TECHNICAL INFORMATION (Not to exceed **three** pages)
Provide sufficient data and short clear statements of outcomes.

The wheat landrace, Mocho de Espiga Branca, had previously been identified to accumulate high (barley levels) of shoot sodium and yet suffered no negative effect on its salinity tolerance. Given barley has a higher salinity tolerance than bread wheat, if barley type salinity tolerance mechanisms could be introduced into bread wheat, this would improve yield stability in saline fields.

Previous SAGIT funded projects used speed breeding to develop a Mocho de Espiga Branca × Gladius recombinant inbred line (RIL) mapping population (UA317), which was then characterized in greenhouse conditions for 37 phenotyping traits (including plant biomass, plant growth, ion content and grain production) under control and salinity stress (UA418). A number of genetic loci were identified linked to plant growth under salinity; sodium (Na^+), potassium (K^+) and Chloride (Cl^-) accumulation in leaves; plant biomass and yield. In addition, money was successfully leveraged by Dr Pearson from the Yitpi foundation to use next generation sequencing technologies to genotype the entire RIL population and generate a genetic map to facilitate quantitative trait loci mapping in this SAGIT project.

The aim of this project was to evaluate whether these identified genetic regions could improve performance in the field.

Seed multiplication

Initially, seed from the RIL population underwent seed multiplication for field trials in the summer of 2019/2020, for field trials in 2020. Unfortunately, due to poor growth conditions in the summer nursery, the seed quality was poor. An added complication was encountered that some RILs flowered 2 months after other lines, due to having detrimental flowering time genes inherited from Mocho de Espiga Branca. A variation to the project allowed us to multiply seed at our own cost in greenhouses at the Waite Campus in 2020 in preparation for field trials in 2021. During the 2020 seed multiplication, we took the opportunity to record the development of the RILs, specifically focusing on time to flowering and height of the plant. RILs which flowered 10 days after Gladius or were substantially taller than Gladius (>100cm) were not selected for field trials in 2021. In the end 64 unique RILs with similar height and flowering time to Gladius were selected for field trials. These RILs were segregating for the high shoot Na^+ tolerance trait. This would allow direct comparison of yield between plants which accumulated high shoot Na^+ without other confounding factors.

Figure 1. Mocho de Espiga Branca × Gladius recombinant inbred lines growing at Roseworthy

Field trial - methods

A mild to moderate saline field site near to the University of Adelaide's Roseworthy campus was used. This field site had on average an ECe of 2.35 dS/m, with enough salinity to affect plant growth and yield but not enough to kill sensitive lines. We also wanted to explore the possibility that under certain low rainfall conditions, the accumulation of Na^+ in plant tissue, may assist with growth under drought conditions, as the higher Na^+ accumulating lines may be better adapted to uptake water from a dry paddock.

The 64 RILs and Gladius were sown in two-row breeders plots, surrounded by a border of durum wheat (Saintly). Mocho de Espiga Branca was not sown in the field, due to it having considerable differences in height and flowering time to the 64 selected RILs and the Gladius parent. Lines were sown using a randomised unequal replicate block design. Prior to sowing DAP+Zn%, Ultramax, Treflan and Avadex Xtra pre-sowing treatments were applied. Sowing occurred in late May 2021 into damp soil, which was followed by a significant rainfall event 3 days later. Establishment counts and soil conductivity measurements of the individual plots (using an EM38) were taken 4 weeks later. Soil cores were obtained across the range of the EM38 measurements (at 20% intervals) and sent to APAL for analysis to quantify pH, soil carbon, soil texture, nutrient/element composition as well as the presence of subsoil constraints, such as salinity. Growth and development of the lines was followed over the growing season. Further EM38 measurements and soil cores were obtained at flowering, to determine if the salinity had changed during the growing season. At flowering, the penultimate leaf before the flag leaf on the main tiller (Flag leaf-1) was taken for ion analysis using a Flame Photometer (Na^+ and K^+) and a Chloridedometer (Cl^-). Prior to harvest, plant height was recorded, as well as the presence of awns (another trait segregating in the population). Plots were harvested and the seed from each plot weighed. The weight of <2mm screening was also determined. Using the known EM38 measurements below each plot, the performance of each line was standardized to the average concentration of Na^+ in the trial area, to allow comparison between NILs and Gladius grown across the paddock.

