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Benzylglucosinolate, Benzylisothiocyanate, and Myrosinase Activity in Papaya Fruit during Development and Ripening

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Papaya is a climacteric fruit that has high amounts of benzylglucosinolates (BG) and benzylisothiocyanates (BITC), but information regarding levels of BG or BITC during fruit development and ripening is limited. Because BG and BITC are compounds of importance from both a nutritional and a crop yield standpoint, the aim of this work was to access data on the distribution and changes of BG and BITC levels during fruit development and ripening. BG and BITC levels were quantified in peel, pulp, and seeds of papaya fruit. Volatile BITC was also verified in the internal cavity of the fruit during ripening. The influence of the ethylene in BG and BITC levels and mirosinase activity was tested by exposing mature green fruits to ethylene and 1-methylcyclopropene (1-MCP). The highest BG levels were detected in seeds, followed by the peel and pulp being decreased in all tissues during fruit development. Similarly, the levels of BITC were much higher in the seeds than the peel and pulp. The levels of BG for control and ethylene-treated fruit were very similar, increasing in the pulp and peel during late ripening but not changing significantly in seeds. On the other hand, fruit exposed to 1-MCP showed a decrease in BG amount in the pulp and accumulation in seed. The treatments did not result in clear differences regarding the amount of BITC in the pulp and peel of the fruit. According to the results, ethylene does not have a clear effect on BITC accumulation in ripening papaya fruit. The fact that BG levels in the pulp did not decrease during ripening, regardless of the treatment employed, and that papaya is consumed mainly as fresh fruit, speaks in favor of this fruit as a good dietary source for glucosinolate and isothiocyanates.

KEYWORDS: Benzylglucosinolate; benzylisothiocyanate; myrosinase activity; papaya fruit

INTRODUCTION

Glucosinolates are sulfur-rich compounds found in plants with potential benefits for human health and controlling agricultural pests. This class of compounds comprises almost 120 compounds with a sulfonated moiety, a β-D-thioglucose group in combination with a variable amino acid-derived side chain (R group) as the typical structure. Glucosinolates are mainly found in the Capparales order, which includes the Brassicaceae family, and specifically in *Carica papaya*, a representative of the Violales order (*1, 2*).

Although glucosinolates themselves are stable and inactive, the products of their breakdown, especially isothiocyanates, are bioactive compounds acting as insect repellents, bactericides, nematicides (*3*−*5*), and putative anticarcinogenics in humans (*6*−*12*). Isothiocyanate is produced from the unstable aglycone moiety of the glucosinolate following glucose release by the action of β-thioglucosidase (EC 3.2.1.147) or myrosinase. As a result, myrosinase plays an important role in the production of the bioactive isothiocyanate molecule. In normal plant tissues, the enzyme is brought into contact with its substrate mostly after physical damage to the tissue by mechanical action, pests, mastication, freeze−thawing, etc. However, although the enzyme is concentrated in the myrosin cells, the production of isothiocyanates under conditions of preservation of cellular integrity is possible by symplastic transportation of the glucosinolates to the myrosin cells, as discussed by Andreasson et al. (*13*).

In *C. papaya*, the occurrence of benzylisothiocyanates (BITC) was first reported in the seeds (*14*) and pulp (*15, 16*) of the fruit. The precursor benzylglucosinolate (BG) was detected in all of the plant tissues (*17*−*19*), being the only glucosinolate found in this species (*19*). Some studies had attributed a protective effect of BG and BITC against the deposition and viability of fruit fly eggs and larvae (*20, 21*). In plants,

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glucosinolate production seems to be responsive to several biotic and abiotic stresses and also to molecules that are usually involved in signaling for disease resistance, such as salicylic acid, jasmonic acid, and ethylene (*3, 22*−*25*).

Papaya is a climacteric fruit with a typical rise in respiration as a consequence of the autocatalytic production of ethylene during ripening. This ethylene increase accounts for several changes in sensorial attributes, such as pulp firmness, color, and taste. In addition, the amount of compounds beneficial to human beings, such as carotenoids, can be adversely affected by the supplementation or depletion of ethylene (*27*). As mentioned above, isothiocyanates are valuable bioactive compounds due to their proposed anticarcinogenic effects, and the papaya fruit could be an alternative source of isothiocyanate in the human diet. However, information on the amounts of BG and BITC in papaya fruit tissues is limited, and the effects of procedures employed during postharvest handling are not known. Because BG and BITC are molecules of importance from both a nutritional and a crop yield standpoint, the aim of this work was to access data on the distribution and changes of BG and BITC levels and myrosinase activity in fruit that underwent either noninduced ripening or exposure to exogenous ethylene or to the ethylene antagonist 1-methylcyclopropene (1-MCP).

MATERIALS AND METHODS

Plant Material. Papaya fruit (*C. papaya* cv. Golden) was obtained directly from a producer in the cities of Sooretama and Linhares, Espírito Santo, Brazil (19°15′S, 40°10′W, 25 m altitude).

