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Short communication

# Cell wall-related enzymatic activities and transcriptional profiles in four strawberry (*Fragaria x ananassa*) cultivars during fruit development and ripening

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ABSTRACT

Fruit softening during ripening is mainly a consequence of solubilization and depolymerization of cell wall components mediated by the action of a complex set of enzymes and proteins. In the present work, we performed a comparative study of the changes in physiological properties, cell wall-associated polysaccharide contents and expression of cell wall-related genes during different fruit developmental stages of four strawberry (*Fragaria* x *ananassa* Duch.) cultivars (Camarosa, Cristal, Monterey, and Portola). The four cultivars showed different fruit firmness values being Camarosa and Cristal the firmest, Monterey the softest, and Portola with an intermediate value between them. Additionally, in order to obtain a correlation between mRNA transcriptional levels and fruit firmness with physiological properties, we have analyzed the abundance of five mRNAs (*FaEXPA2, FaEXPA4, FaXTH1, FaXTH2* and *FaPG1*). We have found a correlation between fruit firmness and mRNA abundance levels for *FaEXPA2, FaEXPA4, FaXTH1* and *FaPG1*. For these four mRNAs we have observed higher transcript levels in the softest cultivar (Monterey) than in the other two firmer cultivars (Cristal and Camarosa) at the 50% ripe or ripe stage. Finally, the results showed that exist a correlation between cell wall-modifying enzymes, physiological properties and firmness, which would explain the fruit softening process that reduces post-harvest life.

## 1. Introduction

Strawberry is among the most widely consumed fruits in the world. The octoploid cultivated strawberry (*Fragaria* × *ananassa*) is a economically important fruit that in 2012 its growing area was 241,000 ha, and fruit production was 4,516,810 tonnes (http://www.fao.org/home/en/). The coast of Maule Region in southwestern zone of Chile, along with the Metropolitan Region are the two main strawberry growing areas in Chile with 80% of the production (http://www.odepa.gob.cl/). In this sense, the most important cultivars of *Fragaria* × *ananassa* planted in the Metropolitan Region are Albion and Monterey, while in Maule Region is Camarosa followed by Monterey, Portola and Cristal cultivars (http://www.odepa.gob.cl/).

In the freshly fruit production, texture is an important attribute for consumer acceptability, and it is related with fruit softening during ripening. Commercial strawberry fruit exhibit a rapid softening during ripening, however differences between strawberry cultivars or species has been reported with contrasting softening rates (Rosli et al., 2004; Dotto et al., 2006; Villarreal et al., 2008; Figueroa et al., 2010). Fruit softening that takes place during ripening has been associated to the degradation or disassembly of the different cell wall components (Brummell and Harpster, 2001). Cell wall disassembly is related to loss of the xyloglucan-cellulose network and pectin solubilization (Brummell 2006). These processes increase cell wall porosity, which in turn enhance the access of proteins and enzymes to their substrates for degradation (Brummell 2006). Rosli et al. (2004) evaluated changes in the cell wall composition of three *F.* × *ananassa* cultivars, reporting clear differences in the amount and degree of pectin depolymerization and suggesting that pectin metabolism plays an important role in strawberry fruit softening.

Several enzymes related to pectin metabolism in *F.* × *ananassa* have been related to the softening, such as polygalacturonase (PG) (Redondo-

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Nevado et al., 2001; Villarreal et al. 2008; Figueroa et al., 2008), pectate lyase (Medina-Escobar et al., 1997; Jiménez-Bermúdez et al., 2002; Benítez-Burraco et al., 2003; Figueroa et al., 2008), β-galactosidase (Trainotti et al., 2001), pectin methylesterase (Castillejo et al., 2004; Figueroa et al. 2010) and expansins (EXPs) (Dotto et al. 2006, Figueroa et al. 2009). Additionally, rhamnogalacturonate lyase 1 (FaRGLyase1) enzyme has been reported as an relevant enzyme in the cell-wall middle lamellae degradation (Molina-Hidalgo et al., 2013), while two xyloglucan endotransglycosylase/hydrolase (XTH1 and XTH2) enzymes have been involved in the remodeling of plant cell wall hemicelluloses in strawberry fruit ripening (Opazo et al. 2010; Nardi et al., 2014).

Softening is an important trait to consider in the fruit postharvest life and quality. In this sense, comparison studies between different strawberry cultivars is still scarce. Thus, the aim of this work was to compare the cell wall components of the four strawberry cultivars Camarosa, Crystal, Monterey and Portola grown in Maule Region, Chile, To assess this aim we analyzed the relationships between the mRNA abundance levels of cell wall-related genes, total enzymes activity, and fruit firmness of the fruit belonging to the four cultivars during development and ripening.

## 2. Materials and methods

### 2.1. Plant material

Fruits from *F.* × *ananassa* cultivars Camarosa, Crystal, Monterey and Portola were harvested from plants grown in the same commercial orchard in Pelluhue, Maule Region, Chile (latitude 35°50'00"S; longitude 72º38'00"W). Harvested fruits were immediately transported to the laboratory (Institute of Biological Sciences at Universidad de Talca) under cold conditions. Fruit were classified into four different developmental stages according to weight and color of the receptacle (Figure S1), and named as: large green fruit (G); white fruit (W); 50% red fruit (50%); and ripe fruit (R) according to previous reports by Rosli et al. (2004) to Camarosa, Pajaro and Toyonaka cultivars. A total of 60–70 fruits were collected from each developmental stage of each cultivar.