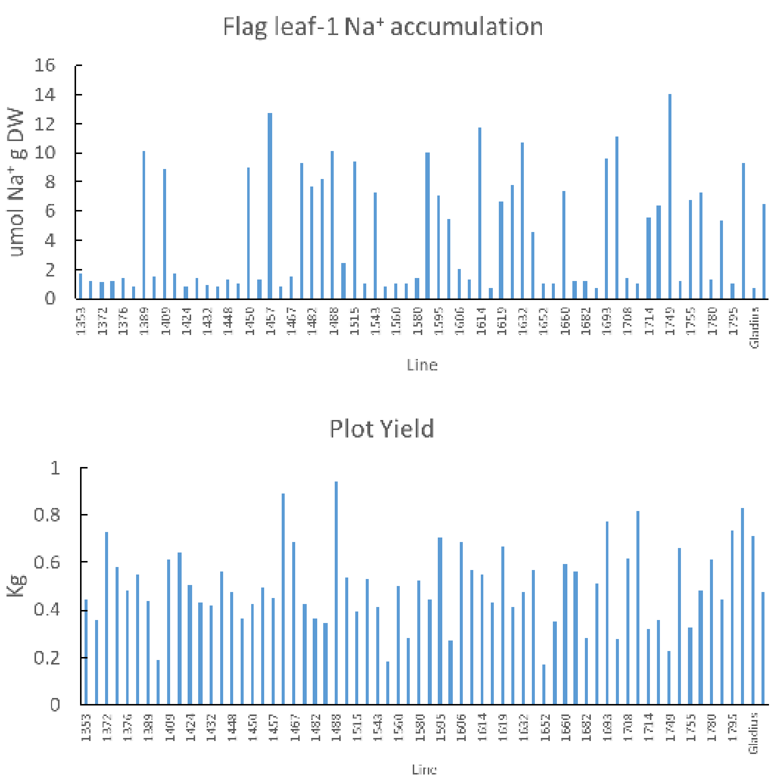

Figure 2. A) Leaf Na^+ concentration and B) yield in a selection of 64 Mocho de Espiga Branca × Gladius recombinant inbred lines (RILs) grown in a mild/moderate saline site at Roseworthy, SA. RILs were selected for field trials based on having similar flowering times and height to Gladius. Lines were grown in 2 row breeder's plots

Field trial – results

The Na^+ concentration in the plots ranged from 280 to 514 mg/kg (Ece = 1.3 to 3.9 dS/m), with an average concentration of 399 mg/kg (Ece = 2.35 ds/m). All RILs had a very similar time to flowering as Gladius, however there was still some variability in RIL height (72 – 100cm), compared to an average Gladius height of 76cm. It was very clear that the population was segregating for leaf Na^+ accumulation with RILs accumulating either low (0.67 to 2.41 μmol Na^+ g^{-1} DW), or high leaf Na^+ (4.5 to 14.0 μmol Na^+ g^{-1} DW) (Figure 2). Gladius by comparison accumulated 0.72 μmol Na^+ g^{-1} DW. Yield in the plots varied from 0.173 kg per plot to 0.939 kg per plot. Note the yield has not been scaled up to ha^{-1}, as we were using two row breeder's plots which has more spacing between lines/rows that would normally be observed in a field.