First Experiment—*Fruit Development.* Fruits were harvested at 30, 60, 90, 120, and 150 days after anthesis (daa). Papaya-150 daa were left to ripen spontaneously in a 240 dm^3 chamber with controlled temperature and humidity (25 ± 0.1 °C and 95%, respectively) and were sampled daily. Samples consisted of at least 10 fruits each, in which the peel, pulp, and seeds were separated, frozen in liquid N_2, and stored at −80 °C.

Second Experiment—*Fruit Ripening under Ethylene and 1-MCP Treatments.* Fruit were harvested at color break to one-fourth yellow (∼150 daa). Soon after their arrival in the laboratory (2 days after harvest), the fruits were randomly divided into three groups. Papayas from the control group were stored as described above. The other two groups were treated with either ethylene or 1-MCP. For ethylene treatment (2 days after harvest), the papayas were left in a 240 dm^3 chamber and exposed to a concentration of 100 ppm (100 $\mu L\ L^{-1}$) ethylene in a synthetic air mixture in a constant flow-through system for 2 (for gas saturation) and 10 h in a closed system. The third group was left in a 334 dm^3 chamber and treated with 1-MCP (2 days after harvest) at a concentration of 100 ppb (100 $nL\ L^{-1}$) for 12 h in a closed system. The 1-MCP gas was generated by the dissolution of approximately 18 mg of EthylBloc (Floralife, Inc.) powder (0.14% active ingredient) in 5 L of distilled water inside the box containing the papayas. After both treatments, the fruits were ventilated and left to ripen in separate chambers under the same conditions of temperature and humidity as described for the control group. On the basis of respiration and ethylene profiles, performed as described by Fabi et al. (*26*), fruits were sampled, and the peel, pulp, and seeds were divided, frozen in liquid N_2, and stored at −80 °C. Each sample consisted of at least four fruits.

BG Extraction and Analysis. BG was extracted from 125 mg of the tissue pulverized using a mortar and pestle with liquid N_2. The powdered tissue was transferred to centrifuge tubes, and 1 mL of 70% methanol + 0.1% trifluoroacetic acid (TFA) was added to each tube. A 50 μL aliquot of 12 mM sinigrin [internal standard (IS) prepared in 70% methanol + 0.1% TFA] was added to the samples. The homogenates were shaken for 20 min at 70 °C and left to stand until equilibrated to room temperature. The samples were centrifuged at 13000*g* for 10 min at room temperature, and the supernatants were collected and filtered through a membrane (0.45 μm) into injection vials. The extracts were injected in a HP1100 chromatographic system (Hewlett-Packard) equipped with a Luna C_{18} column (Phenomenex, 250 mm × 4.6 mm, 5 μm) at 30 °C, and the analytes were separated using the following solvents: A, 0.1% (v/v) TFA in deionized water; and B, 0.1% (v/v) TFA in methanol. The elution gradient was as follows: 100% A for 10 min; 80% A and 20% B from 10 to 15 min; 50% A and 50% B from 15 to 25 min; 100% B from 25 to 35 min; 100% B kept until 45 min; changed to 100% A and kept until 55 min. The flow rate was 1.0 mL min^{-1}, and the detection was done using a diode array detector monitoring the effluent at 228 nm.

BITC Extraction and Analysis. The extractions of BITC from papaya tissues were done according to Brown et al. (*27*). Hexane was the solvent that yielded the highest levels of BITC in tests carried out with the papaya fruit. The tissues from peel, pulp, and seed samples were pulverized using a mortar and pestle in liquid N_2. Approximately 125 mg of the powdered tissue was transferred to a 1.5 mL microcentrifuge tubes, and 1 mL of hexane was added. The BITC was extracted under agitation at room temperature for 1 h. After centrifugation (10000*g* for 10 min at 25 °C), the supernatants were filtered through a membrane (0.45 μm) into injection vials.

Aliquots of 1 μL of each extract were injected in a gas chromatograph (Hewlett-Packard model 5890) equipped with a SPB-50 column (Supelco, 30 mts × 0.25 mm i.d, 0.25 μm film thickness). The injection port was kept at 250 °C, and a ramp temperature program was run at 70 °C (initial) increasing 4 °C min^{-1} to 150 °C. The samples were injected in splitless mode, and the helium carrier gas flow was 1 mL min^{-1}. The detection was done in a mass selective detector (Hewlett-Packard model 5973) with an ion source operating in 70 eV. The calculations were done based on a calibration curve constructed with BITC standard solutions from 10 to 500 $\mu g\ mL^{-1}$.

Volatile BITC Extraction and Analysis. Volatile BITC from the papaya was analyzed from extracted compounds found in the central cavity of the fruit. For this experiment, a polyethylene cannula was introduced through a small opening made with a sterile cork borer in the peduncle region of the fruit. The external part of the cannula was closed with a rubber septum, and a solid phase microextraction fiber (Supelco, 100 μm of polydimethylsiloxane) was introduced through it and allowed to be exposed to the volatiles accumulated in the central cavity of the fruit. After 1 h of exposure, the volatiles were desorbed by heating at the injection port (250 °C) in the same gas chromatograph system employed for the analyses of BITC extracted from the tissues. The desorption time in the injector was 5 min, and the chromatographic conditions were the same as the analysis cited above. This procedure was repeated with a total of five fruits, and the fruits were kept with the sampling cannulas throughout the experiment. The analysis was done every day during ripening.

