FINAL REPORT 2015

PROJECT CODE : S12/05

PROJECT TITLE
Cost-effective selection of high beta-glucan using molecular markers

PROJECT DURATION

Project Start date	1 July 2012
Project End date	30 June 2015

PROJECT SUPERVISOR CONTACT DETAILS

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Project Code	
Project Type	

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PROJECT REPORT

Executive Summary

- Genes contributing to high β-glucan content in barley grain are known and this project aimed to isolate the same sequences from oat varieties Yallara and Mitika which are known to differ in their grain β-glucan content.
- In oats, more than 15 versions of the main candidate β-glucan gene, CslF6, have been identified. While there are distinct differences between the identified members of the CslF6 gene family copies, the comparison of these individual gene sequences between Yallara and Mitika varieties has shown that they are highly conserved and therefore there is limited gene sequence variation from which to design molecular markers that can be applied in breeding. This highlights the genetic complexity of the control of grain β-glucan content in oat, relative to barley.
- In comparison to the known β-glucan sequences from barley and rice, the oat CslF6 is of different sequence and structure.
- This project has provided important information that can guide the next steps for research in this area. This should include the screening of mapping populations to determine the genetic location of loci controlling grain β-glucan in oat and further investigation into underlying biological differences between high and low β-glucan lines, such as cell wall structure and composition. Screening of oat germplasm to identify alternative sources of high β-glucan would also complement these approaches.

Project Objectives

The primary aim of this proposal is to develop molecular tools to screen for high β-glucan content in the National Oat Breeding Program. Due to the time and high cost involved in determining β-glucan content, the grain of only a few advanced breeding lines is routinely being assessed. The late selection doesn't capture promising lines in the early breeding stages and the total potential of oat germplasm is not being exploited. The development of a molecular marker for β-glucan content would allow cost effective screening for this important trait in the National Oat Breeding Program.

Overall Performance

The present work has shown that the control of oat grain β-glucan content is likely to be more genetically complex relative to barley than was expected. The results show that a reverse genetics candidate gene approach was not successful in identifying the gene responsible for increased β-glucan content seen in the oat variety Yallara. In oat, more than 15 β-glucan gene sequences have now been identified, they may represent duplicated copies of genes similar to those controlling β-glucan content in barley, or additional genes in oat that are not present in barley. Furthermore, in comparison to the known β-glucan sequences from barley and rice, the oat gene is of different sequence and structure.

Despite the identification of many copies of β-glucan-like genes in oat, gene sequence differences were not detected between high and low β-glucan lines meaning that the development of markers to associate those β-glucan gene copies with β-glucan content was not possible. This is an important result as it guides the directions of future research in this area in oats to enable tools that can improve the efficiency of breeding for this important quality characteristic.

Personnel involved: Tim Sutton (commenced 24th November 2014), John Harris, Klaus Oldach (resigned from SARDI June 2014), Judy Cheong.

Key Performance Indicators (KPI)

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
First oat β-glucan gene isolated and complete sequence obtained	Y	
All six target β-glucan genes isolated from oat	Y	
Gene comparisons between high and low β-glucan lines completed	Y	
First markers developed that predict high β-glucan content	N	No cultivar specific sequence variation was identified which is the basis for molecular marker development.
Early and advanced generation breeding lines assessed with CCN and β-glucan markers	N	Due to the lack of a molecular marker, a different approach was taken whereby a Yallara x Mitika mapping population was phenotyped for β-glucan content by two methods i.e. flow injection and megazyme. CCN markers have been screened across a set of advanced breeding lines and information provided to the oat breeder.
Write and submit Final Report	Y	

Technical Information

Two genes are believed to be the main candidates in β-glucan biosynthesis in barley grain, CslF6 and CslF9. Searches of the database for oat expressed sequence tags presently only yield sequence for a CslF6 homologue and not CslF9. Based on this

sequence information the coding sequence for the CslF6 gene was obtained from Yallara and Mitika. Comparison with barley and rice revealed a 93% and 85% amino acid conservation, respectively. Comparison between oat cultivars revealed 100% sequence conservation.

Cloned sequences of the Yallara and Mitika CslF6 coding sequence revealed a putative single nucleotide polymorphism (SNP). Attempts to develop KASP and CAPS markers failed suggesting the SNP is either present in both cultivars or was the result of a sequencing error, or was due to the unsuitability of surrounding sequence, respectively.

