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	**UA418**
PROJECT TITLE (10 words maximum)	
Phenotypic evaluation of a wheat RIL population for salinity tolerance	

PROJECT DURATION *These dates **must** be the same as those stated in the Funding Agreement.*			
Project start date	1/09/2018		
Project end date	28/02/2020		
SAGIT Funding Request	**2018**	**2019**	**2020**

PROJECT SUPERVISOR CONTACT DETAILS *(responsible for the overall project)*		
Title:	**First Name:**	**Surname:**
Assoc Prof Dr	Stuart Allison	Roy Pearson
Organisation:	The University of Adelaide	
Mailing address:		
Telephone:		Email:
Mobile:		

ADMINISTRATION CONTACT DETAILS *(responsible for all administrative matters relating to project)*		
Title:	**First Name:**	**Surname:**
Organisation:	The University of Adelaide	
Mailing address:		
Telephone:		**Email**:
Mobile:		

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. The mechanisms and genes behind Mocho de Espiga Branca ability to tolerate the accumulation of high shoot Na^+ have to be identified prior to using marker assisted selection to introduce them into Australian cultivars.

A Mocho de Espiga Branca × Gladius wheat recombinant inbred line (RIL) population was characterized for its salinity tolerance using the Smarthouse based analysis platform at the Australian Plant Phenomics Facility, The Plant Accelerator. Plants were grown under both control and salt stressed conditions with 37 phenotypic traits, including plant biomass, plant growth, ion content and grain production measured. Using a previously developed genetic map, 465 genetic loci were identified relating to the performance of the plants (228 loci under control and 237 loci under salt stress). These included genetic loci linked to plant growth under salinity, sodium, potassium or chloride accumulation in leaves, plant biomass and yield.

With promising new genetic loci identified, the population will now undergo field evaluation in 2021 to determine if these can improve performance in the field

Project objectives

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

1. Identify novel mechanisms (how the plant can move/exclude salt inside it) of salinity tolerance in a SAGIT funded bread wheat mapping population, Mocho de Espiga Branca (Mocho) × Gladius to identify traits linked to enhanced growth.
2. Identify the genetic regions (regions of the plants DNA) underpinning these salinity tolerance traits, to select lines for evaluation in the field and for future breeding programs.
3. Based on greenhouse results, generate wheat lines which contain either the salt tolerance or salt sensitive genetic region for future field evaluation.

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), Assoc Prof Stuart Roy (supervisor of phenotyping; project lead from 1st January 2020), Dr Takashi Okamoto (growth of plants, phenotyping and analysis), Dr Jessey George (processing of experimental material and analysis), Dr Julian Taylor (Biometry Hub, genetic analysis)

As detailed below the project successfully met aims 1 and 2, however, only partially delivered aim 3.

This project was able to successfully leverage data and knowledge from other projects. Dr Pearson was successful in obtaining funding from the Yitpi Foundation to cover the costs of the genotyping of the RIL population using next generation sequencing. This enhanced our ability to detect genetic loci linked to salinity tolerance traits in this project. A Chinese International Scholarship PhD student supervised by Dr Pearson and Assoc Prof Roy, identified the main mechanism and gene behind the high sodium accumulation in Mocho de Espiga Branca (a defective protein which transports sodium), knowledge which was used in this project.

We have been able to identify a number of novel tolerance mechanisms in the population, with heritability results suggesting that in future the introduction of these into breeding programs would be successful. We were able to identify 465 genetic loci linked to 37 phenotypic traits, 237 of these loci

were linked to traits in salt stressed plants. Continued analysis of these results will help to identify those loci with the greatest potential to enhance yield under saline conditions.

There were a couple of delays encountered in 2019 regarding the phenotyping of the RIL population in the Plant Accelerator. The first due to another project being run in The Plant Accelerator running over time and the second due to lighting parameters for our experiment incorrectly set up set up by the Plant Accelerator staff. This set the project back by several months. The delays meant that the data analysis could not be completed by the end of 2019 as initially planned, however, this was completed by April 2020.