Enzyme Extraction and Activity Assay. The protein extraction for the β-thioglucosidase activity assay was optimized according to Botti et al. (*28*) and Hara et al. (*29*). The myrosinase activities were determined by the release of glucose from sinigrin (*28, 30*). The reactions were done with 50 μL of enzymatic extract and 50 μL of 10 mM sinigrin and were incubated at 30 °C for 30 min. The reactions were stopped by the addition of 25 μL of 0.6 M perchloric acid. The glucose released was determined by the glucose oxidase/peroxidase/ABTS method, according to Bergmeyer (*31*).

RNA Isolation and Cloning. Total RNA was extracted as described previously (*32*). Five grams of frozen papaya seeds was triturated under liquid N_2, and following phenol and chloroform extractions, the RNA was precipitated with LiCl. The pellet was recovered in DEPC-treated water, and after ethanol precipitation, the RNA was resuspended in TE buffer.

Five micrograms of RNA isolated from the seeds of the papaya fruit was reverse-transcribed using "SuperScript First-Strand Synthesis System for RT-PCR" (Invitrogen) kit and oligo $(dT)_{12-18}$ primer, according to the manufacturer's instructions. Amplification of the cDNA fragment was performed in a 25 μL PCR reaction using degenerated sense [*Cp_myro_S1*; 5′-CCT TT(C/T) GTT AC(A/T) CT(G/T) TT(C/T) CA(C/T) TGG GA-3′] and reverse [*Cp_myro_R1*; 5′-ATA TCC CTT (A/G)CA (A/G)AA (C/G)TC (A/G)TA (A/G)TT GTC-3′] primers. Amplified cDNAs were resolved by agarose-gel electrophoresis and purified using "GFX PCR DNA and Gel Band Purification" kit (Amersham Biosciences).