A rare variant of intron II, found only in one clone from Mitika, also proved fruitless after further investigation. A KASP assay failed to distinguish any parental differences and a nested PCR from both Yallara and Mitika yielded products suggesting the variant is to be found in both parents.

In total, approximately 130 clones of Yallara and Mitika CslF6 intron II were sequenced which revealed, to date, 15 different versions of intron II of CslF6 in oat. These formed 7 structurally related gene copies (Figure 1).

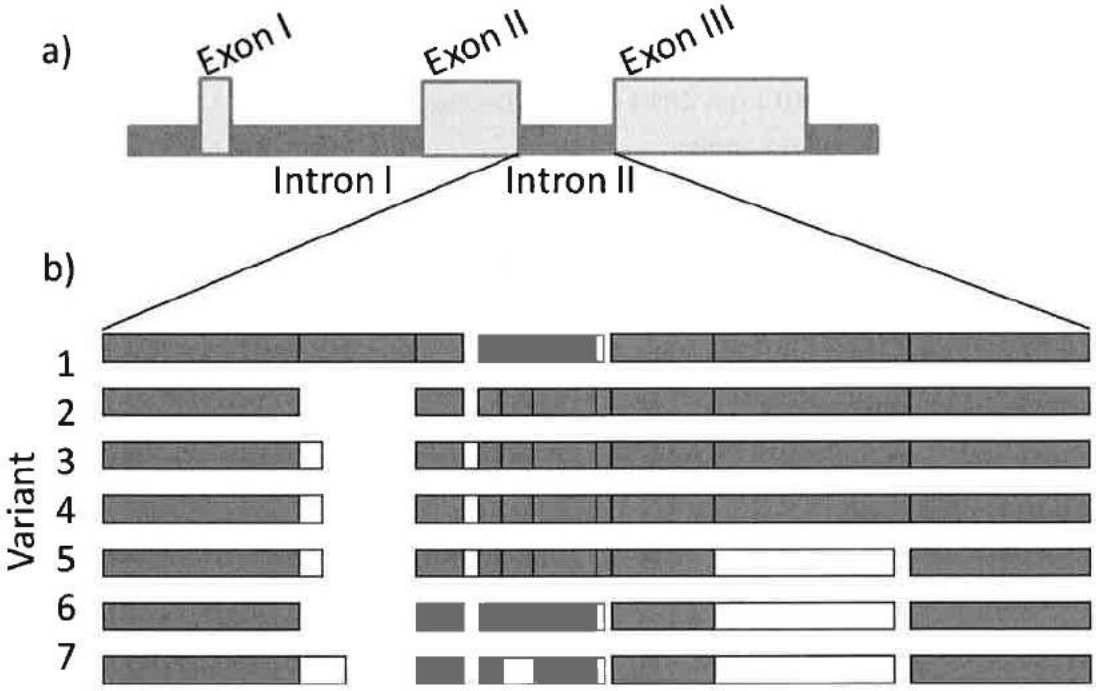

Figure 1: Gene structure of the β-glucan gene CslF6

a) the exon (pale grey) and intron (dark grey) pattern of the β-glucan gene from oat. Exons are highly conserved as they encode for the CslF6 protein whereas introns can be highly variable as they are edited out prior to protein synthesis.
b) general structure of the seven identified intron II variants based on approximately 15 distinct versions of the oat β-glucan gene CslF6. Introns are aligned into blocks of similarity and colour coded to imply similarity at each block, either white or dark grey.

An alternative forward genetics strategy was explored whereby a QTL investigation of a field grown Yallara x Mitika mapping population was initiated for grain β-glucan

content. GBS data and a genetic map for Yallara by Mitika had been previously generated at an F3 stage (GRDC grant DAS00133). Unfortunately, only 50 of the single seed descent lines from the F3 map had persisted to the F6 stage that was grown in the field. However, the megazyme β-glucan assay kit, that has been used to determine the barley grain β-glucan content QTL, was used to determine β-glucan content of the 50 lines (Figure 2). In addition, the flow injection method employed by AEGIX was also pursued and gave results with little consistency when compared with the megazyme assay (Figure 2). This discrepancy suggests that as an industry standard the validity of the flow injection method may require further validation for specificity towards β-glucan.

