FUNCTIONAL ANALYSIS OF PECTIC RG-I IN TOMATO AND STRAWBERRY FRUIT

Grant number: 625270
Funding scheme: FP7-MC-IEF
Coordinator fellow: Sara Posé-Albacete
Name, title and organisation of the scientist in charge of the project's coordinator:
Dr. J. Paul Knox, Centre for Plant Sciences, University of Leeds, Leeds, UK

AIM OF THE PROJECT

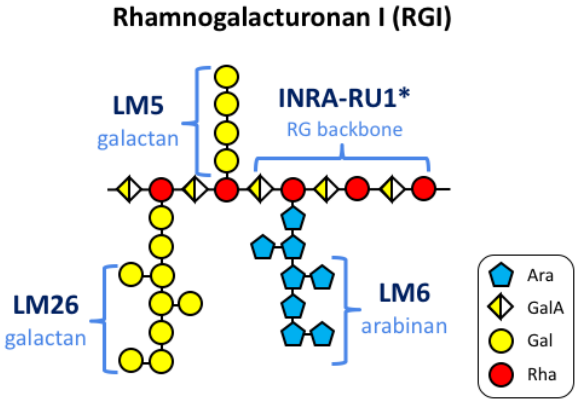
The aim of this project was to characterise the structure and function of RG-I polysaccharides during fruit development and ripening and its contribution to fruit texture. In order to address this objective, we followed a glycomic/phenomic approach based on the immuno-detection of RGI components by monoclonal antibodies generated in the host laboratory. Our initial hypothesis was that the disruption of cell wall disassembly in fruits will contribute to improve their textural properties with longer fruit shelf-life, reduced mechanical bruising during the postharvest period and more resistance to plant pathogens.

BACKGROUND

Despite the early discovery of plant cell walls by Robert Hooke in 1665, much is still unknown about their relationship with textural properties of important plant organs/products such as fruits and vegetables. This lack of progress is due to the high structural complexity of cell wall glycans and the technical challenges to their study.

Fruit texture and shelf life are a key priorities from a horticultural and commercial standpoint. Fleshy fruits, such as tomatoes and strawberries, are characterized by a soft flesh that becomes less firm with progressive ripening. Therefore, improvement of fruit texture to reduce spoilage during ripening and postharvest is of great commercial importance. This topic constitutes one of the main objectives of breeding programs, using both, traditional and biotechnological approaches.

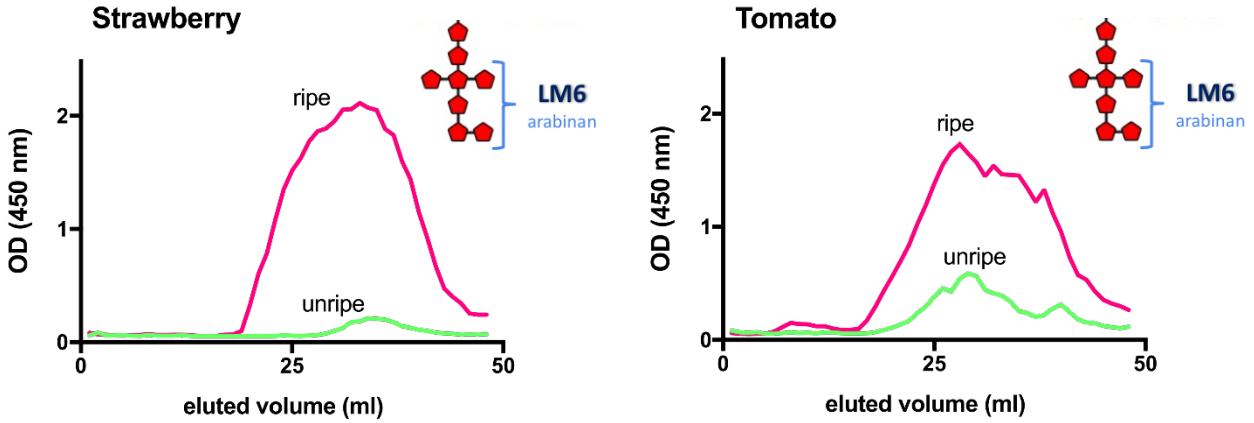
It is widely accepted that cell wall modifications responsible for the softening involve the disassembly and degradation of the cell wall components that are interwoven in a complex matrix. These cell wall modifications are common to the ripening of most fleshy fruits, although each species has specific patterns of cell wall disassembly. However, texture changes related to cell wall architecture and the genes involved in the disassembly that accompany ripening are still not well understood. Among the main cell wall components implicated in texture, this project focused on the immuno-analysis of rhamnogalacturonan-I (RG-I) components to study the structures and roles of these pectins. RG-I polysaccharides are highly variant and structurally complex branched polymers as shown in the diagram, where the main antibodies used and their epitopes are also included (INRA-RU1 was kindly provided by MC Ralet).