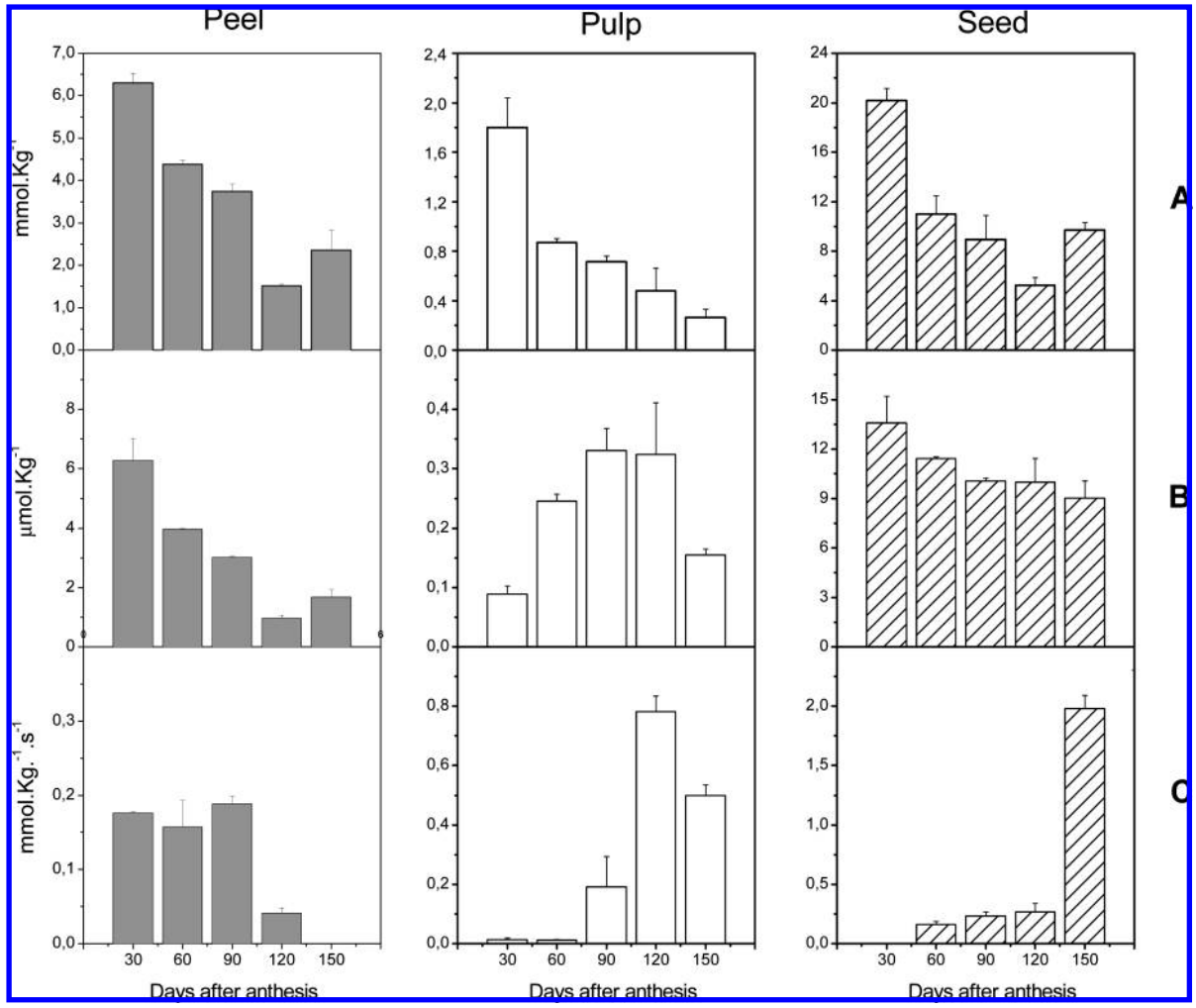

Figure 1. BG (**A**), BITC (**B**), and myrosinase activity (**C**) profiles in peel, pulp, and seeds of papaya fruit during development (fruit samples from the first experiment).

Gel-purified cDNAs were subcloned into a pCR4-TOPO vector using the TOPO TA Cloning kit (Invitrogen). DNA inserts were sequenced using the "Thermo Sequenase Cy5 Dye Terminator" and the ALF*express* II sequencing instrument (Amersham Biosciences). Sequences were analyzed using the Alfwin2.10 (Amersham Pharmacia Biotech) software, and the homology search on the GenBank was performed by online-based BLAST (*33*).

Northern Blotting Analysis. Total RNA (20 μg) from the seeds of the papaya fruit from the first experiment was size-fractionated by denaturing electrophoresis on a formaldehyde-agarose gel (*34*). The Northern blotting analysis was performed as previously described by Nascimento et al. (*32*).

RESULTS

BG and BITC Levels on Seeds, Pulp, and Skin during Fruit Development. Papayas were sampled at 30, 60, 90, 120, and 150 days after flowering, and the amounts of BG, BITC, and myrosinase activity were analyzed in the peel, pulp, and seeds. According to **Figure 1**, the highest BG levels were detected in seeds, followed by the peel and pulp, being at generally decreased levels in all of the tissues during fruit development. Similarly, the levels of BITC were much higher in the seeds than the peel and pulp. However, while BITC levels paralleled those of BG in the peel and seed, the amounts of isothiocyanate in the fruit pulp increased during development, peaking around 120 daa. Because BITC is produced after release of the aglycone moiety by myrosinase, the enzyme activity in the tissues was also evaluated. However, no suggestive correlation was observed between myrosinase activity and BITC levels, except in the pulp, where maximum activity was observed at 120 days. There was a 10-fold increase in activity in the seeds at late development, but no significant differences in BG or BITC levels.

BG and BITC on Fruit Tissues Following Treatments with Ethylene and 1-MCP. The levels of BG and BITC were also evaluated for fruit treated with either ethylene or 1-MCP and also for those allowed to ripen naturally. **Figure 2** presents the changes in BG amounts for papaya pulp, peel, and seeds after the treatments. The overall appearance of the curves for control and ethylene-treated fruit was very similar, showing an increase in BG levels for the pulp and peel during late ripening but no significant changes in levels for the seeds. On the other hand, fruit exposed to 1-MCP showed a decrease in BG amounts in the pulp and an accumulation of BG in the seeds. The treatments did not result in clear differences regarding the amount of BITC in the pulp and peel of the fruit (**Figure 3**). During ripening, the levels in the pulp did not change significantly, while the peel of fruit from all treatments showed an accumulation of BITC. For fruit treated with 1-MCP, BITC levels were higher than what was observed after ethylene treatment, which is in agreement to the observed changes in BG levels. Myrosinase activity in seeds was also similar among the different treatments. According to **Figure 4**, the enzyme activity increased during