### 2.2. Fruit quality determinations

For each cultivar and developmental stages 20 fruits without external damage were analyzed for weight (g) and firmness. Firmness was measured using a texture analyzer (model CT3, Brookfield Engineering Labs., USA) using a 1-mm diameter cylinder probe. Each fruit was punctured at the equatorial region on opposite sides and expressing the results in Newton (N). Then fruit was cut into pieces, frozen in liquid nitrogen and stored at -80 °C until use. A bulk of tissue sample was prepared from each developmental stage per cultivar.

For the determination of soluble solid content (SSC), titratable acidity (TA) and pH, 2 g of frozen tissue from each replicate were ground with liquid nitrogen, homogenized in 5 ml distilled water and filtered through miracloth. SSC was determined in the fruit juice at 20 °C using a hand-held temperature compensated refractometer (Atago, Tokyo, Japan) and expressed as ºBrix. The pH was measured in the juice using a pH-meter (model pH 20, HANNA Instruments, USA). TA was determined by diluting the remaining juice in distilled water (1/10, v/v), and titrating an aliquot of 13 ml with 20 mM NaOH to pH 8.2 with a digital burette (Jencons, UK) and expressed as g citric acid per 100 g of fresh weight (FW). The determination was carried out in triplicate, and the results were expressed as the SSC/TA ratio.

### 2.3. RNA isolation and reverse transcription

Total RNA was isolated from frozen fruit and different plant tissues using CTAB method based on Salvatierra et al. (2010). Contaminant genomic DNA was removed using TURBO DNA-free™ Kit (Ambion, Life Technologies) according to the manufacturer's procedure. RNA integrity and concentration were determined by agarose gels and by ND-1000 UV spectrophotometer (Nanodrop Technologies, Montchanin, DE, USA), respectively. Three independent RNA extractions were carried out from each frozen pool of samples from different fruit developmental stages per cultivar. First-strand complementary DNA (cDNA) synthesis was performed using a First Strand cDNA Synthesis Kit (Fermentas Life Science, Glen Burnie, MD, USA) following the manufacturer's instructions.

### 2.4. Transcripts abundance analysis by real time quantitative PCR (qRT-PCR)

The mRNA abundance of *F.* x *ananassa* expansins A (*FaEXPA*s), xyloglucan endotransglycosylase/hydrolases (*FaXTH*s) and polygalacturonase 1 (*FaPG1*) genes were measured by qRT-PCR analysis. Reaction and quantification were performed following the procedure described by Ramos et al. (2012). Primers used for qPCR analysis are listed in Table S1. Amplification reactions were performed using KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal (Kapabiosystems) in a Step One Real-Time PCR system (Applied Biosystems) according to the manufacturer's instructions. Amplification conditions were: 95 °C for 10 min; 40 cycles of 95 °C for 15 s, 60 °C for 1 min; and a melting curve from 55 °C to 95 °C at 0.3 °C increments. Serial dilutions of amplified PCR products were used as standard templates to assess the PCR efficiency for each primer pair (Table S1), using a cDNA from fruit samples as template. Each reaction was performed in triplicate, and a negative water control was included in each run, and normalized against the expression level of *F.* x *ananassa* glyceraldehyde-3-phosphate-dehydrogenase 1 (*FaGAPDH1*) gene. Fluorescence was measured at the end of each extension step. Data were analyzed using the Excel (Microsoft) macro GENEX v1.10 (gene expression analysis for iCycler iQ® Real-Time PCR Detection System, v1.10, 2004; Bio-Rad Laboratories), using the methods derived from the algorithm of Vandesompele et al. (2002).

### 2.5. Activity assays of cell wall-modifying enzymes

Total xyloglucan endotransglycosylase (XET) activity was assayed with a colorimetric method based on the measure of overall XG-degrading activity (XDA) in the presence ($XDA_{OS}$) and absence ($XDA_0$) of XG-subunit oligosaccharides (XGOs) according to Sulová et al. (1995) with the adaptation of Opazo et al. 2010. The $XDA_0$ activity was measured in a reaction mixture of 200 µL total volume consisting of 0.2 mg tamarind xyloglucan (Megazyme, Ireland), and 300 µg protein extract in 40 mmol $L^{-1}$ sodium acetate buffer (pH 6.0). $XDA_{OS}$ was measured in the presence of 0.1 mg $mL^{-1}$ XGOs (Megazyme) in the reaction mixture. The mixture was incubated at 37 °C during 1 h. For negative control and blank tubes, sodium acetate buffer (instead of protein extract) and XGOs were used, respectively. To each aliquot, 300 µL of 20% (w/v) $Na_2SO_4$ and 30 µL of $I_2$–KI solution (0.5% $I_2$ + 1% KI) were added and the tubes were allowed to stand for 30 min in the dark at room temperature. The optical density of the samples was measured at 620 nm against the blank. The net XG-transglycosylating activity (XETA), expressed as arbitrary units (a.u.), and XG-degrading activity (XDA), expressed as mg of xyloglucan hydrolyzed by mg of protein in the absence of XGOs, were calculated according to Opazo et al. 2010.

The methodology to measure total polygalacturonase (PG) activity was measured to the method described by Gross (1982) and adapted by Villarreal et al. (2008) and Figueroa et al. (2010). Frozen strawberries (10 *g*) were homogenized in an Omnimixer with 30 mL of the following buffer: 0.05 mol L − 1 sodium acetate/acetic acid, 1% (w/v) PVPP, pH 6.0. The mixture was centrifuged at 12,000 x g for 30 min and the supernatant was discarded. The pellet was washed twice with 30 mL of buffer A (0.05 M sodium acetate/acetic acid pH 6.0). Then, the sample was centrifuged at 12,000 x g for 30 min, the supernatant was discarded and the pellet was extracted with 30 mL of buffer A containing 1 mol