Importantly, we were able to categories the RILs depending on their performance into 5 groups (Figure 3):

1) Lines which accumulated low leaf Na^+ and had similar yield to Gladius, an Australian cultivar which accumulates low amounts of Na^+.
2) Lines which accumulated high concentrations of leaf Na^+ and were salt sensitive. These lines had a yield half as much as Gladius (salt sensitive phenotype). These plants are defective in controlling the movement of Na^+ to the shoot and be missing Mocho de Espiga Branca's unique salt tolerance mechanisms.
3) Lines which accumulated low levels of leaf Na^+ AND had poor yield (poor growth phenotype and poor salt tolerance).
4) Lines that accumulated high levels of leaf Na^+ and had similar yield as Gladius (good phenotype). These lines will have Gladius' good quality traits, and one or more of Mocho de Espiga Branca's unique salt tolerance mechanisms
5) Lines that accumulated high levels of leaf Na^+ and had a yield greater than Gladius (very good phenotype). These plants will have Gladius' good quality and yield traits but have all of Mocho de Espiga Branca's salt tolerance mechanisms.

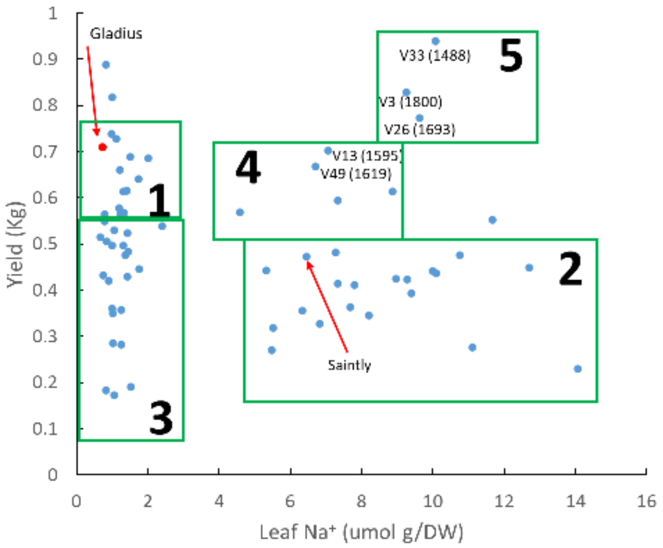

Figure 3. The five major groupings of 64 lines based on their leaf Na^+ accumulation and yield. **1)** Lines with a similar phenotype to Gladius (good yield, low leaf Na^+); **2)** Lines which accumulate high leaf [Na^+] and have poor yield; **3)** Lines which accumulate low leaf Na^+ and have poor yield); **4)** Lines which accumulate leaf [Na^+] >10× more than Gladius and have similar yield; **5)** Lines which accumulate leaf [Na^+] >10× more than Gladius and have better yield.

Lines MG1488 (V33), MG1800 (V3) and MG1693 (V1693) were the best performing, accumulating high leaf Na^+ concentrations (10× greater than Gladius) and showed a 5 – 28% yield improvement. These lines have the unique salinity tolerance mechanism from Mocho de Espiga Branca, which allow them to accumulate high levels of Na^+ and have a better yield. In addition, given the conditions at the Roseworthy site were only moderately saline, it is hypothesized that the yield improvement of these lines was due to their ability to use the higher leaf Na^+ concentration to lower leaf water potential and extract more water from the soil, thus improving their growth. While there will be an upper limit as to how much Na^+ these lines are able to accumulate before it is detrimental to the plant, there is the possibility that in addition to having higher salinity tolerance, these plants will be particularly suited for improving yield in drier (drought) years.

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

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

Mocho de Espiga Branca is a wheat landrace with unique salinity tolerance mechanisms that allow it to accumulate over 10× as much shoot Na^+ as Australian elite varieties of bread wheat. However, being a landrace, it has many undesirable traits including (but not limited to a long time to flowering and plant height). The salt tolerance trait(s) of Mocho de Espiga Branca needs to be studied and transferred intoAustralian cultivars. In previous SAGIT projects (UA317 & UA418) a novel recombinant inbred line population was produced between Mocho de Espiga Branca and Gladius.

Phenotyping of this population in the greenhouse identified a number of genetic loci linked to enhanced performance under salinity stress.