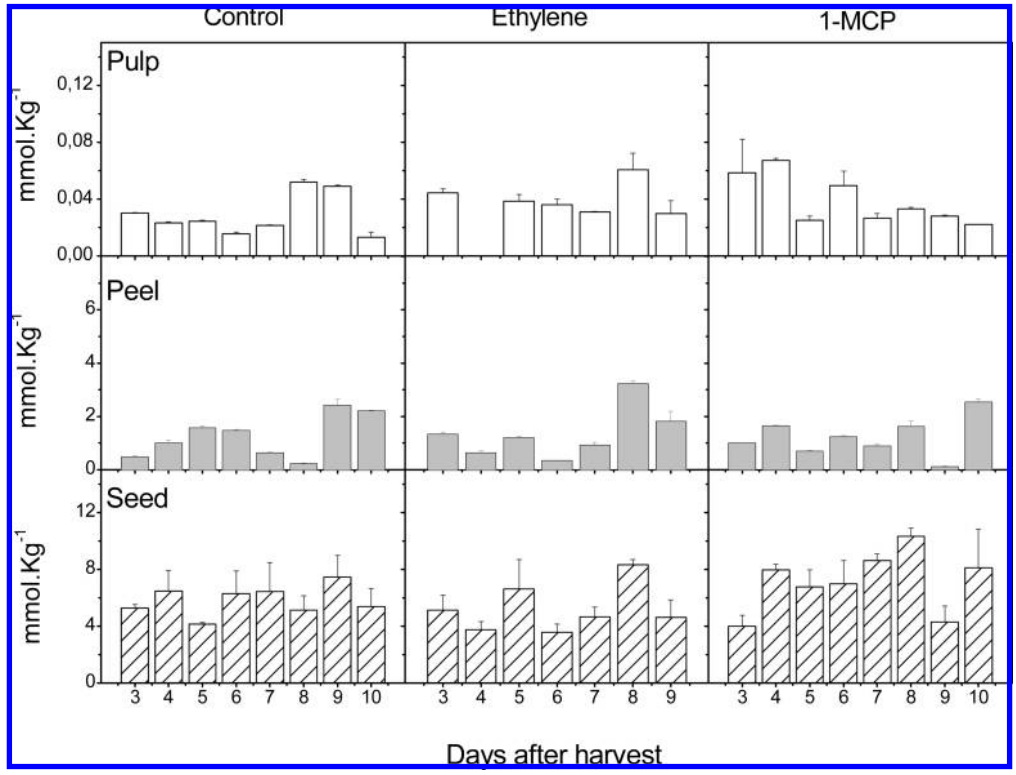

Figure 2. BG profiles in peel, pulp, and seeds of papaya fruit submitted to ethylene and 1-MCP treatments during postharvest ripening.

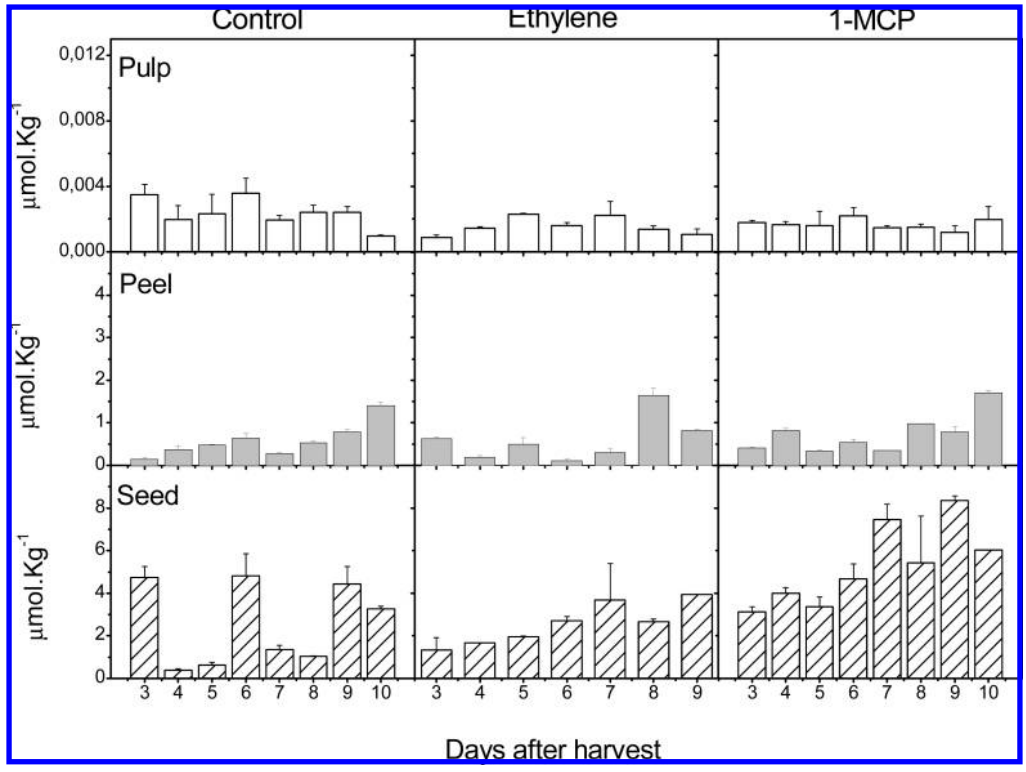

Figure 3. BITC profiles in peel, pulp, and seeds of papaya fruit submitted to ethylene and 1-MCP treatments during postharvest ripening.

ripening, peaking at late stages for both control and ethylene-treated fruit. Surprisingly, the activity increased even in seeds from fruit that were prevented from ripening by 1-MCP treatment.

BITC in the Internal Cavity of Papaya Fruit during Ripening. Because BITC is a volatile compound produced largely in the seeds of the papaya, the amount of BITC in the internal cavity of the fruit was determined (**Figure 4B**). The levels of BITC in the internal cavity of control and 1-MCP-treated fruit were consistently decreased during ripening. In contrast, the exposure to exogenous ethylene seemed to cause a transient burst in BITC released into the internal cavity of fruit that underwent that treatment.