Aim 3 was only partially completed. In 2019 seed multiplication of the RIL population in the greenhouse took place in parallel with the Plant Accelerator phenotyping experiment. Enough seed was produced for planting in a summer nursery to obtain enough seed for field trials in 2020 (SAGIT project UA419). However, issues with segregation of undesirable landrace traits (such as long flowering time) in some of the lines, and extreme summer temperatures, resulted in poor seed set from many of the lines. To correct for this, the material will again undergo seed multiplication in 2020 under more favorable conditions. They will be used in SAGIT funded field trials in 2021 (UA419). A variation to project UA419 to delay field trials to 2021 has been granted. The lines which have undesirable traits in the field (delayed flowering time, head shattering) will not be multiplied for field trials.

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.**
Line selection and statistical design of greenhouse phenotyping experiment	Yes ☒ No ☐	
Perform smarthouse based analysis at the Plant Accelerator Facility on the salinity tolerance of the Mocho × Gladius mapping population, with plants moved to greenhouses and grown to yield	Yes ☒ No ☐	
Winter bulking of lines for field experiments	Yes ☒ No ☐	
Summer/Autumn bulking of lines for field experiments	Yes ☐ No ☒	Summer bulking commenced in the birdnetted area on the Waite campus however, issues with extreme heat significantly affected yield from plots. This was compounded in some lines which had undesirable traits, such as too long to get to flowering or head shattering
Perform QTL analysis of results to determine regions of the genome that allow the plants to be more salinity tolerant	Yes ☒ No ☐	
Select plant lines for future field experiments	Yes ☒ No ☐	
	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.

A previous SAGIT funded project UA317 used speed breeding to develop a Mocho de Espiga Branca × Gladius recombinant inbred line (RIL) mapping population. 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.

Methods

210 Mocho de Espiga Branca × Gladius RILs were selected at random from the larger 320 RIL population for phenotyping under control and saline conditions. They were grown in the facilities of Australian Plant Phenomics, Plant Accelerator, using both traditional greenhouses and the Accelerator's Smarthouse. A partially replicated design was constructed (42 lines duplicated, 168 unduplicated), with plants from the same line undergoing different stress treatments grown next to each other to minimize special effects in either the greenhouse or Smarthouse.

Uniform seed from each RIL line were imbibed in water at room temperature for 4hrs, then in the dark for a further three days prior to sowing. Three seed from each line were sown in a single plastic pot filled with University of California soil, peat and clay loam. Plants were arranged in a greenhouse as per the statistical design. At the emergence of the second leaf, plants were thinned to one uniformed sized plant per plot. At the emergence of the 3rd leaf, pots were loaded on to the conveyer belt in The Plant Accelerator's Smarthouse (Figure 1), where they were weighed daily and automatically watered to maintain water soil content at 17% (w/w). At the emergence of the 4th leaf (19 days after planting) the plants were treated with either 0 or 150 mM NaCl. Pots were continued to be watered to maintain soil content at 17% (w/w) to keep salinity levels constant.

Figure 1. Mocho de Espiga Branca x Gladius RILs lines growing at the Plant Accelerator 2019.

Using the non-destructive imaging system (LemnaTex 3D Scanalyzer) in the Smarthouse, the shoot area of each plant was determined from before salt treatment (16 days after planting) until 32 days after planting. This allows measurements of biomass and growth rates over time. After the final imaging time point, the 4th leaf from the main tiller was harvested for ion analysis by flame photometer (Na^+ and K^+) or a chloride analyser (Cl^-). Plants were removed from the Plant Accelerator Smarthouse and returned to a greenhouse where they were grown through to seed production. The statistical design was maintained in the greenhouse and the pots were continually watered to weight (17% w/w) to maintain consistent water and salt levels. Continual observation of plant development, such as time to flowering, were maintained. At anthesis (flowering) the leaf below the flag leaf (Flag-1) was removed for ion analysis as before. Once the plants had completed grain fill, destructive

measurements of traits including plant height, biomass, spike weight, number of productive tillers, grain weight and grain number were obtained.

In addition to the main experiment described above, the same 210 Mocho de Espiga Branca × Gladius RILs were grown in a separate greenhouse for seed multiplication. One plant per pot was sown in coco peat and the plants watered accordingly. Observations on plant development were recorded, such as time to flowering. Grain was collected from these lines and sown in a summer nursery for seed multiplication for field trials. Small 5 row, 1 m long plots were sow. Standard agronomic practice was used to grow the plants.

