Finding the LMA needle in the wheat haystack proteome

GRDC Project
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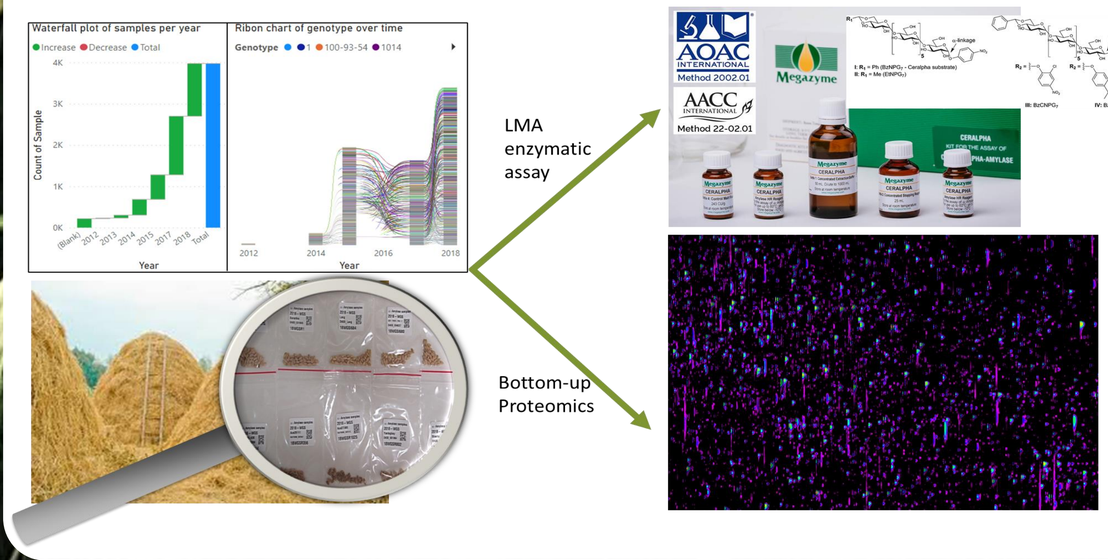
Introduction

High isoelectric point (pI) α-amylase is normally synthesized after maturity in wheat grains when they may sprout in response to rain or germinate following sowing the next season's crop. Late maturity α-amylase (LMA) is a wheat genetic defect causing the synthesis of high pI α-amylase in the aleurone as a result of a temperature shock during mid-grain development or prolonged cold throughout grain development. In LMA-affected grains, the activated enzyme prematurely degrades the starch leading to grain discount, which incurs a loss of profit for the producers. Whilst the physiology is well understood, the biochemical mechanisms involved in grain LMA response remain unclear.

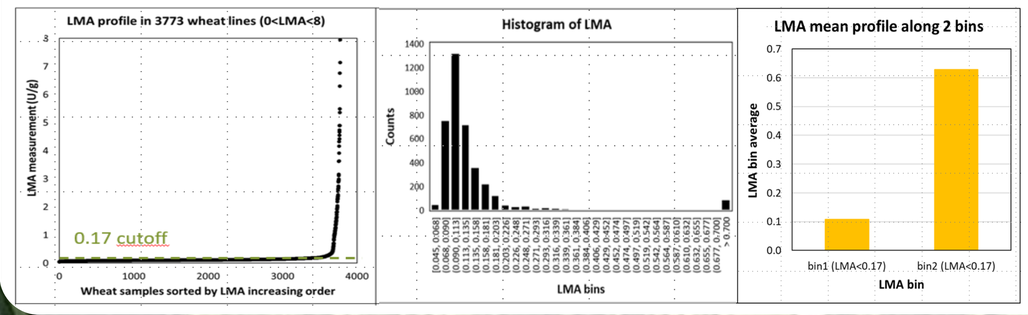
Study design

4,061 grain samples from 858 wheat genotypes sourced from all over the world, grown/harvested in Horsham (VIC) from 2012-2018 and stored in optimal conditions were subjected to LMA assays and bottom-up proteomics (BUP).