Expression of Papaya Myrosinase. Degenerated primers based on the sequences of myrosinase enzymes from *Arabidopsis thaliana* (P37702 and BT002458), *Brassica napus* (CAA79989 and Q00326), and *Sinapis alba* (P29092 and P29738) were used to amplify a 975 bp cDNA from papaya

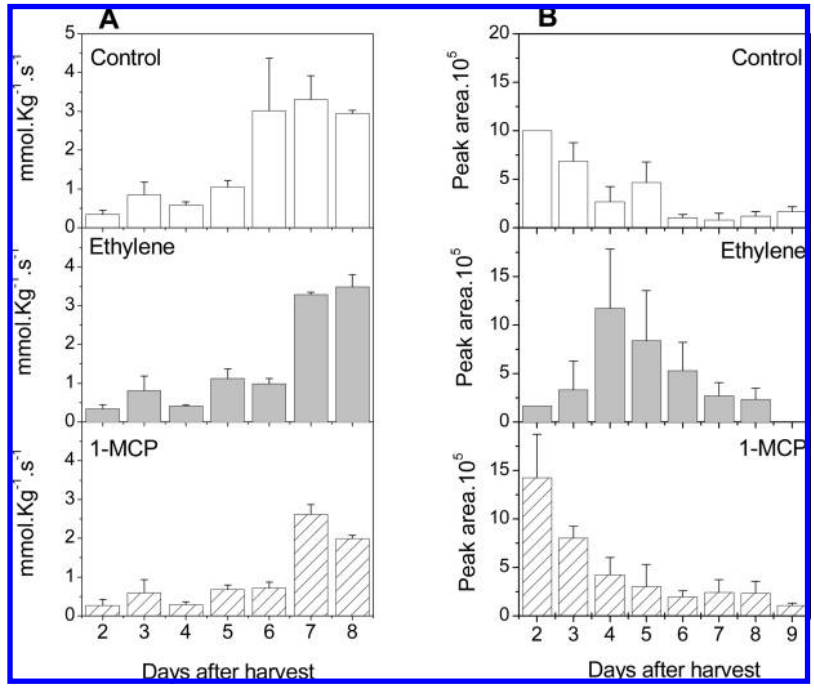

Figure 4. Profiles of myrosinase activity (**A**) in seeds and BITC (**B**) in the internal cavity of papaya fruit submitted to ethylene or 1-MCP treatments during postharvest ripening.

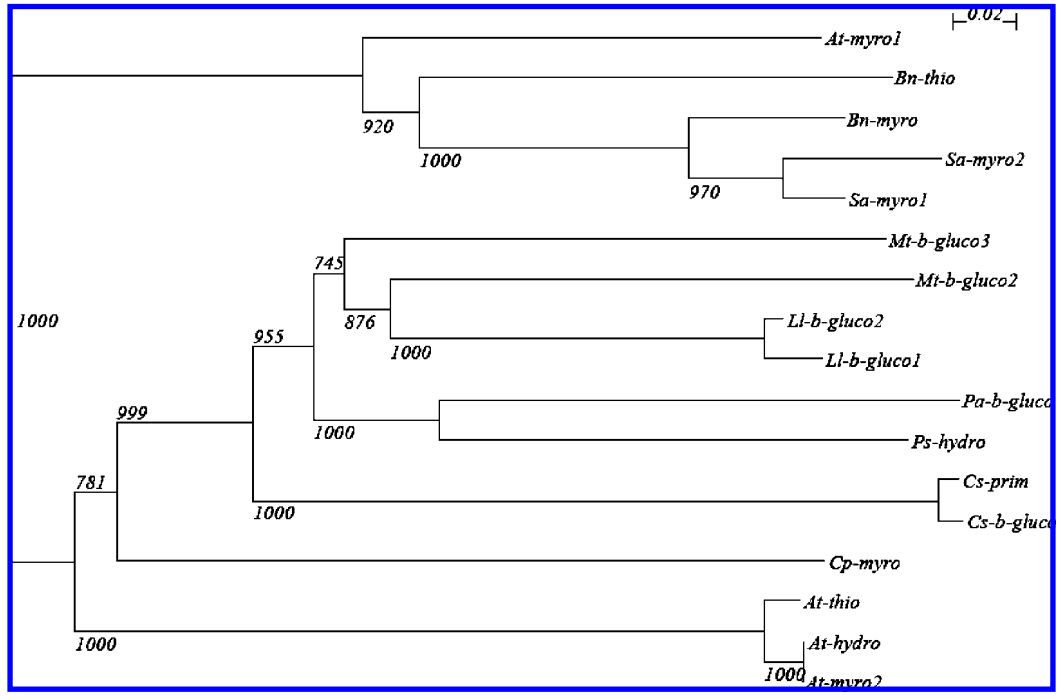

Figure 5. Unrooted phylogram encompassing glucosidases (thioglucosidases and myrosinases) from plants. The phylogenetic tree was calculated using the neighbor joining method (1000 bootstrap replicates) based on the ClustalW alignment of deduced amino acids sequences. Bootstrap values are indicated below the branches, with the scale bar meaning 0.02 residue substitutions per site. Glucosidases sequences used are from *A. thaliana* (*At-myro1*, P37702; *At-thio*, BT002458; *At-myro2*, AAG52628; and *At-hydro*, NP_175558), *B. napus* (*Bn-thio*, CAA79989; and *Bn-myro*, Q00326), *S. alba* (*Sa-myro1*, P29092; and *Sa-myro2*, P29738), *Leucaena leucocephala* (*Ll-b-gluco1*, ABY48758; and *Ll-b-gluco2*, ABY84677), *Medicago truncatula* (*Mt-b-gluco2*, ABW76287; and *Mt-b-gluco-3*, ABW76288), *Prunus serotina* (*Ps-hydro*, AAA93234), *C. sinensis* (*Cs-b-gluco*, CAK97604; and *Cs-prim*, BAC78656), *Prunus avium* (*Pa-b-gluco*, AAA91166), and *C. papaya* (*Cp-myro*, EU642644).