Results

A large range in phenotypes was observed in the population grown in The Plant Accelerator. This included distinct groups of plants which accumulate high levels of leaf Na^+ irrespective of whether they were grown in 0 or 150 mM NaCl (the plants are even accumulating the very low amounts of Na^+ present as background in control soil). Work by a PhD student, who has been investigating the mechanisms behind this phenotype in parallel with this SAGIT project, has identified that a single mutation in a key gene (*TaHKT1;5-D*) which transports Na^+ through the plant results in high leaf Na^+ concentrations in plants with the Mocho de Espiga Branca version of the gene. While this is exciting in itself, it does not tell us the mechanism as to how plants with the Mocho de Espiga Branca version of the gene survive having such high leaf Na^+. Analysis of the gentic loci (quantitative trait loci, QTL) which are linked to other phenotypic traits will help elucidate the novel salinity tolerance in Mocho de Espiga Branca.

Using the 37 phenotypic traits measured in the Plant Accelerator, in combination with the genetic map containing 3490 molecular markers, generated by Dr Pearson's Yitpi Foundation grant, 465 QTL were found in the population. Under control conditions, 228 loci were identified as being linked to the differences in the performance of Mocho de Espiga Branca and Gladius. Under saline conditions, 177 loci were identified as being linked to the differences in the performance of Mocho de Espiga Branca and Gladius, with a further 60 loci identified as being linked to differences in salt tolerance between the cultivars (Figure 2).

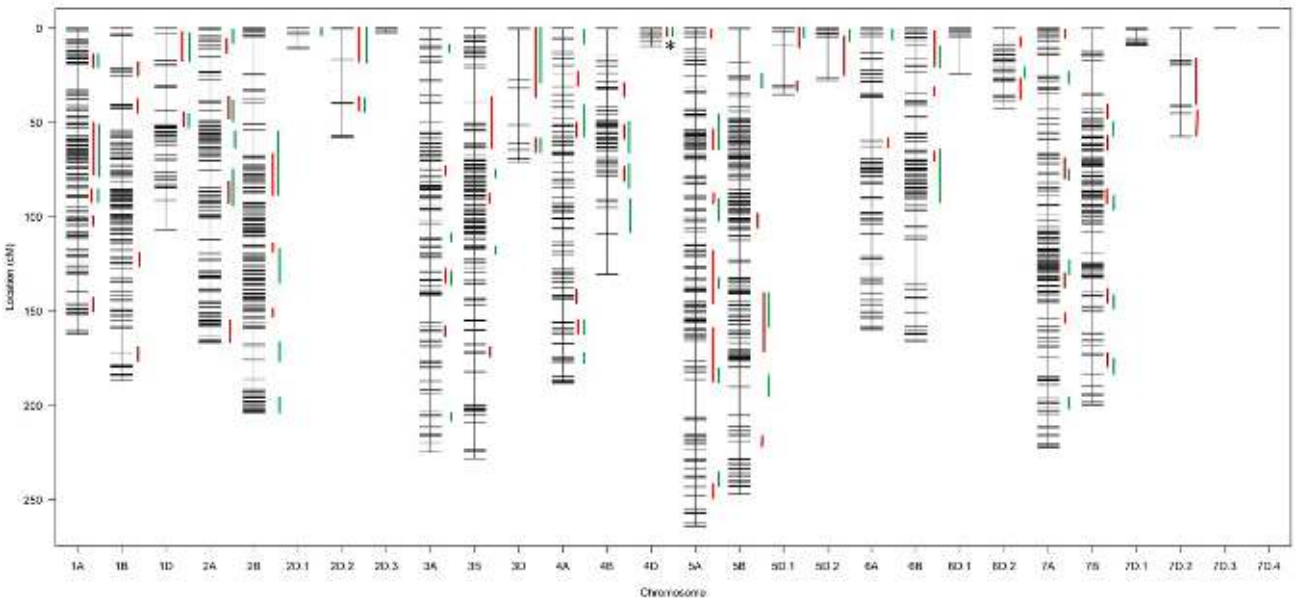

Figure 2. Graphical representation of the wheat genome and the location of the quantitative genetic loci (QTL) for 37 phenotypic traits. For simplicity the location of all 465QTL for individual traits have not been shown, instead the region on the wheat chromosome which is linked to any QTL under 0 mM (red bars) or 150 mM (green bars) NaCL are shown. The location of the *TaHKT1;5-D* gene, which has been shown to have a large effect on leaf Na^+ concentration is shown with a * on chromosome 4D.

