

CELL WALL DISASSEMBLY IS DELAYED BY RHAMNOGALACTURONATE  
LYASE GENE SILENCING: POTENTIAL ROLE IN FRUIT FIRMNESS

S. Posé<sup>a,b</sup>, P. Ric-Varas<sup>a</sup>, J. Schüchel<sup>c</sup>, F.J. Molina-Hidalgo<sup>d</sup>, R. Blanco-Portales<sup>d</sup>, J. Muñoz-Blanco<sup>d</sup>, P. Knox<sup>b</sup>, A.J. Matas<sup>a</sup>, M.A. Quesada<sup>d</sup>, J.A. Mercado<sup>a</sup>

<sup>a</sup>Instituto de Hortofruticultura Subtropical y Mediterránea 'La Mayora', IHSM-UMA-CSIC, Departamento de Biología Vegetal, Universidad de Málaga, Málaga, Spain

<sup>b</sup>Centre of Plant Sciences, University of Leeds, LS2 9JT, Leeds, United Kingdom

<sup>c</sup>Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, DK-1871 Frederiksberg, Denmark

<sup>d</sup>Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba, 14071, Córdoba, Spain

<sup>e</sup>Departamento de Biología Vegetal. Universidad de Málaga, 29071, Málaga, Spain

Strawberry fruits greatly reduce their quality due to softening during ripening with economically important losses. Texture changes of fleshy fruits during ripening are mainly due to middle lamellae dissolution, cell-to-cell adhesion losses and wall weakening of parenchyma cells by the coordinated action of several cell wall enzymes. Pectin degradation has been proven a key factor in strawberry softening by functional analysis of several pectinase genes (polygalacturonase, pectate lyase and  $\beta$ -galactosidase). The complexity and highly dynamic nature of pectins remains a challenge to fully elucidate structure-function relationships of pectins. In this work, we present the functional analysis of two independent strawberry transgenic lines with more than 95% silencing of a rhamnogalacturonate lyase gene (*FaRGLyase1*). Firmness of ripe fruit was significantly higher in both transgenic lines than in the control. Cell walls from these fruits were extracted and analyzed by glycan microarray profiling. This high-throughput technique allows a wide screening of cell-wall glycan occurrence based on the detection of specific cell wall oligosaccharide epitopes by monoclonal antibodies and reveals profiles which can be used as potential fingerprints specific for a singular organ and/or developmental stage. Our microarray results showed that the silencing of *FaRGLyase1* reduced degradation of several rhamnogalacturonan-I related epitopes, as expected. Additionally, comparison of transgenic cell walls from ripe fruits with those extracted from control fruits at different developmental stages (green, white and red) by hierarchical clustering, demonstrated a higher similarity of transgenic fruit cell walls with the control cell walls from fruits at the white stage. Glycan microarray profiles revealed less degraded fruit cell walls as result of *FaRGLyase1* down-regulation which could contribute to the increased firmness of transgenic fruits.

**Acknowledgements:** This research was supported by FEDER EU Funds and the Ministerio de Economía y Competitividad of Spain (grant reference AGL2017-86531-C2-1-R), a Marie Curie IEF within the 7th European Community Framework Programme (reference: PIEF-2013-625270) and a postdoctoral fellowship from Malaga University (E-29-2017-0215684) for SP and a FPI fellowship (BES-2015-073616) to support PR-V.