LMA profile

0 < LMA activity < 8 u/g flour but heavily biased towards very small values. Arbitrary cutoff of 0.17 u/g flour to categorise samples into up and down-regulated profiles.

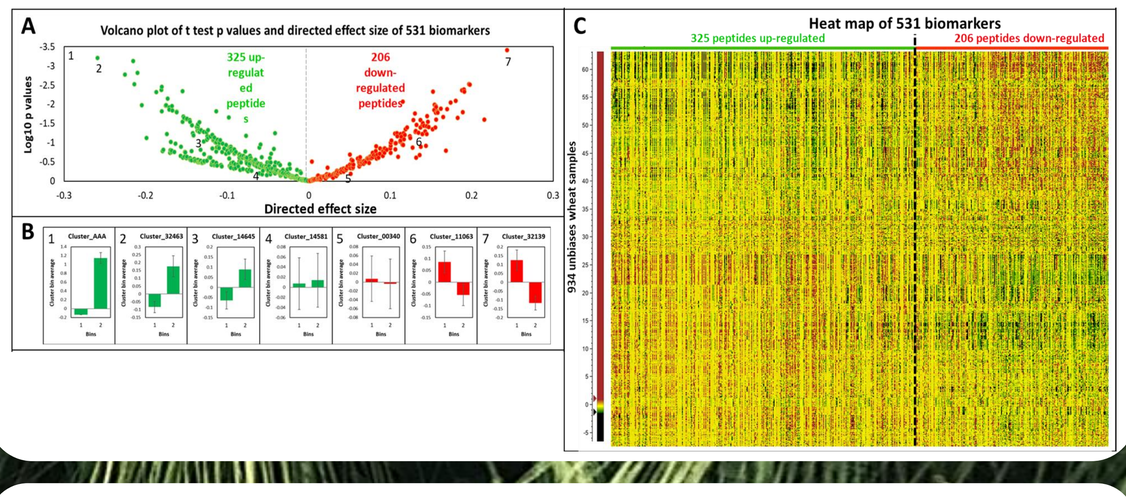


Stats to determine LMA biomarkers

32,346 peptides were reproducibly quantified.
531 LMA biomarkers selected with at least one of the following criteria:

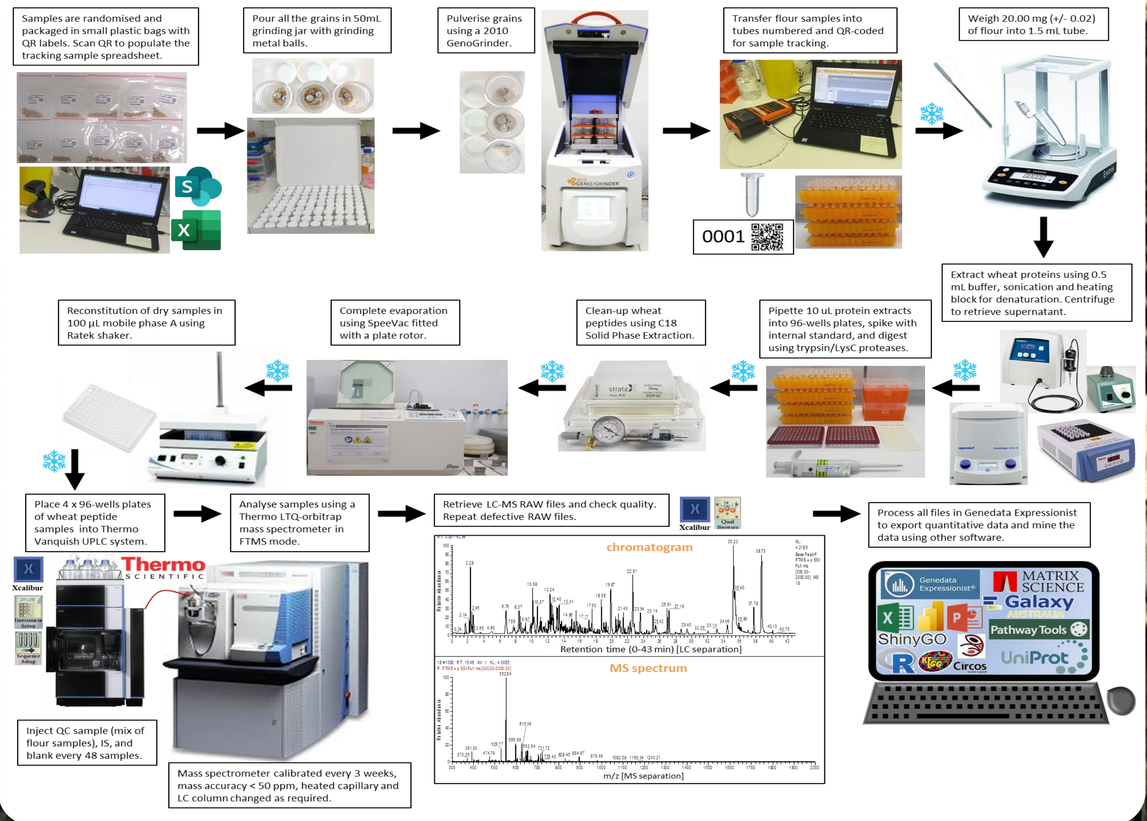
- Linear model p-value < 5% (494 peptides), and/or
- Correlation>15% (28 peptides), and/or
- SOM group (4,3) = 26 peptides, and/or
- K-means group 14 (93 peptides), and/or
- Divisive HCA Group 1915-1947 (33 peptides)