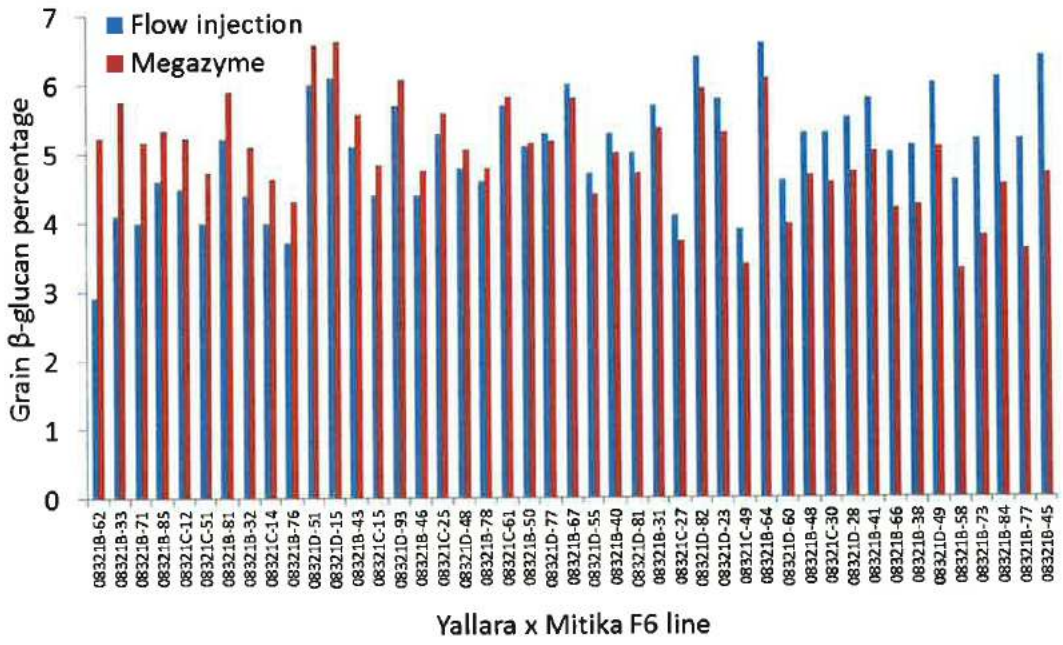

Figure 2: Oat grain β-glucan content percentage as determined by flow injection and megazyme assay

QTL mapping was attempted, unfortunately, no significant QTL were identified likely due to the few lines ultimately phenotyped and the GBS map being based on F3 plants with residual heterozygosity.

Conclusions Reached &/or Discoveries Made

β-glucan is an important trait for breeding in oats, and has gained much attention in Australia and internationally. The high cost, relatively low throughput, and technical challenges of phenotyping for β-glucan content in grain make it prohibitive currently for a breeding program to screen all germplasm early in the breeding cycle when the numbers of lines are large. This, potentially, is resulting in the loss of lines with high β-glucan content from the program, and limiting the breeding program's ability to exploit diversity for this trait amongst large and diverse germplasm collections. The availability of molecular markers would give the breeding program a competitive

advantage and benefit growers who would have access to marketable high β-glucan varieties.

In barley, two genes have been associated with high β-glucan in grain. This project aimed to investigate the orthologous (similar) sequences in oat, identify variety specific variation, correlate this with β-glucan content and then develop markers that can be applied in breeding. This project has shown that the control of β-glucan content in oats is more genetically complex than in barley, based on the identification of as many as 15 different CslF6 genes versions. They may represent duplicated copies of genes similar to those controlling β-glucan content in barley, or additional genes in oat that are not present in barley. Varietal specific difference in gene sequence that may correlate with grain β-glucan content was not able to be identified. Whilst we cannot rule out the possibility that CslF6 is involved in variation in grain β-glucan content in oat, the results support the need for further investigation into the CslF6 gene members, and indeed other members of the Csl family in oats. Furthermore, the results suggest that an approach based on transferability of knowledge from one species to another in this case will only partially assist us to achieve the goal of markers for selection in breeding.