Some of these loci have a large effect on the phenotype of the plant, others a more minor effect and will perhaps require stacking with other minor traits to improve the salinity tolerance of wheat.

Importantly, it was found that 29 of the 37 phenotypic traits measured were hereditable and could be used in breeding programs. This included traits for leaf Na^+, K^+ and Cl^- accumulation, plant growth, plant biomass and water use efficiency. QTL hotspots, where different phenotypic traits were linked to similar regions of the genome were identified on chromosomes 1A, 1B, 1D, 2A, 2B, 2D 3D, 4A, 5A, 5D and 6B. The hotspots on 1A, 1D, 2B, 3D, 4A, 5A and 5D were found under both control and salt stressed conditions, suggesting that these are QTL controlling differences in phenotype between Mocho de Espiga Branca that are independent of treatment. Novel QTL which were only identified under salt treatment were found on chromosomes 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 5B, 6A and 7A, suggesting that these regions are involved in specific adaptations to salinity stress. In addition to the already described *TaHKT1;5-D* gene, genetic regions involved in ion accumulation were identified on chromosomes 1B, 2A, 2B, 3A, 3D, 4A, 4B, 5A, 5B, 5D, 6A, 6D, 7A and 7D. QTL relating to growth and water use efficiency were identified on 1A,1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 6B, 7A and 7B. QTL relating to yield were identified on 1A, 1B, 1D, 2A, 2B, 3A, 3B, and 6B.

Currently, the genetic loci linked to plant performance in saline conditions correspond to large amounts of DNA in the plant genome. The key steps going forward will include verification of these results in field grown material (as will be done in SAGIT project UA419) and narrowing down the size of these genetic loci to the specific gene(s) responsible for the phenotype. The latter will facilitate the development of molecular markers which could be used for marker assisted selection in pre-breeding and breeding programs. Further analysis will also take place using the data already generated to remove compounding effects due to the differences in the RIL parent cultivars' time to flowering and height.

In the Accelerator, the greenhouse seed multiplication, and summer nursery seed multiplication experiments observations of flowering time were taken. The RIL parent Gladius can flower up to one month earlier than the Mocho de Espiga Branca parent, and this can be seen in the RIL populations, with lines flowering either like one parent or the other. The greenhouse seed multiplication exercise was successful in generating enough seed for multiplication trials in the summer nursery. Unfortunately, the very warm and dry summer had a severe effect on the yield of the summer nursery trials, with many producing small amounts of seeds. There were also issues with the aforementioned flowering time, with some plants flowering too early and shedding seed, others flowering late and others not flowering at all. This will prove problematic when measuring the yield of the lines in the field (UA419). In light of the various issues around seed multiplication for field trials in 2020, a decision was made to postpone field trials of the lines until 2021 and re-grow lines with similar flowering times in the greenhouse in 2020.

CONCLUSIONS REACHED &/OR DISCOVERIES MADE (Not to exceed one page)
Please provide concise statement of any conclusions reached &/or discoveries made.

The phenotyping and quantitative trait loci mapping of a Mocho de Espiga Branca RIL population has identified a number of novel salinity tolerance mechanisms and genetic loci. These loci are linked to a number of phenotypic parameters including ion accumulation, plant growth and yield in saline conditions. A total of 465 QTL were identified in this population by growing them in both control and saline stressed conditions. Of these 465 QTL, 237 were identified under saline conditions. Heritability analysis suggests that it would be possible to introduce loci that would improve ion tolerance, growth and yield into elite germplasm.