seeds (Cp-myro; accession EU642644; protein_id ACC95418). According to the phylogenetic tree (**Figure 5**), the putative myrosinase from papaya seems to be more closely related to the enzyme from *A. thaliana* (53% identity) and *Camellia sinensis* (50% identity). As presented in **Figure 6**, the papaya sequence has conserved motifs that correspond to the catalytic nucleophile of glucosidases (thioglucosidases and myrosinases). A significant increase in the abundance of papaya myrosinase transcript at harvesting (150 daa), followed by a discrete decrease during ripening, was observed when Cp-myro was used as a probe in the Northern blots. According to **Figure 7**, the observed changes in transcription were well-correlated to myrosinase activity detected in the seeds during ripening.

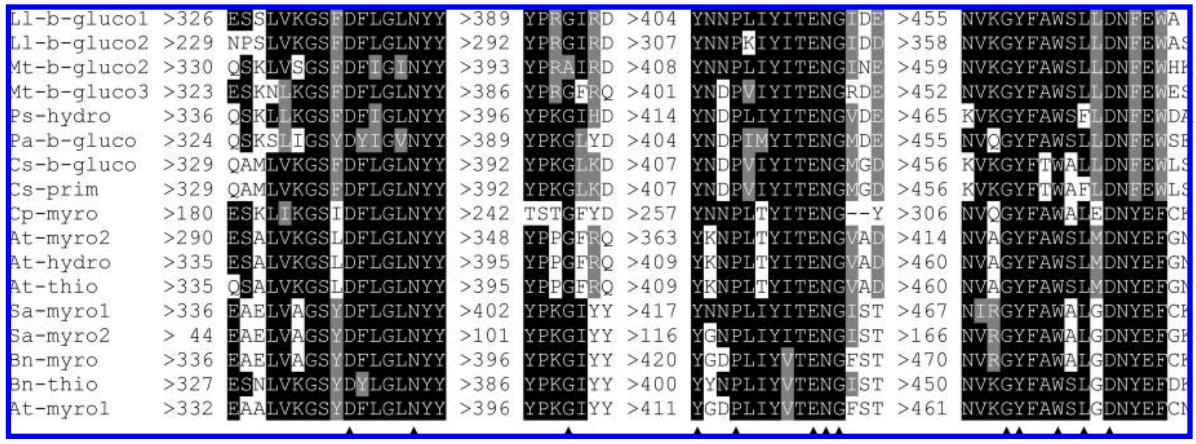

Figure 6. Conserved motifs representing the catalytic nucleophile of glucosidases (thioglucosidases and myrosinases). The sequences and the GenBank accession numbers are the same as described for the phylogenetic tree (**Figure 5**). The identical amino acids are highlighted with black boxes and white letters; the similar amino acids are highlighted with gray boxes and white letters; nonconserved amino acids are highlighted with white boxes and black letters. The amino acid count is from left to right, after the symbol >. The conserved residues corresponding to the catalytic nucleophile are indicated with black triangles.

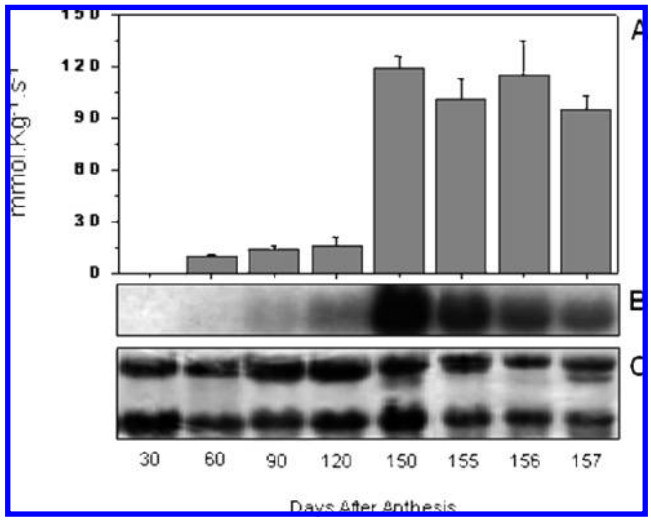

Figure 7. Patterns of myrosinase activity (**A**), myrosinase mRNA accumulation (**B**), and total RNA (**C**) of papaya seeds during fruit development and ripening (samples from the first experiment). Electrophoresis conditions, probe hybridization, enzyme extraction, and activity assays are described in the Material and Methods. The ethidium bromide staining gel of RNA samples confirmed equal loading based on spectrophotometic quantification. The myrosinase activity was represented as the amount of of hydrolyzed glucose (mmol) produced by 1 kg of fresh fruit by the second (mmol kg^{1-} s^{-1}).