Nowadays the availability of high-throughput techniques, such as genomics, underpin a new era for Big Data in Science. The application and analysis of such large amounts of information is not facile and must be combined in parallel with a detailed analysis of the functions of genes/proteins in relation to less tractable biomolecules such as glycans and ultimately cell, tissue and organ attributes. This last part of the analysis looking at the properties of biological organisms, named phenomics, is a final step of Big Data included in our project approach as is crucial for maximizing Big Data application and usefulness.

MAIN RESULTS

Using sets of monoclonal antibodies that can bind to specific domains of RG-I and in combination with separation techniques, RG-I analysis during fruit ripening in tomato and strawberry fruit cell walls identified novel specific features in each fruit species as shown by the arabinan (LM6 epitope) anion-exchange chromatography/epitope detection profiles depicted below from soluble cell wall fractions. These results indicate increased solubility and varying acidities of arabinan-rich RG-I in both fruits as an aspect of cell wall disassembly during fruit ripening. Additionally, a new antibody named LM26 which recognizes branched galactan in RG-I appears as a specific structure only in the ripe stage of strawberry fruits.



Regarding the analysis of genes related to the remodeling of RG-I pectin, the functional analysis of strawberry transgenic lines with a rhamnogalacturonan lyase gene silenced showed RG-I less degraded at the cell wall level, which seems to correlate with firmer texture at fruit level, supporting the initial hypothesis about the importance of RG-I in fruit texture.

To gain further insight into RGI roles in fruit texture, a cell wall immuno-analysis of four fruits, including apple and aubergine in addition to strawberry and tomato was also undertaken. Those four fruits were selected based on both, a contrasting firmness and phylogenetic relations (tomato (soft texture) and aubergine (firm texture) belong to the Solanaceae family, whilst apple (firm texture) and strawberry (soft texture) belong to the Rosaceae family) and this study presented broad patterns of pectin and other matrix polysaccharide heterogeneity that will be core information for future studies. A novel key feature identified was the presence pectin-xyloglucan-heteroxylan complex as a distinctive attribute of aubergine cell walls that is likely to be related to fruit firmness.

Finally, a high throughput glycome microarray analysis of pectin epitopes and related cell wall polysaccharides in a set of strawberry lines (including cultivars, ripening stages and transgenic lines of cell wall related silenced genes) revealed correlations between different transgenic genotypes, cell wall degrading enzymes and developmental fruit stages. This work is providing insights into how plant cell walls in fruits are able to be functional and play roles in the mechanical properties of the fruit in spite of structural and compositional alterations.

CONCLUSIONS

The outputs of this project widen the knowledge about RG-I as a key cell wall component influencing textural properties in high value crops with fleshy fruits and underpin the application of R&D advances into crop breeding programs towards a more sustainable agricultural management and healthier foods, which are key priorities of Horizon2020.

Contact details: J.P.Knox@leeds.ac.uk