Proteogenomics to explore a common weed for the presence of gluten-like proteins

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Background

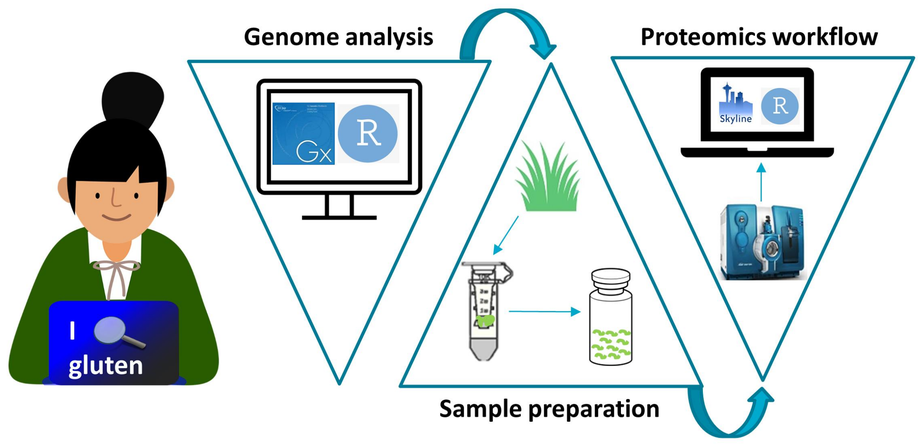
Why gluten proteins?
- **Main storage proteins in cereal grains**
- **Cause adverse immune response in people with Coeliac Disease[CD] (>70million)**
- **There is a need to find gluten containing grains/weeds to avoid cross contamination**

Why Ryegrass?
- **Most common weed in Australia, grown alongside with crops**
- **Member of the grass family (Poaceae) wherein the storage proteins comprise gluten-like proteins**

Food cross-contamination

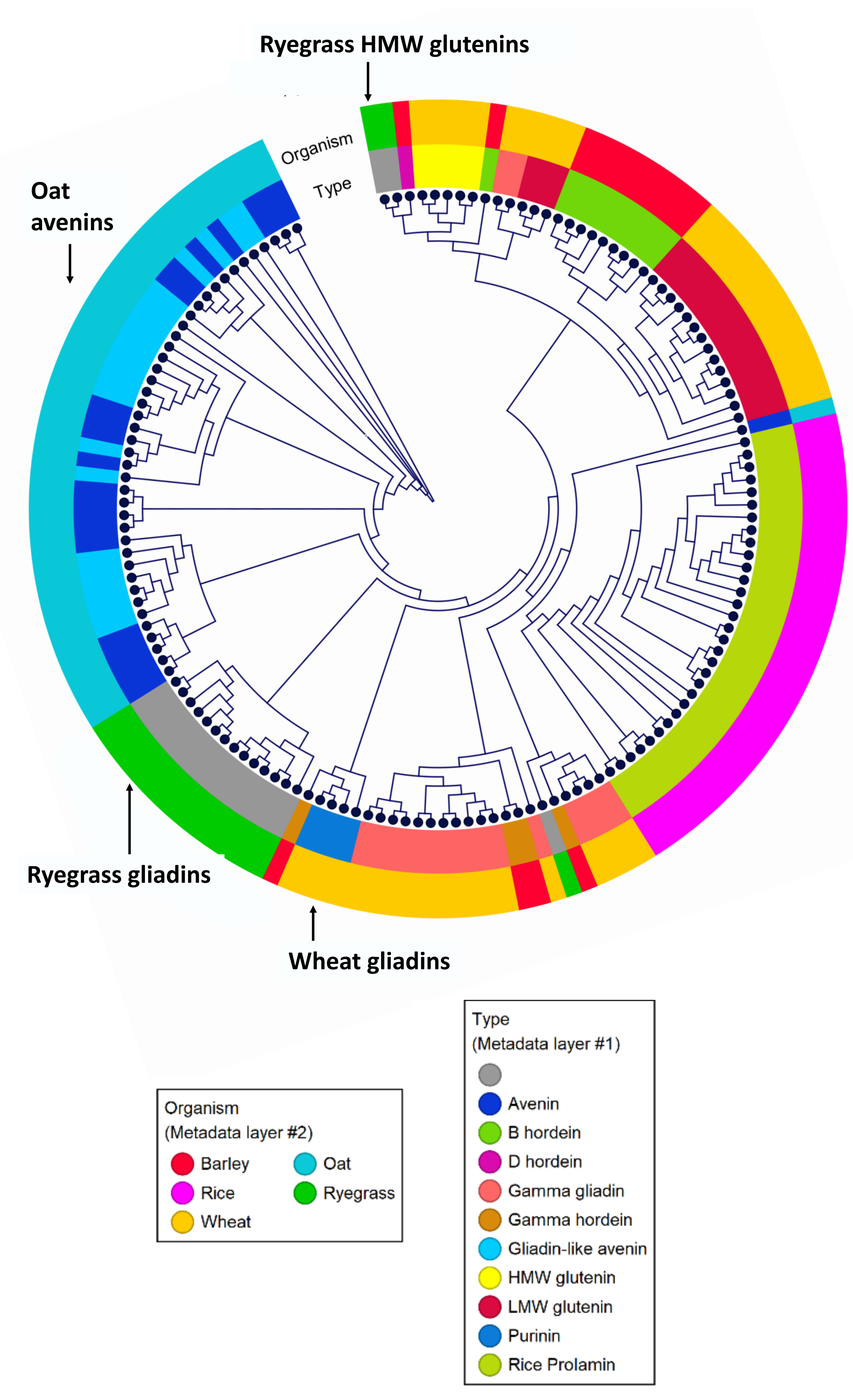
Materials & Methods

- **Database Search: *Lolium perenne* genome**
- **Genome analysis: Epitope search on PacBio assembly of *L. perenne;* manual annotation of gluten-like protein genes; BLAST to identify gluten-like protein types**
- **Sample preparation of two ryegrass species, perennial ryegrass *L. perenne* and annual ryegrass *L. multiflorum***
- **Developed of quantitative assay to detect gluten-like proteins using 6500 QTRAP mass spectrometer (SCIEX)**



