RESEARCH NOTE

Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 44 (2007) 179–183
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Research Note

Characterization of wound-regulated cDNAs and their expression in fresh-cut and intact papaya fruit during low-temperature storage

Yasar Karakurt [a,*], Donald J. Huber [b]

[a] *Suleyman Demirel Universitesi, Ziraat Fakultesi, Isparta 32260, Turkey*
[b] *Horticultural Sciences Department, PO Box 110690, University of Florida, Gainesville, FL 32611, USA*

Received 22 December 2005; accepted 7 December 2006

Abstract

Fresh-cut papaya fruit undergo rapid tissue deterioration during storage. A comparative gene expression study was conducted to isolate genes differentially expressed upon fresh-cut processing by means of mRNA differential display RT-PCR. Differential display analysis was performed on intact and fresh-cut papaya fruit stored in parallel for 12 h at 5 °C with three different one-base-anchored oligo dT and eight arbitrary primers. Confirmation of true positive bands was performed by northern blotting. Fourteen differentially expressed cDNAs ranging from 154 to 777 bp were cloned, sequenced and compared to GenBank sequences. The partial cDNAs showed significant homologies to signaling pathway genes, membrane proteins, cell-wall enzymes, proteases, ethylene biosynthetic enzymes, and enzymes involved in plant defense responses. Northern blot analysis with probes of each of the partial clones revealed that most of the genes corresponding to partial cDNAs were expressed in a fresh-cut dependent manner during 8 days storage. The transcripts for PC18-5, PA19-3 and PC17 were not detectable on northern blots. The results suggest that fresh-cut processing induces the expression of proteins involved in membrane degradation, free radical generation, and enzymes involved in global stress responses.

Keywords: cDNA cloning; Differential display; Fresh-cut; Papaya fruit

1. Introduction

A role for both cell walls and membranes in the rapid deterioration of fresh-cut papaya fruit has been proposed (Karakurt and Huber, 2003). This results from enhanced softening, solubility and depolymerization of polyuronides, and increased activities of polygalacturonase, α and β-galactosidase, lipoxygenase, and phospholipase D in fresh-cut compared with intact fruit stored under identical conditions. These effects were noted as early responses to fresh-cut processing.

Plants respond to mechanical wounding with the induction of numerous genes. For instance, many genes including those involved in defense responses are induced by mechanical wounding in *Arabidopsis* (reviewed in Reymond and Farmer, 1998) and cacao leaves (Bailey et al., 2005). The expressions of many of these genes are induced by treatment with jasmonic acid (JA) or its precursor oxophytodienoic acid. These compounds are essential in vivo regulators of defense gene expression (Reymond and Farmer, 1998). Other signals and stimuli also lead to the expression of genes in wounded plant tissues, although the involvement of growth regulators such as ethylene (Bailey et al., 2005) and abscisic acid (Birkenmeier and Ryan, 1998), and of electrical signals (Wildon et al., 1992) and water stress/hydraulic pressure (Malone and Alarcone, 1995) are still unclear.

The objective of this study was to characterize differentially expressed genes induced in response to fresh-cut processing (wounding) in papaya fruit by means of mRNA differential display (Liang and Pardee, 1992). The identification of genes involved in the deterioration of fresh-cut tropical fruit might have significance for extending their shelf-life, and provide information of value to long-term goals of designing or selecting cultivars through genetic transformation with genes conferring resistance or tolerance to deterioration. The gene expression profiles of fresh-cut fruit were compared to those of intact fruit stored under identical conditions. This approach was employed to facilitate identification of genes regulated by wounding (fresh-cut processing) versus

* Corresponding author. Fax: +90 2371693.
E-mail address: karakurty@hotmail.com (Y. Karakurt).

0925-5214/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2006.12.002

those responsive to the low temperature (5 °C) employed for storage.

2. Materials and methods

Papaya (*Carica papaya*, var. Sunrise Solo) fruit originating from Belize were obtained from Brooks Tropicals, Homestead, FL, USA. Fruit were sorted for uniformity and freedom from defects, washed with tap water, and rinsed with chlorinated water containing 150 μL L^{-1} free chlorine as were the cutting surfaces and cold room interior prior to cutting. The fruit were stored at 20 °C until they reached 60–70% yellow skin color. Fresh-cut processing of the post-climacteric fruit was performed as described by Karakurt and Huber (2003). The tissue pieces were randomized and stored in vented plastic containers for 12 h, 1, 2, 4 and 8 days at 5 °C. Intact fruit at the same physiological ripening stage were used as controls and were stored under the same temperature/time regimes to address the influence of fresh-cut processing on gene expression independently of low-temperature storage. At the specified storage intervals, fruit pieces were removed from storage, immediately frozen in liquid nitrogen, and analyzed as described below. At each sampling period, intact fruit were peeled, cut into pieces and immediately frozen.

