Biological Systems: Open Access

Lauer et al., Biol Syst Open Access 2017, 6:1
DOI: 10.4172/2329-6577.1000178

Short Communication Open Access

Genetic Variation in Barley (1→3,1→4)-β-Glucan Endohydrolases: A Short Commentary

J Lauer*, J Eglinton, S Cu and R Burton

School of Agriculture, Food and Wine, The University of Adelaide, South Africa

***Corresponding author:** Juanita Lauer, School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1 Glen Osmond SA 5064, Tel: +61 8 8313 6738; Fax: +61 8 8313 7129; E-mail: juanita.lauer-smith@adelaide.edu.au

Received date: March 23, 2017; **Accepted date:** April 03, 2017; **Published date:** April 11, 2017

Copyright: © 2017 Lauer J, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Short Communication

Complete degradation of starchy endosperm cell wall (1→3,1→4)-β-glucan during germination is an essential requirement in barley varieties selected for malting and brewing. Residual malt β-glucan from incomplete degradation of endosperm cell walls during the malting process is associated with increased wort viscosity that can slow filtration and reduce brew house efficiency. New barley varieties commercially released in Australia intended for malting are extensively evaluated by the Malting and Brewing Industry Barley Technical Committee (MBIBTC) before achieving malting accreditation from Barley Australia. The MBIBTC evaluates the nominated barley varieties following industry procedures to examine; malt extract, diastatic power, Kolbach Index, viscosity, wort β-glucan and apparent attenuation limit. Failure for the barley variety to fulfil the malting specifications consistently over two seasons of commercial scale evaluation will result in a downgraded recommendation to food or feed barley.

The Australian variety Hindmarsh that was commercially released in 2007 is a recent example of a variety intended for malting that failed malt accreditation in 2010. Malting accreditation was not recommended because of unacceptably high wort β-glucan and viscosity levels in the final year of evaluation [1]. Hindmarsh had proven to be strong agronomically, performing well in lower rainfall regions, and it continues to be adopted strongly by farmers with over two million tonnes of production in each of the 2013, 2014 and 2015 seasons (Eglinton pers comm). Although not accredited as a malting variety, Hindmarsh has found some market acceptance in high adjunct brewing and is suitable for Japanese shochu production [2]. As a result, Hindmarsh grain prices are usually $5-$10 per tonne above feed and $20-$30 lower than the currently preferred malting varieties, Commander, Buloke and Scope [3]. On the assumption that 50% of production achieves first grade malt specifications these values equate to $20-$30 million direct losses in farm gate value. The reliable production of malt with low β-glucan could prevent problems associated with borderline viscosity rather than managing viscosity by changing brew house processes or by adding exogenous enzymes and this would be of value to the industry. Consistently acceptable viscosity levels could potentially be achieved if defined and specific genetic variation governing this trait was available for efficient selection in breeding programs.

The focus of this study "Variation in barley (1→3,1→4)-β-glucan endohydrolases reveals novel allozymes with increased thermostability" [4] was the identification and characterisation of novel barley (1→3,1→4)- β -glucan endohydrolase (β -glucanase) alleles from *Hordeum vulgare* ssp. spontaneum. Currently, β-glucanase alleles are not targeted in breeding for malting barley; however β-glucanase activity is commonly assayed. The potential outcome of this study was to identify novel β-glucanase alleles with economic significance for implementation into breeding programs. Genetic diversity of cultivated barley has been significantly reduced through domestication limiting the potential for genetic gain in many traits within elite germplasm. *H. spontaneum* has become a reliable genetic resource for allele mining of rare alleles for the genetic improvement of cultivated barley. Past research has identified a novel thermostable β-amylase allele in *H. spontaneum* which has been implemented into breeding programs for malt quality improvement [5,6]. Significant variation in β-glucanase activity and other malt quality traits have also previously been identified in *H. spontaneum* [7]. However, phenotyping for complex malting quality traits in *H. spontaneum* is likely to be confounded by gross differences in grain morphology and germination behaviour that can obscure the significant variation in the trait of interest. Methodologies that counter these effects have been developed using Advanced Backcross populations and Nested Associated Mapping populations that manage the effect of genetic background. The development of these populations involve the introgression of novel alleles from *H. spontaneum* donor lines into elite breeding lines for high resolution quantitative trait loci (QTL) discovery [8,9]. The F1 progeny produced from the elite and *H. spontaneum* lines are backcrossed into the elite breeding line for two to three generations to reduce the frequency of undesirable donor alleles followed by serial self-fertilisation or doubled haploid production to produce homozygous lines. QTL discovery methods are able to exploit novel alleles from exotic germplasm in the mapping populations for crop improvement. However, the development of these populations are costly and time consuming due to the number of lines generated and limit the number of *H. spontaneum* accessions that can be exploited. Consequently, only a fraction of the genetic diversity of *H. spontaneum* is examined increasing the possibility of missing rare alleles.