In the current study we evaluated a selection of Mocho de Espiga Branca × Gladius RILs in a mild/moderately saline field to determine whether it was possible to have good or enhanced yield with a novel high Na^+ accumulating salinity tolerance mechanism. We identified 3 RILs which could accumulate significantly higher leaf Na^+ than Gladius (>10×) which also had a 5-28% yield improvement. We also identified a further 5 RILs which accumulate significantly higher leaf Na^+ than Gladius (>10×).

INTELLECTUAL PROPERTY

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

We have produced three lines from the Mocho de Espiga Branca × Gladius RIL population (MG1488 (V33), MG1800 (V3) and MG1693 (V1693)) with enhanced salinity tolerance and enhanced yield. A further 5 lines (MG1409 (V39), MG1595 (V13), MG 1619 (V49), MG 1650 (V18), MG 1660 (V22)) have similar yields to Gladius but with enhanced salt tolerance. With molecular markers designed to relevant salt tolerance traits, these 8 RILs could be crossed to current elite germplasm, such as Calibre and Vixen to introduce enhanced salt tolerance into current material.

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.

- Salinity stress has been estimated to result in up to $1.3B loss to Australian agriculture. In South Australia up to 50% of the cropping area can be affected by transient salinity
- Mocho de Espiga Branca is a wheat landrace with the ability to accumulate 10× as much shoot Na^+ than Australian cultivars but has other qualities not desired in Australian wheat (time to flowering and plant height)
- Novel lines from a population produced by crossing Mocho de Espiga Branca with the Australian cultivar Gladius were evaluated for their salinity tolerance in the field
- Lines with high salt tolerance (accumulating 10× as much leaf Na^+ compared to Australian varieties) with improvements in yield (>5%) were identified.
- Further development of these lines, by introducing the salt tolerance trait into current elite Australian germplasm, would allow the development of germplasm better adapted to subsoil constraints found in South Australian farms.
- Further evaluation of the material at different sites over different years, as well as pre-breeding to introduce the salt tolerance traits into germplasm suitable for breeders to use in their breeding programs is required. Additional funding will be sought from sources such as the GRDC.
- Stuart Roy hosted the SA Crop Science Society on October 7th 2021 at the field trial site and described the aims of the project and the work towards a wheat variety that can accumulate leaf Na^+ without detrimental effects. The results from this project have been discussed with SARDI's Agronomy and Crop Improvement groups and with the GRDC.

POSSIBLE FUTURE WORK

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

Seed of MG1488 (V33), MG1800 (V3) and MG1693 (V1693), along with Gladius was provided to Stefan Schmitt of Ag Consulting & Research (http://agconsultingco.squarespace.com) for including in a field trial on the Yorke Peninsula in 2022. Enough material was provided for some single row plots. The evaluation of these lines at a different site in a second year will help establish the stability of the trait and the repeatability of the performance of those lines observed in Roseworthy in 2021. Seed multiplication will allow the lines to be evaluated in additional field trials in later years.

Further QTL analysis of the population will continue to identify the genetic regions responsible for enhanced tolerance and yield in the RILs. This will facilitate the development of molecular markers for use with marker assisted selection to introduce salt tolerance traits into commercial crops.

We are in discussion with the South Australian Research and Development Institute (SARDI) about a combined approach to the Grains Research and Development Corporation (GRDC) for further funding of this project. SARDI have similar bread wheat material (from a difference genetic source) that can also accumulate high levels of shoot Na^+. A combined approach would evaluate both the material described in this project and SARDI's material, across multiple field sites, while developing a breeding strategy to develop pre-breeding plant material and markers which can be used by Australian breeders. In addition, the University of Adelaide and SARDI have also been engaging with the Department of Primary Industries and Regional Development (WA) about the possibility of getting funding for continuing this work from the Australian Centre for International Agricultural Research (ACIAR), as this material has potential for improving productivity in countries such as Pakistan and Bangladesh. Results from an ACIAR project would be used to benefit Australian growers.