Cell Wall Ultrastructure and Xylan Distribution in ‘Bluecrop’ Highbush Blueberry (Vaccinium corymbosum) Fruits during Ripening

Sinath Chea1†, Duk Jun Yu1,2†, Junhyung Park1, Sung Hoon Jun1, and Hee Jae Lee1,2*
1Department of Plant Science, Seoul National University, Seoul 08826, Korea
2Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea
*Corresponding author: heejlee@snu.ac.kr
†These authors have equally contributed to this work.

Abstract

Fruit firmness is largely determined by cell wall, cell shape, cell-to-cell adhesion, and tissue anatomy. This study was conducted to investigate cell wall ultrastructural changes and xylan distribution during ripening and softening of ‘Bluecrop’ highbush blueberry (Vaccinium corymbosum) fruits. Fruits at pale-green, reddish purple, and dark-purple stages, which represented large green, turning point, and ripe stages, respectively, were used in this study. Cell walls and middle lamella were degraded, while plasma membranes were separated from primary cell walls at the reddish purple and dark-purple stages. The immunofluorescence labelling of xylan was abundant at the pale-green stage once the fruits were firm. However, the labelling became weaker at the reddish purple and dark-purple stages, and it was not well confined to cell wall areas, implying cell wall alteration at late ripening stages. These results demonstrated that the cell wall, middle lamella, and xylans were extensively degraded during the ripening and softening of ‘Bluecrop’ highbush blueberry fruits.

Additional key words: fruit firmness, fruit softening, immunolocalization, middle lamella, plasma membranes

Introduction

Firmness is one of the important fruit textures that are mainly determined by cell size and shape, cell wall thickness and strength, cell-to-cell adhesion, and tissue property and arrangement (Allan-Wojtas et al., 2001; Cornuault et al., 2018). During fruit ripening, the softening occurs as a consequence of multiple cellular processes that may have undesirable effects on fruit quality and storage (Brummell, 2006; Chea et al., 2019; Shin et al., 2020). Thus, the observation of cell microstructure and ultrastructure using modern microscopy techniques can provide qualitative and comprehensive information required to understand anatomy-related softening processes (Allan-Wojtas et al., 2001).

Fruit cell walls are structurally complex and may be synthesized during ripening (Brummell, 2006; Takizawa et al., 2014). Specific monoclonal antibodies could be used to detect the distribution of...
major epitopes of pectins and hemicelluloses in the cell wall during ripening. Cell wall monoclonal LM10 antibody was used to detect the (1,4)-\(\beta\)-xylan epitope (Handford et al., 2003; Sutherland et al., 2009) using either confocal or fluorescence microscopy. Such information is important for understanding cell wall remodeling-related softening processes and for interpreting the results obtained from the traditional fractionation of cell wall and analysis of monosaccharides.

In blueberry fruits, little is known about the alteration in cell wall ultrastructure. Xylan is a major hemicellulose present in blueberry fruits (Vicente et al., 2007), but its distribution and role during fruit softening remain unknown. Moreover, no information is available regarding the xylan distribution using cell wall monoclonal antibodies throughout fruit development and ripening in blueberry fruits. In this study, cell wall ultrastructure was investigated and xylan was visualized to obtain information regarding cellular structural changes and the role of xylan during ripening and softening in ‘Bluecrop’ highbush blueberry \((Vaccinium corymbosum)\) fruits.

**Materials and Methods**

**Plant Materials**

Twelve-year-old ‘Bluecrop’ highbush blueberry \((V. corymbosum)\) shrubs were grown in the field at the experimental orchard of Seoul National University, Suwon (37°15'N, 126°98'E), Korea. Fruits were harvested at pale-green, reddish purple, and dark-purple stages, indicating the large green, turning point, and ripe stages, respectively (Fig. 1). Ten fresh fruits at each ripening stage were used for the microscopic observation and immunofluorescence labelling.