325 and 206 peptide biomarkers were up- and down-regulated, respectively.

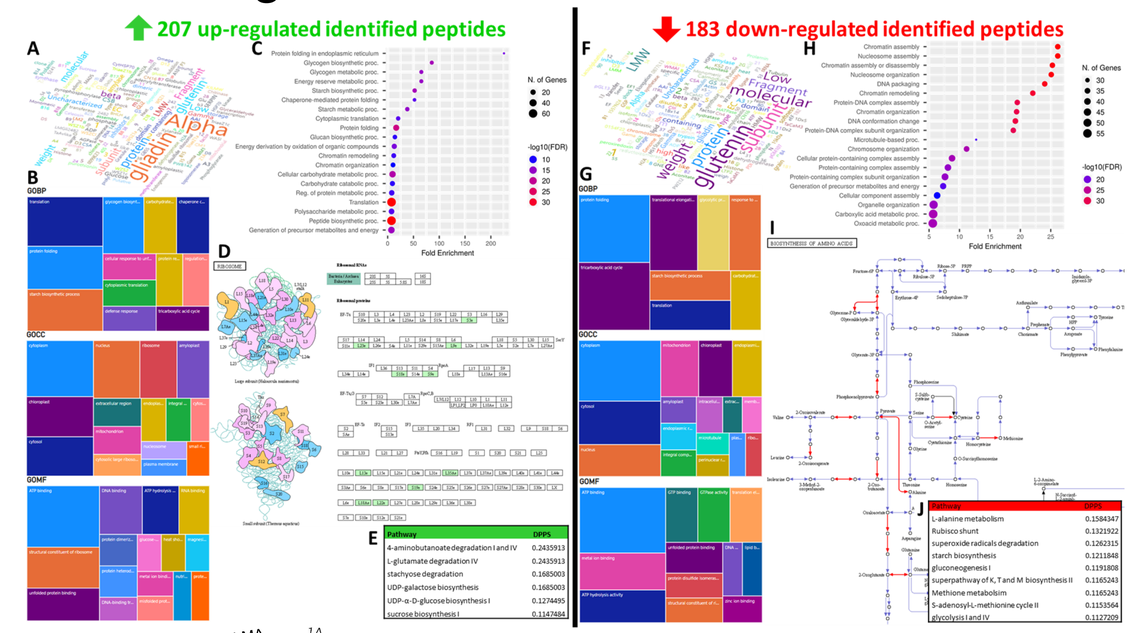


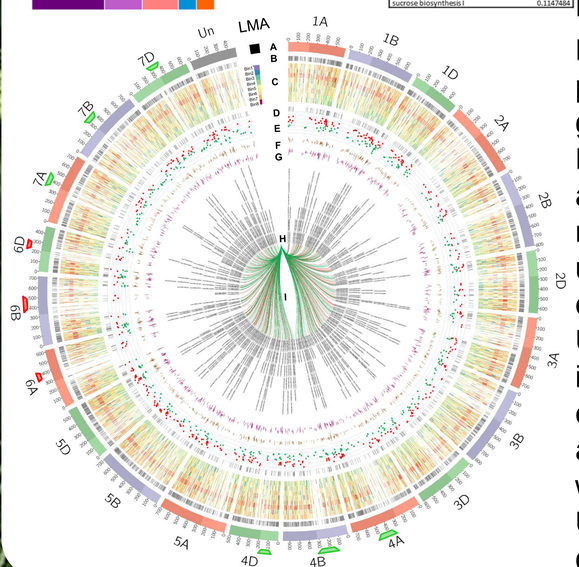
High-throughput BUP workflow

Key elements to minimise batch to batch variation were accurate sample weights (20 +/- 0.2 mg flour), internal standard (IS), quality controls (QCs), and regular LC-MS maintenance.



Data mining of identified biomarkers

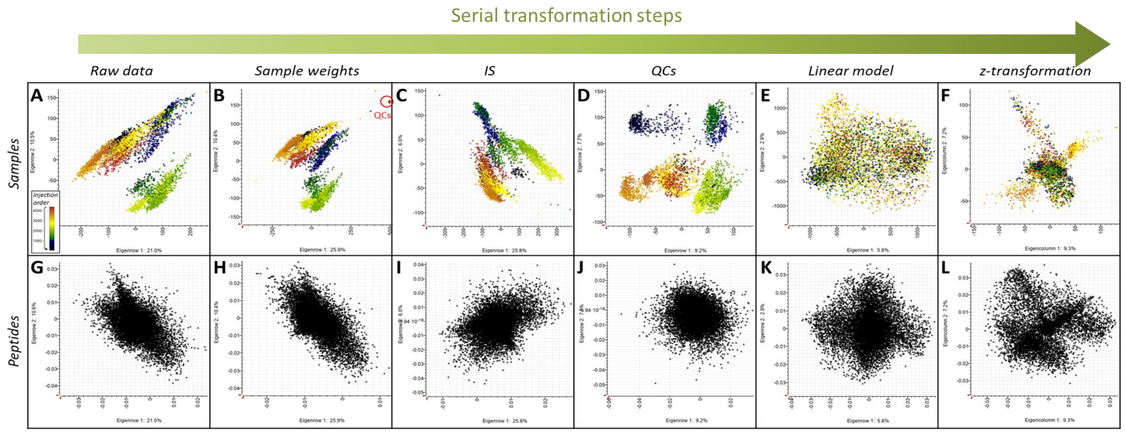




LMA-affected grains activated their primary metabolisms (glycolysis and gluconeogenesis, TCA cycle, along with DNA- and RNA binding mechanisms, and protein translation). The secondary metabolism was also mobilised with the up-regulation of phytohormones, chemical and defense responses. Unsurprisingly, LMA expression greatly impacted grain starch and other carbohydrates with the up-regulation of alpha-gliadins and starch metabolism, while LMW glutenin, stachyose, sucrose, UDP-galactose and UDP-glucose were down-regulated.

Eliminating technical variation

Serial transformations were needed to eliminate technical variation, including applying a linear regression and retrieving the residuals. The last z-transformation was needed to achieve data normalisation for statistical analyses.



Conclusion

To our knowledge, this is not only the first proteomics study tackling the wheat LMA issue but also the largest plant-based proteomics study to date. This work demonstrates that proteomics deserves to be part of the wheat LMA molecular toolkit and should be adopted by LMA scientists and breeders in the future.