This project has provided important knowledge that guides the next steps for research in this area in oats. Alternative strategies can now be considered. These would include genetic mapping in populations that segregate for β-glucan content to identify associated regions of the genome. This approach will enable the development of linked molecular markers that could be applied to breeding, and also provide an opportunity to investigate which of the Csl gene family members is most likely to be involved with high β-glucan content. Whilst the genome of oat is not sequenced, we can infer information on gene content in localized regions in oat from information derived from other species that have sequenced genomes such as rice and barley.

Intellectual Property

During the project, a large number of candidate β-glucan gene sequences (more than 15) were identified, many of which possess novel sequences. However, until these sequences can be associated with increased or decreased β-glucan content in oats the value of this intellectual property would likely be limited.

Application / Communication of Results

- In comparison to the known β-glucan sequences from barley and rice, the oat CslF6 gene is of different sequence and structure.
- More than 15 versions of the *CslF6* gene controlling β-glucan content have been identified in oat, highlighting the genetic complexity of the control of this trait in this species relative to barley where more is known.
- While there are distinct differences between the identified members of the *CslF6* gene family copies, the comparison of these individual gene sequences between selected high and low β-glucan oat varieties has shown a high level of conservation and limited sequence variation , which is necessary for the development of molecular markers.
- These results have shown that a complementary approach is warranted so that association can be made between the many identified β-glucan gene sequences

and increased β-glucan content in oat grain. This could involve the screening of existing mapping populations and undertaking genetic analysis, or the development of new mapping populations between lines with contrasting β-glucan content. This information would be used to target the specific copies of the β-glucan genes that determine high β-glucan content in oat, then providing a basis for molecular marker development.

Progress of the project has been regularly communicated to the leader of the National Oat Breeding Program, which is where the molecular tools can be applied to improve selection. Close linkages to the breeding program ensure that the project has access to relevant oat germplasm in our study and that we pass on the latest development in the project. Through the National Oat Breeding Program, information on the β-glucan research in oats is communicated to growers at events such as field days and to industry through meetings with collaborative partners such as Uncle Tobys.

POSSIBLE FUTURE WORK

The outcomes of this project have provided some basic and essential information to guide the future direction of research that will enable the cost efficient selection of high β-glucan oats and derive a competitive advantage for the South Australian grains industry. During this work we have learned that the control of β-glucan content in oat grain is more complex than assumed from knowledge in barley, and to the extent that was possible in this project, variation in identified β-glucan gene sequences was not able to be correlated with high β-glucan content.

A number of future approaches and partnerships can be considered to advance this research.

The approach in this project was to leverage existing information in barley to identify likely candidate genes controlling β-glucan content in oat. This cross-species reverse genetics approach has been demonstrated to work for other traits in cereals, but in this case it appears to have been complicated in oat by the fact that β-glucan content appears to be under more complex control than in barley, or controlled by genes other than those in the *CslF6* family. Importantly, this is useful information as it suggests that a QTL analysis approach utilising mapping populations would be warranted. Resources are currently available that can be considered for such an approach, which include an F6 Yallara x Mitika recombinant inbred line (RIL) population. Analysis of β-glucan content in such a mapping population would enable:

- Verification that population shows significant heritable differences in β-glucan content, a prerequisite for mapping the location of a gene controlling the trait
- Verification of chromosomal location of loci controlling β-glucan content in oat, and use of synteny with rice and barley to determine if *CslF6* genes may still be involved. Identification of loci involved will permit downstream approaches that can fine map the critical regions, identify the genes involved potentially and generate markers for breeding.
- As part of this process, candidate β-glucan genes can be validated in a grain developmental series. This would involve the sampling of grain tissue at critical stages of development, and investigating the expression of candidates to

determine if gene regulation is correlated with changes in grain β-glucan content.

To complement this type of approach, it would be important to investigate further the underlying biological differences between high and low β-glucan lines, such as cell wall structure and composition. The project team has recently established collaboration with Associate Professor Rachel Burton of the ARC Centre of Excellence in Plant Cell Walls. Dr Burton has significant experience in cereal cell wall biology, being involved with the initial work on β-glucan in barley that this project was based on. An ongoing collaboration is being developed that will provide the potential to understand more about the underlying biological differences between high and low β-glucan oat varieties. Screening of diverse oat germplasm to identify alternative sources of high β-glucan lines that can be studied and utilised in breeding programs would complement these approaches.

AUTHORISATION	
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