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Transcription analysis of softening-related genes during postharvest of papaya fruit (*Carica papaya* L. 'Pococí' hybrid)

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ABSTRACT

Fruit of *Carica papaya* L. (papaya) has several post-harvest problems mainly caused by quick softening that reduces its shelf life. This softening is directly related to degradation and modification of cell wall oligosaccharides. Therefore, it is important to study, understand and, eventually, regulate the softening process of this fruit to increase its shelf life. This work aims at looking for correlations between transcription patterns of four genes potentially involved in papaya fruit softening with postharvest treatments and the softening process. Papaya fruit ('Pococí' hybrid) were treated with ethylene (275–300 mL L^{-1}), 1-methylcyclopropene (1-MCP, 300 nL L^{-1}), or not treated, as control. Fruit were subsequently stored for eleven days at 18–20 °C and 95% relative humidity. During the evaluation period, firmness (N) and color (CIE L^*, a^* and b^*) of pulp and peel were determined; pH, titratable acidity (TA) and total soluble solids (TSS) of pulp were also measured; in addition, transcription patterns of polygalacturonase, endoxylanase, pectinesterase and expansin genes were determined by real time PCR. Treatments showed differences in terms of firmness, color, pH, TA, ripening index and accumulation of transcripts of some genes. Transcription of polygalacturonase and endoxylanase genes correlated negatively with firmness of pulp and peel; whereas pectinesterase gene was positively correlated with peel firmness. No correlation with transcription of the expansin gene analyzed was found. Our results also suggest that polygalacturonase and endoxylanase correlated negatively with papaya fruit firmness and that 1-MCP treatment repressed and reduced the expression of these two genes, respectively. According to these results, silencing genes that encode polygalacturonases or endoxylanases might be a potential strategy to confirm their crucial role on papaya ripening.

1. Introduction

Papaya (*Carica papaya* L.) is a crop of great economic importance worldwide. Papaya fruit is consumed fresh in many countries and has a high potential to be industrialized (Fuentes and Santamaría, 2014). Despite the importance of this crop and the breeding efforts that have been conducted, the marketing of papaya fruit has limitations related to post-harvest constrains. The rapid fruit softening during ripening increases susceptibility to mechanical damage and to pathogen infections, reducing shelf life and increasing post-harvest losses (Chen et al., 2007 and references therein). Therefore, studying the softening process of this climacteric fruit, and understanding the genes involved in cell wall disassembling, could give clues to increase its post-harvest life.

Fruit softening has been related to the degradation or modification of cell walls by hydrolases and other cell wall associated proteins (Chen et al., 2007; Figueroa et al., 2009; Sañudo-Barajas et al., 2008b; Shiga et al., 2009; Thumdee et al., 2010). Enzymes like polygalacturonase, pectin methylesterase or pectinesterase, glucanase, endoglucanase, galactosidase,

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xylosidase and endoxylanase have been reported to be involved in papaya cell wall disassembly (Chen et al., 2007; Lazan et al., 1995; Razali et al., 2007; Thumdee et al., 2007, 2010). In addition, expansins, non-enzymatic cell wall proteins, have been linked to the process of fruit softening in highland papaya, *Vasconcellea pubescens* (Cosgrove, 2005; Gaete-Eastman et al., 2009). Some of these enzymes and proteins have been reported to be sensitive to the plant hormone ethylene during fruit ripening, which also controls gene expression (Bennett and Labavitch, 2008; Mwaniki et al., 2005; Storch et al., 2015). In this sense, climacteric fruit showed a peak in ethylene production, concomitant with a rise in CO_2 level. This fact regulates ripening-related events, including enzymes involved in softening (Barry and Giovannoni, 2007). The use of 1-methylcyclopropene (1-MCP), an ethylene perception blocker, has proved to be a useful tool to study ethylene dependence of ripening-related events (Blankenship and Dole, 2003; Freiman et al., 2015; Manenoi et al., 2007; Zhu et al., 2015). Therefore, ethylene and/or 1-MCP treatments can be employed to experimentally accelerate and/or delay the ripening process of papaya fruit, in order to measure the expression of key genes and identify their possible roles in the softening process. Simultaneously, changes in common physical and chemical post-harvest parameters (such as firmness, color, pH, total acidity and total soluble solids) must be measured to reflect progress through fruit ripening process stages and confirm changes associated to the treatments.

Several genes coding for enzymes involved in papaya fruit softening have been previously identified and isolated (Chen and Paull, 2003; Devitt et al., 2006; Fabi et al., 2009, 2010; Gaete-Eastman et al., 2009; Manenoi and Paull, 2007a). Solubilization of pectins by polygalacturonase (*cp*PG1) and solubilization of hemicellulose by endoxylanase (*cp*EXY1) are now considered to play key roles in papaya pulp softening, although the expression of the former occurred after most softening had already happened (Fabi et al., 2014).

Nevertheless, confusion still exists about the role of pectin methylesterase or pectinesterase (PME1 and PME2) on the softening process (Fabi et al., 2010, 2012, 2014; Lazan et al., 1995; Razali et al., 2007; Thumdee et al., 2007, 2010). Furthermore, little information exists about the expression of expansin genes and their role on *C. papaya* fruit ripening (Fabi et al., 2012).