Results

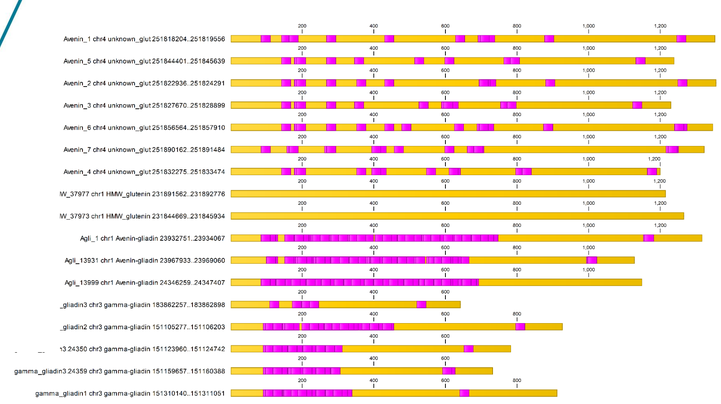
- **Gluten protein-like coding regions were found on chromosomes 1, 3 and 4 of *L. perenne***
- **A total of 17 gluten-protein-like gene models were found across the genome with >90% epitope sequence similarity**
- **Phylogenetic tree analysis reveals two ryegrass sequences similar to high molecular weight (HMW) glutenins**
- **Based on sequence similarity ryegrass gliadins position is between oats avenins and wheat gamma-gliadins**
- **Fifteen peptides were monitored with LC-MRM-MS**
- **Peptide intensities are different between two ryegrass species**



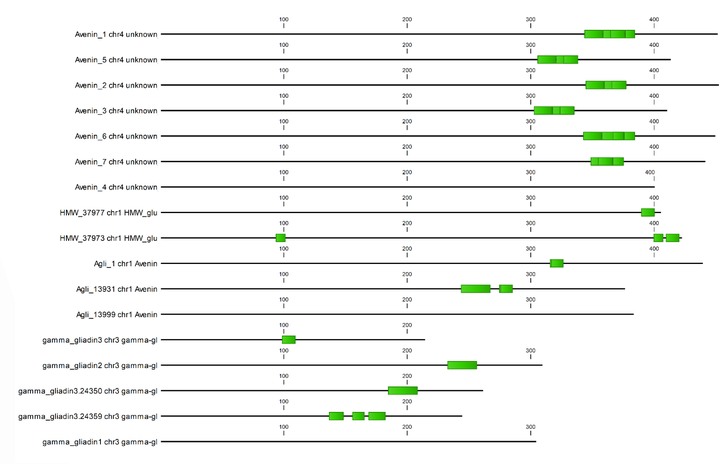
Conclusion

- **Precise annotation of gluten-like proteins in the ryegrass genome**
- **Identification of putative epitope regions in gluten-like proteins from ryegrass**
- **Developed MRM method to validate gluten-like proteins and compared their abundances between ryegrass species**
- **Future research will use immune assay to confirm their immunogenicity**

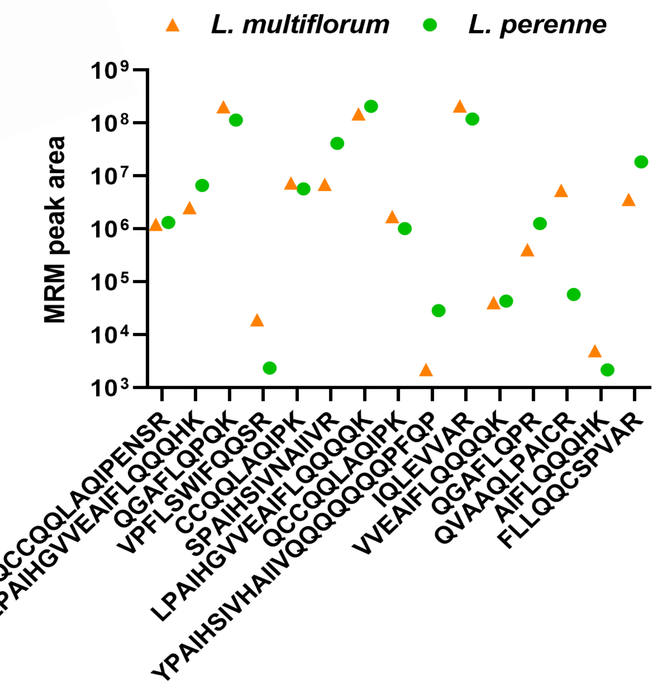
Gluten protein-like coding genes and putative epitope regions (pink)



Monitored peptides (Green)



MRM-MS results



FOR FURTHER INFORMATION

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