Total RNA was isolated as described by Strommer et al. (1993) and used for both Northern analysis and mRNA differential display. Total RNA extracted from intact and fresh-cut fruit stored for 12 h was used for differential display analysis. Total RNA (30 μg) was treated with DNase I in order to eliminate genomic DNA contamination (GenHunter).

Two microliters (0.1 μg/μL) DNA-free total RNA was reverse transcribed with MMLV reverse transcriptase using subsets of specific 1-base-anchored oligo (dT) primers (H-T11G, H-T11A, or H-T11C) that recognize different fractions of the total poly (A)+ RNA population as described (GenHunter). The resulting cDNA was amplified with a combination of the fluorescein-labeled-anchored oligo(dT) primer and a 13-base pair (bp) primer of an arbitrary sequence (one of H-AP17-H-AP24, GenHunter) by a polymerase chain reaction with Taq DNA polymerase (Qiagen) in a thermal cycler (Techgene, Cambridge, UK) as described (GenHunter). Amplified cDNAs were separated on a 6% denaturing polyacrylamide DNA sequencing gel (4 mm) containing 7 M urea as described (Sambrook et al., 1989). The gel was scanned on a fluorescence Imager with 525 nm filter following manufacturer's instructions (FMBIO). Differentially expressed bands of interest were cut from the gel and re-amplified as described (GenHunter).

Thirty microliters of the PCR samples were run on a 1.5% agarose-TBE gel and stained with ethidium bromide. After confirmation of the size of re-amplified PCR products with their sizes on the original DNA sequencing gel, the re-amplified cDNA probes were extracted from the agarose gel using QIAEX kit according to manufacturer's instructions (Qiagen). The extracted cDNA was labeled with Digoxygenin dUTP using DIG-High Prime labeling kit following manufacturer's instructions (Roche). The DIG-labeled probes were used to verify the differential gene expression and the change in gene expression through 8 days of storage by Northern blot analysis as described below with 20 μg total RNA.

For Northern blot analysis, total RNA (20 μg) was fractionated on a 1.5% agarose-formaldehyde gel (1.5% agarose, 20 mM MOPS, 5 mM sodium acetate (pH 7.0), 1 mM EDTA, 1% formaldehyde, 0.1 μg/mL ethidium bromide) and then transferred onto a nylon membrane (Roche, Indianapolis, IN, USA) by capillary transfer in 10× SSC buffer (1.5 M NaCl, 0.3 M sodium citrate, pH 7.0). After 12 h transfer time, the membrane was rinsed briefly in 2× SSC to remove excess salt, and placed in a UV cross-linker (312 nm, Bioslink). Blots were pre-hybridized in DIG-Easy solution and then hybridized with the DIG-labeled probe according to manufacturer instructions (Roche). Following hybridization, washing and detection were carried out as described (Roche). Equivalence of RNA loadings was checked by examination of the gel over a UV transilluminator (Fisher Scientific, Pittsburgh, PA, USA).

After confirmation of differential gene expression, the re-amplified PCR products were cloned via either PCR-TRAP (GenHunter) or TA Cloning System (Invitrogen). Plasmids containing the insert of interest were prepared using Wizard plus SV Miniprep kit following manufacturer's instructions (Promega). The plasmids were sequenced (ICBR, University of Florida) with fluorescent dideoxy terminator method of cycle sequencing on a Perkin-Elmer, Applied Biosystems Division (PE/ABd) 373A or 377 automated DNA sequencer, following Abd protocols (McCombie et al., 1992). All consensus sequences were generated using the Sequencher Software from Gene Codes (Ann Arbor, MI). The sequences were compared to the nucleotide and protein sequences available in the database using BLAST programs with default settings (Altschul et al., 1997).

3. Results and discussion

Fresh-cut papaya fruit show significant softening by day 8 of storage (Karakurt and Huber, 2003). Analysis of changes in enzyme patterns in fresh-cut versus intact fruit during 8 days of storage (Karakurt and Huber, 2003) revealed that the activity of α- and β-galactosidases, lipoxygenase, phospholipase D, and polygalacturonase were enhanced in response to fresh-cut processing (wounding), suggesting an early targeting of the membrane and cell wall in the rapid softening and deterioration of fresh-cut fruit as compared with intact fruit. In the present study, parallel comparisons of fresh-cut and intact (control) fruit differential display (DD-PCR) profiles revealed differences indicative of a wound-induced alteration of fresh-cut fruit RNA population.

Table 1 summarizes the cDNAs isolated from the fresh-cut fruit stored for 12 h at 5 °C by the differential display strategy. Of the 91 differentially expressed fragments, a total of 14 cDNA products of interest were excised, successfully re-amplified, and cloned. To determine the expression patterns of each of the isolated clones and to correlate their expression patterns to the softening and deterioration of fresh-cut fruit observed during 8 days of storage (Karakurt and Huber, 2003), RNA was extracted from intact and fresh-cut fruit stored for 1, 2, 4 and 8 days at 5 °C and used for Northern blot analysis (Fig. 1).