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Characterization of CRISPR Mutants Targeting Genes Modulating Pectin Degradation in Ripening Tomato

By: Wang, DD (Wang, Duoduo)^[1]; Samsulrizal, NH (Samsulrizal, Nurul H.)^[2]; Yang, C (Yang, Cheng)^[3]; Allcock, NS (Allcock, Natalie S.)^[4]; Craighon, J (Craighon, Jim)^[1]; Blanco-Ulate, B (Blanco-Ulate, Barbara)^[5]; Ortega-Salazar, I (Ortega-Salazar, Isabel)^[5]; Marcus, SE (Marcus, Susan E.)^[6]; Bagheri, HM (Bagheri, Hassan Moeiinyan)^[1]; Perez-Fons, L (Perez-Fons, Laura)^[7] ...More

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PLANT PHYSIOLOGY

Volume: 179 Issue: 2 Pages: 544-557

DOI: 10.1104/pp.18.01187

Published: FEB 2019

Document Type: Article

[View Journal Impact](#)

Abstract

Tomato (*Solanum lycopersicum*) is a globally important crop with an economic value in the tens of billions of dollars, and a significant supplier of essential vitamins, minerals, and phytochemicals in the human diet. Shelf life is a key quality trait related to alterations in cuticle properties and remodeling of the fruit cell walls. Studies with transgenic tomato plants undertaken over the last 20 years have indicated that a range of pectin-degrading enzymes are involved in cell wall remodeling. These studies usually involved silencing of only a single gene and it has proved difficult to compare the effects of silencing these genes across the different experimental systems. Here we report the generation of CRISPR-based mutants in the ripening-related genes encoding the pectin-degrading enzymes pectate lyase (PL), polygalacturonase 2a (PG2a), and beta-galactanase (TBG4). Comparison of the physicochemical properties of the fruits from a range of PL, PG2a, and TBG4 CRISPR lines demonstrated that only mutations in PL resulted in firmer fruits, although mutations in PG2a and TBG4 influenced fruit color and weight. Pectin localization, distribution, and solubility in the pericarp cells of the CRISPR mutant fruits were investigated using the monoclonal antibody probes LM19 to deesterified homogalacturonan, INRA-RU1 to rhamnogalacturonan I, LM5 to beta-1,4-galactan, and LM6 to arabinan epitopes, respectively. The data indicate that PL, PG2a, and TBG4 act on separate cell wall domains and the importance of cellulose microfibril-associated pectin is reflected in its increased occurrence in the different mutant lines.

Keywords

KeyWords Plus: PRIMARY-CELL WALLS; ANTISENSE DOWN-REGULATION; COVALENT LINKAGE; PECTATE LYASE; FRUIT; POLYGALACTURONASE; POLYSACCHARIDES; EXPRESSION; CELLULOSE; XYLOGLUCAN


Author Information

Reprint Address: Seymour, GB (reprint author)

 Univ Nottingham, Sch Biosci, Loughborough LE12 5RD, Leics, England.


Addresses:

 [1] Univ Nottingham, Sch Biosci, Loughborough LE12 5RD, Leics, England


 [2] Int Islamic Univ Malaysia, Dept Plant Sci, Kulliyah Sci, Kuantan 25200, Pahang, Malaysia

[3] Shanxi Acad Agr Sci, Inst Vegetable Res, Taiyuan 030031, Shanxi, Peoples R China

 [4] Univ Leicester, Ctr Core Biotechnol Serv, Electron Microscopy Facil, Leicester LE1 7RH, Leics, England

 [5] Univ Calif Davis, Plant Sci Dept, Davis, CA 95616 USA

 [6] Univ Leeds, Fac Biol Sci, Ctr Plant Sci, Leeds LS2 9JT, W Yorkshire, England

 [7] Univ London, Sch Biol Sci, Plant Mol Sci, Egham TW20 0EX, Surrey, England

E-mail Addresses: graham.seymour@nottingham.ac.uk

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|--|--------------|
| Biotechnology and Biological Sciences Research Council | BB/M025918/1 |
| Innovate UK | |

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Publisher

AMER SOC PLANT BIOLOGISTS, 15501 MONONA DRIVE, ROCKVILLE, MD 20855 USA

Journal Information

Impact Factor: [Journal Citation Reports](#)

Categories / Classification

Research Areas: Plant Sciences

Web of Science Categories: Plant Sciences

Document Information

Language: English

Accession Number: WOS:000457486700015

PubMed ID: 30459263

ISSN: 0032-0889

eISSN: 1532-2548

Other Information

IDS Number: HJ8YT

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