Modulation of Tomato Fruit Texture by Silencing Cell Wall Structure-Related Genes

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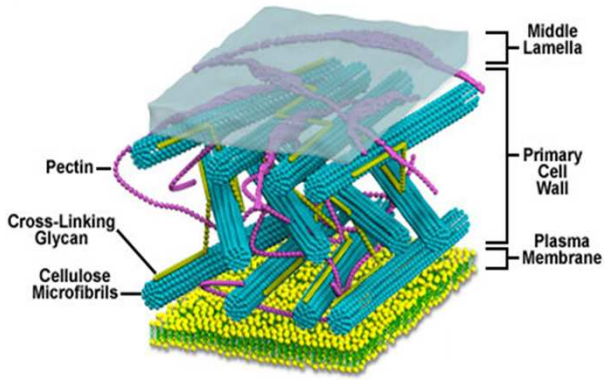
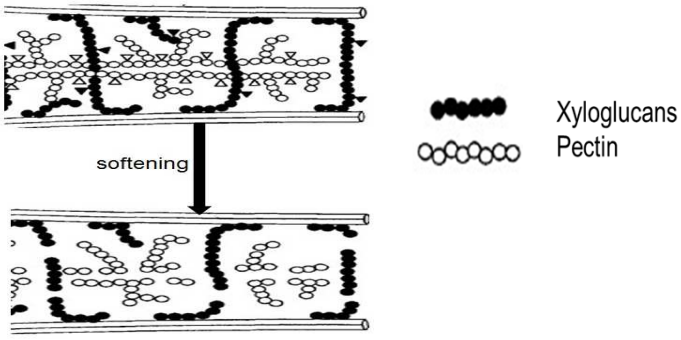
Introduction

Cell wall structural remodelling including solubilisation and depolymerisation of pectin polysaccharides, and depolymerisation of hemicelluloses normally occurs during the process of tomato fruit softening, which is a consequence of the combined action of multiple gene products involved in modulation of cell wall structure. The tomato genome sequence contains more than 700 gene models annotated as having cell wall-related functions; of these around 50 are expressed during fruit development and ripening (Tomato Genome Consortium, 2012; Supplementary Section). However, only a small proportion of these genes are highly up-regulated during the ripening process. We will investigate the role of the most highly ripening-related genes and the concerted action of multiple genes in different gene families involved in the texture changes of tomato fruit.



Structure of Plant Cell Wall



Schematic representation of remodelling of cell wall during softening

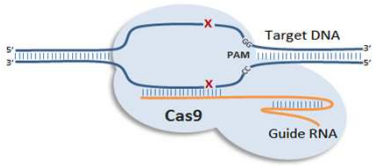
Aims and Objectives

The aim of the study is to understand the biological basis of fruit ripening associated with fruit texture. This is important because if we can slow texture changes in the absence of effects on other aspects of ripening, shelf life can be improved without negative impacts on other quality attributes. This will help reduce postharvest waste and therefore have a direct impact on issues related to Global Food Security.

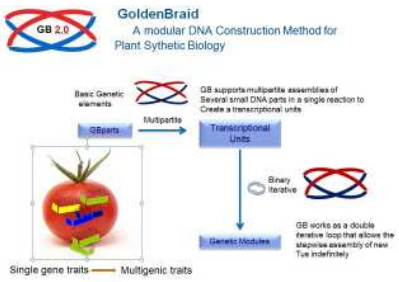
Methods

Generating single-gene transgenic lines for cell wall structure-related genes by using CRISPR technology

CRISPR/Cas9— an RNA based



Generating multiple gene constructs by using GoldenBraid with RNAi or CRISPR



Results and Conclusion

First generation transformed (T0) plants were generated by Agrobacterium-mediated transformation and T0 plants were confirmed by PCR to carry an integrated transfer DNA (T-DNA) from the introduced CRISPR/Cas9 constructs. The CRISPR/Cas9-induced mutations were analyzed using PCR/PE assay combined with genotyping by sub-cloning and sequencing. Results indicated that the CRISPR/Cas9 system was efficient in tomato for generating DSB-induced target gene editing. Germ line transmission and heritability analysis of CRISPR/Cas9-generated mutations indicated that gene mutations could be passed to the next generation.

Future Work

- Gene expression, enzyme activity analysis and detailed phenotyping especially cell wall analysis and immunocytochemistry
- Generating multi-gene RNAi transgenic lines

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