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

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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 β-galactanase (TBG4). Comparison of the physiochemical 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 β-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.

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