**Ultrastructural Observation by Transmission Electron Microscopy**

Fruit flesh tissues \((3 \times 4\text{-mm diameter pieces})\) at each stage were excised and fixed in 2% \(p\)-formaldehyde and 0.25% glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered saline (PBS, pH 7.4). The fixed samples

![Fig. 1. Ripening stages of ‘Bluecrop’ highbush blueberry fruits used in the study. PG, pale-green; RP, reddish purple; DP, dark-purple.](image_url)
were washed three times in distilled water for 10 min each, post-fixed with 2% OsO₄ (Sigma-Aldrich) for 2 h, and washed again in distilled water. After dehydration in a graded series of ethanol solution (30, 50, 70, 80, 90, 100, 100, and 100%), the samples were immersed twice in 100% xylene (Sigma-Aldrich) and then infiltrated in LR white resin (Sigma-Aldrich) for 5 h by exchanging the solution every hour for the first 2 h. The infiltrated tissues were then embedded in LR white resin and polymerized at 55°C for 48 h. Ultrathin sections (approximately 85 nm thick) were cut using an ultramicrotome (EM UC7, Leica Microsystems, Wetzlar, Germany), mounted onto Formvar-coated copper grids, and stained with uranyl acetate and lead citrate for 30 and 10 min, respectively. Cell wall ultrastructures in the tissues were observed using a transmission electron microscope (JEM-1010, Jeol, Tokyo, Japan) at 80 kV.

**Immunofluorescence Labelling of the Xylan Epitope**

LM10 antibody (PlantProbes, Leeds, UK) was used to detect the (1,4)-β-xylan epitope in the cell wall. Fruit segments as described above were excised, fixed, and immunolabelled as described by Sutherland et al. (2009) and Ng et al. (2013). Samples not embedded in LR white resin were visualized for autofluorescence under UV excitation using a confocal laser scanning microscope (SP8 X, Leica Microsystems). Samples without primary antibody (200 mL of goat anti-rat IgG AlexaFluor 488 (Life Technologies Corp., Carlsbad, CA, USA) diluted 1:600 in PBS) were also examined as a control.

**Results and Discussion**

**Alteration of Cell Wall Ultrastructure during Ripening**

Fruit parenchyma cell walls exhibited well-integrated structures at the pale-green stage. As ripening progressed, however, the cell wall and middle lamella were degraded (Fig. 2). During late ripening, cell walls were wavy and partially

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**Fig. 2.** Transmission electron micrographs of the parenchyma cell wall of 'Bluecrop' highbush blueberry fruits at the pale-green (A, B), reddish purple (C), and dark-purple (D) stages. CM, cell membrane; ML, middle lamella; PCW, primary cell wall. Scale bars indicate 500 nm for A, C, and D, and 2 µm for B.
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Immunolocalization of the Xylan during Ripening

There are various structures of xylans, non-substituted or substituted, with a backbone of $\beta$-(1,4)-linked xylose residues (Brummell and Schröder, 2009). These xylans are variably distributed within plant species, cultivars, and tissues (Brummell and Schröder, 2009) and have also been reported to be present in various fruit parenchyma cells, including guava (Marcelin et al., 1993) and blueberry (Vicente et al., 2007). In the present study, immunofluorescence labelling of xylan in the fruit cell walls was more intense at the pale-green stage, but the labelling became weaker as ripening progressed (Fig. 3). The labelling at the reddish purple and dark-purple stages was not well confined to the cell wall areas presumably due to broken cell walls at these stages that release cell wall components to other parts of cells. Since LM10 is specific to xylans with short side chains of either $\alpha$-L-arabinose or $\alpha$-D-glucuronic acid (McCartney et al., 2005; Brummell and Schröder, 2009), the present results suggest that xylans in ‘Bluecrop’ highbush blueberry fruits are present as low substituted types.

The cell wall strength is determined by the cross-linkages among hemicelluloses with celluloses (Bennett and Labavitch, 2008), pectins with celluloses (Wang et al., 2015), and xylans with pectins (Cornuault et al., 2018). Since xylan is the major hemicellulose in the cell wall of blueberry fruits (Vicente et al., 2007), its degradation contributes to the weakening of the cross-linkage, leading to softening as observed in some fruits, including papaya (Manenoi and Paull, 2007; Brummell and Schröder, 2009; Iniesta-González et al., 2013), apple (Gwanpua et al., 2016), and blueberry (Chea et al., 2019). Therefore, the present results suggest that the early softening of ‘Bluecrop’ highbush blueberry fruits is associated with xylan degradation.

Fig. 3. LM10 immunolabelling of xylan in the parenchyma tissues of ‘Bluecrop’ highbush blueberry fruits during ripening. PG, pale-green; RP, reddish purple; DP, dark-purple; IS, intercellular space; PCW, primary cell wall. Scale bars indicate 50 µm.
Literature Cited