Seed continues to be multiplied for these lines in anticipation of sowing them in a saline paddock in 2021 (UA419). Analysis of the field material will determine which salinity tolerance traits and QTL are translatable from the greenhouse to the field, and will guide future pre-breeding activity to introduce salt tolerance traits into elite Australian wheat cultivars

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

Phenotypic data for 37 traits of 210 Mocho de Espiga Branca × Gladius RILS grown under control is available for use by other researchers. We have multiplied seed of the RIL population which can be made available to other researchers. As Mocho de Espiga Branca is a novel landrace, it is possible that it could provide a novel source of genetic diversity for other abiotic and biotic tolerance mechanisms

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
- A new bread wheat mapping population has been used to investigate mechanisms for enhancing the tolerance of wheat to saline soils
- A large number of genetic loci linked to a number of salinity tolerance traits, including plant growth, ion accumulation and yield, were identified using the advanced phenotyping facilities at the Australian Plant Phenomics Facility, The Plant Accelerator
- Seed multiplication is underway for future field trials of this population in a saline field site. Results from these field trials will identify the best candidate loci to include in future pre-breeding trials

The work on this project has been presented at the annual SAGIT update in July 2019. Aspects of this project have been presented at the Gordon Research Conference for Salt and Water Stress in Plants, New Hampshire, USA and the 1st International Wheat Congress, Saskatoon, Canada. Through Assoc Prof Roy's affiliation with the Australian Research Council (ARC) Industrial Transformation Research Hub for Wheat in a Hot and Dry Climate, aspects of this work have been discussed with wheat breeders (AGT, Intergrain and LongReach PB). Finally, it is our intention to publish the data from this project in an open access journal, so that the results are freely available to researchers, breeders and growers.

POSSIBLE FUTURE WORK

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

Data analysis will continue to refine the QTL results and to identify best bet candidates for enhancing salinity tolerance for future study. Selection of loci will depend on the level of effect the loci has on the plant phenotype, the ability to fine map the genetic region and the availability of RIL lines with both heterozygosity or genetic recombination in those regions. It is envisioned that the research program will be split in two, with one aspect taking a more basic scientific approach to understand the mechanisms behind salinity tolerance, while an applied approach will determine which alleles are best for incorporation into Australian wheat germplasm.

Field evaluation of a selection of RILs in a saline paddock will take place in 2021 as part of a SAGIT funded project UA419. This work will help validate whether QTL measured in the glasshouse can be translated to the field. The project will also facilitate the measurement of proper yield parameters in field conditions, rather than in plant pots.

In recent years, cereal salinity projects at the University of Adelaide and the South Australian Research and Development Institute (SARDI) have been identifying very similar mechanisms for enhancing the growth of both wheat and barley under saline conditions. We have been in discussions with researchers at SARDI on how best to combine efforts, combining both our skills in genomics, while making use of their expertise in field trials along with skills at Adelaide University in understanding the mechanisms behind salinity tolerance. We are currently exploring avenues of seeking investment from the GRDC, either directly or through their tender schemes, to further this research. The Mocho de Espiga Branca × Gladius RIL population has been identified as a key research resource in that collaboration. It is envisioned that through such a scheme we can further characterize the Mocho de Espiga Branca × Gladius RIL population in a number of field sites, while identifying the best genetic loci and alleles to introduce into elite Australian breeding lines.

We have recently initiated a collaboration with Prof Robbie Waugh from The James Hutton Institute (Scotland) who has independently identified a similar Mocho de Espiga Branca *HKT1;5* mutation which results in high accumulation of shoot Na^+ in barley. It is likely that similar salinity tolerance mechanisms exists in these barley accessions as in Mocho de Espiga Branca. In collaboration Prof Waugh and The James Hutton Institute we have submitted an application for an ARC Discover project to dissect these tolerance mechanisms in barley further, with a goal to translate those findings into wheat through use of the Mocho de Espiga Branca × Gladius mapping population. With Prof Waugh and a number of European partners, we have also submitted a grant application to the Cofund on Sustainable Crop Production for a project entitled Barley Responses and Adaptation to Changing Environments. The Australian component of this work will investigate whether barley with the Mocho de Espiga Branca *HKT1;5* allele has better performance in the Australian environment.