Most studies about softening, quality and effect of postharvest treatments on papaya fruit have been focused on measuring the activity of the enzymes mentioned above, while fewer reports exists about the dynamics of expression of their genes (Chen and Paull, 2003; Devitt et al., 2006; Fabi et al., 2009, 2010, 2012, 2014; Manenoi and Paull, 2007a). Furthermore, the dynamics of expression of these genes in different fruit tissues (i.e., pulp and/or peel) in response to postharvest treatments has not been previously reported. A better understanding of the transcription patterns of genes involved in fruit softening of *C. papaya* L. 'Pococí' subjected to different postharvest treatments and their correlation with softening are the main aims of this study.

2. Materials and methods

2.1. Plant material

One hundred and fifty 'Pococí' hybrid papaya fruit (*C. papaya* L.) were selected from the production line of a packaging company (Centro Agrícola Cantonal de Guácimo, Limón, Costa Rica) at ripening stage 1, defined as a yellow stripe near the fruit apex (Umaña et al., 2011). All fruit were similar in size (between 1.0 and 1.2 kg, 20 and 24 cm long, 15 and 18 cm diameter) and without any apparent physical damage.

2.2. Post-harvest treatments

Fruit were subsequently disinfected by immersion in 100 mg L^{-1} sodium hypochlorite (Cloro, Lemen de Costa Rica S.A., San José, Costa Rica) for 1 min, followed by immersion in a fungicide solution composed of 250 mg L^{-1} Mirage® 45EC (a.i. prochloraz, Makhteshim Chemical Works Ltd., Beer Sheva, Israel) and 250 mg L^{-1} Mertect® 50SC (a.i. thiabendazole, Syngenta S.A., Bogotá, Colombia). Fruit were allowed to dry at room temperature and then stored in a ripening chamber at 18–20 °C until they reached ripening stage 2, defined as fruit with two yellow areas on their peel (Umaña et al., 2011). Fruit were then separated into three groups as follows: 1. fifty non-treated fruit as control; 2. fifty fruit treated with 275–300 mL L^{-1} gaseous ethylene for 21 h in a chamber equipped with an ethylene generator (ARCO Ethylene Generator Model #100, American Ripener Company Inc., Charlotte NC 28217, USA); 3. fifty fruit treated with 300 nL L^{-1} gaseous 1-MCP (0.14% SmartFresh™ Technology, AgroFresh Inc. and Rohm and Haas Company, Spring House PA 19477-0904, USA) for 21 h in a 400 m^3 hermetic plastic chamber. Selecting the 21 h for the treatments was based on previous unpublished results of the authors. After treatment, the fruit were stored in ripening chambers at 18–20 °C for eleven days.

2.3. Physical and chemical parameters

Firmness and color of five fruit from each group were determined at days 0, 3, 5, 7 and 11, to evaluate the effects of the treatments on the ripening process of papaya over time. Pulp and peel firmness was measured on the intact fruit using a texturometer (TA-TX Plus Texture Analyzer, Stable Micro Systems LTD., Godalming, UK) with a 2 mm diameter stainless steel probe at a speed of 1.5 mm s^{-1} and a depth of 10 mm, as described by Schweiggert et al. (2011, 2012). Two measuring points were chosen in the center of each fruit carpel at the maximum diameter zone. Pulp firmness corresponded to the average force (N) between 4 and 6 mm penetration and peel firmness corresponded to the maximum force (N) registered for 10 mm penetration.

Color was measured with a colorimeter (ColorFlex®, Hunterlab, Hunter Associates Laboratory Inc., Reston VA 20190, USA) in CIElab scale and using a D65 light and 10° viewing angle. Three color parameters were considered for this analysis: color lightness (CIE L^*); color change from green to red (CIE a^*) and color change from blue to yellow (CIE b^*). To measure the peel color, each fruit was placed on the colorimeter and three random measures were made on the exocarp of each fruit. To measure the pulp color, fruit were cut in half and two circle pieces of each half were removed with a metal stainless steel biscuit round cutter (5 cm x 1 cm). These pieces were placed on the colorimeter and three random color measures were made on each.

Total soluble solids (TSS), pH and titratable acidity (TA) of five fruit per group were also determined at the beginning and at the end of the experiment (days 0 and 11) to evaluate the effects of treatments on the ripening process of papaya over time. TSS were determined using a digital refractometer (AR200 Digital Refractometer, Reichert Technologies, Depew NY 14043, USA) as described by the official method 932.12 of the Association of Official Analytical Chemists (AOAC, 2005). pH was determined using a pHmeter (Corning pHmeter 430, Corning Incorporated and Nova Analytics Corporation, Woburn MA 01801, USA) as described by the official method 981.12 (AOAC, 2005). TA was determined as percentage of citric acid as described by the official method 942.15 (AOAC, 2005). All chemical parameters were determined by triplicate for each fruit. Papaya ripening index (RI = PF/TSS; where PF = pulp firmness in N) and TSS/TA ratio were calculated as proposed by Schweiggert et al. (2011, 2012) with the following