A different approach is the examination of a gene of interest by conducting a sequence survey, which is less restricted by sample size. The sequence survey is most powerful when the target gene and protein has been well characterised, such as for β-glucanase. This study examined the exon regions of *HvGlb*1 and *HvGlb*2 in cultivated barley and *H. spontaneum* accessions for the presence of novel β-glucanase alleles. *H. spontaneum* accessions exhibited significantly more polymorphisms than cultivated barley, which is consistent with the loss of genetic diversity through domestication by traditional breeding methods. However, there were fewer polymorphisms identified in *HvGlb*1 in both cultivated barley and *H. spontaneum* accessions compared to *HvGlb*2. The differences in frequency observed in nucleotide and amino acid sequence variation resulted in a total of 20 β-glucanase alleles for potential characterisation. Five new *HvGlb*1 alleles were identified in 11 individuals, three were unique to *H.*

spontaneum and two were unique to landraces. 13 new *HvGlb*2 alleles were identified, one new allele was exclusive to the cultivated varieties examined and 11 were exclusive to *H. spontaneum*.

The sequence survey research of *HvGlb*1 and *HvGlb*2 could be extended to include intron and promoter regions that may be important as they influence the expression levels of the active enzyme. Polymorphisms in intron regions generally occur more frequently than in coding regions with a higher likelihood of insertions and deletions. Differences in the intron region of *HvGlb*1 have been identified previously, however the influence of the *HvGlb*1 and *HvGlb*2 intron regions on enzyme expression remain to be examined. Additionally, expanding the geographical diversity of the *H. spontaneum* accessions from the Fertile Crescent with selections from North Africa, Central Asia and the Caucasus region would increase the probability of identifying additional rare alleles [10].

Identification of large numbers of sequence variants from a sequence survey necessitates the use of predictive modelling tools to prioritise alleles for functional characterisation; these are particularly useful when the structure of the target protein has been solved. Predictive protein modelling tools enable the visual examination of protein structure by homology modelling and stability predictions from calculations of Gibbs free energy changes upon the entropy of protein folding. The combination of structural and stability prediction tools increase the accuracy of the prediction analysis. The current study correctly predicted protein stability changes in EI-c, EII-c, EII-d, EII-e and EII-l. These allozymes all exhibited increased β-glucanase activity after heat treatment in comparison to the reference allozyme, however, only EII-l exhibited increased activity at elevated temperatures. An effect on EI-b stability was not accurately predicted, and this was manifested as a decrease in β-glucanase activity after heat treatment. Incorrect predictions of functional variation limit the success of mining useful new alleles from sequence based surveys. The incomplete understanding of amino acid substitution effects on structure and function is also a significant limitation for genetically modified (GM) and gene editing approaches generating new variation. GM barley varieties are not accepted by brewing companies in any country, however it is possible that gene editing may be considered differently in some jurisdictions. The current study demonstrates that a degree of redundancy is required when using predictive functional analyses as complete dependence on such tools is not reliable even for very well characterised enzymes such as β-glucanase.

This study has identified significant genetic variation in barley (1→3,1→4)-β-glucanase. Further examination of β-glucanase amino acid sequence variation using homology modelling identified candidates for biochemical characterisations. Significant β-glucanase thermostability improvement conferred by contrasting EII allozymes in preliminary experiments has been characterised.

References

1. Baker N (2010) Hindmarsh falls short of malt accreditation. Media Release, Barley Australia.
2. Seednet (2011) Hindmarsh factsheet, Seednet.
3. AWB (2016) AWB daily contract prices.
4. Lauer JC, Cu S, Burton RA, Eglinton JK (2017) Variation in barley (1→3,1→4)-β-glucan endohydrolases reveals novel allozymes with increased thermostability. Theor Appl Genet.
5. Eglinton JK, Langridge P, Evans DE (1998) Thermostability variation in alleles of barley beta-amylase. J Cereal Sci 28:301-309
6. Von Korff M, Wang H, Léon J, Pillen K (2008) AB-QTL analysis in spring barley: III. Identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare* ssp. *spontaneum*). Mol Breeding 21: 81-93.
7. Ahokas H, Naskali L (1990) Variation of α-amylase, β-amylase, β-glucanase, pullulanase, proteinase and chitinase activity in germinated samples of the wild progenitor of barley. J I Brewing 96: 27-31
8. Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92: 191-203.
9. Yu J, Holland JB, McMullen MD, Buckler ES (2008) Genetic design and statistical power of nested association mapping in maize. Genetics and Improvements of Barley Malt Quality. Springer, New York.
10. Steffenson BJ, Olivera P, Roy JK, Jin Y, Smith K, Muehlbauer GJ (2007) A walk on the wild side: mining wild wheat and barley collections for rust resistance genes. Aust J Agr Res 58: 532-544.