DISCUSSION

During papaya development, there was an overall decrease in BG amounts for all three tissues evaluated. However, only in the fruit pulp did the degradation of the BG precursor result in the accumulation of BITC. When the fruits were allowed to ripen either naturally or following exposure to exogenous ethylene or 1-MCP, the BG and BITC profiles were similar, suggesting an accumulation of the BITC product after harvest. No remarkable differences were seen for the three tissues between treatments, except that the accumulation of BITC in papaya seeds seemed to be favored by 1-MCP treatment. In relation to seed myrosinase activity, the activities were more strongly correlated to the overall increase in BITC levels than to any specific changes observed during fruit development.

In general, the amount of BITC was much lower than of the amount of BG during ripening. An explanation for this large difference between the amount of precursor and product in the tissues could be the fact that BITC is a volatile compound. As a result, the BITC produced as a consequence of myrosinase action would be easily released from tissues. In addition, there would be differences in the permeability of the peel, pulp, and seed to the volatile BITC, generating a gradient across the fruit, from seeds to peel. This would also explain why the enzymatic activities were not so well-correlated to the levels of BG or BITC. Another reason for the observed discrepancies would be the loss of BG to other myrosinase-independent degradative pathways or chemical degradation. This difficulty in correlating papaya myrosinase activity to BG and BITC levels is not wholly unexpected, as similar results were also reported in other plant tissues by Bones (*35*) and Rask et al. (*3*). However, irrespective of these factors, the myrosinase activity level detected in the three tissues is still enough to account for the production of the observed levels of BITC. The papaya pulp myrosinase activity was similar to that observed for horseradish (*29*) and much higher than that of *Brassica juncea*, which was considered to be high enough to provide protection against the infection by the pest *Plutella xylostella* (*36*).

Because of the lack of information at the 5′-end of the partial cDNA isolated from papaya seed, it was not possible to check the existence of a peptide signal that would target the enzyme to a secretory pathway. However, the comparison of the partial papaya myrosinase primary sequence showed the presence of conserved residues representing the catalytic nucleophile of myrosinases and the other thioglucosidases, similar to that described by Burmeister et al. (*37*) and Rask et al. (*3*). Therefore, CP-myro could be considered a newly isolated papaya myrosinase sequence. Although glucosidases can have a broad range of substrates, there was a strong positive correlation between the mRNA levels and the consumption of the BG substrate; thus, it can be assumed that expression of the CP-myro protein could play a role in the regulation of myrosinase activity levels in seeds and general BITC production.

Considering the protective effect of BG, the amounts detected in the peel of papaya fruit during development (from 2 to 6 μmol/g) would be close to the lethal dose (6.7 μmol/g) determined to be effective to protect the western mustard (*B. juncea*) against *Spodoptera eridania* (*36*). In relation to the effect of BITC, the levels detected in the peel of undeveloped papaya would be similar to those seen to promote 30% mortality in

eggs of *Otiorhynchus sulcatus*, a grapewine pest (*38*). In fact, Seo et al. (*21*) demonstrated that BITC concentrations of 6.7×10^{-4} mol/kg agar and/or 1.9×10^{-10} mol/L (volatile BITC) caused a decrease of 57, 93, and 93% in oviposition of fruit flies *Dacus dorsalis*, *Ceratitis capitata*, and *Dacus curcubitae*, respectively, although a 10-fold increase in concentration would be needed to reach the same effect in ripe fruit. However, because there were differences in BG and BITC levels in fruit tissues sampled from the same plantation in two consecutive years, it is difficult to say if the observed levels would provide effective protection against fruit flies under noncontrolled conditions.

The production of toxic metabolites, such as glucosinolates, is thought to be part of the plant defensive mechanism, and ethylene seems to play a role in this process (*39, 40*). Surprisingly, exogenous ethylene did not result in any increase in myrosinase activity or BG and BITC levels in the tissues of papaya fruit. On the contrary, the application of 1-MCP seemed to have a discrete positive effect on the accumulation of BITC. Whether the blockage of ethylene detection favored the biosynthesis of BG or inhibited some ethylene-related degradative pathways is still an open question.

Aside from the protective effect against plant pests, BG and isothiocyanates are also argued to be beneficial toward human health, and papaya fruit would be an alternative source of these bioactive compounds. Researchers (*9*) have proposed the use of papaya seeds as a food source of biologically active isothiocyanates, since its content is quite similar to what is found in some *Brassica* vegetables. In fact, the results presented here point to a much higher content of BG and BITC in the fruit seeds, and in addition, there are also significant levels detected in the pulp. On the basis of the average size of a papaya fruit, eating a portion of approximately 200 g of fruit pulp would account for an intake of almost 10 μmol of BG, which is comparable to the levels present in 100 g of fresh broccoli, white cabbage, Brussels sprouts, and cauliflower (*41*). Considering that those vegetables need processing prior to consumption, and the processing conditions usually employed would negatively affect the amounts of native BG and isothiocyanates (*41, 42*), the consumption of a fresh papaya fruit pulp would be clearly advantageous. In addition, we observed that neither the ripening stage of the fruit nor the postharvest treatments resulted in any remarkable differences in the amounts of BG in the fruit pulp.

The results presented in this manuscript suggest that ethylene does not have a clear effect on BITC accumulation during papaya fruit ripening, in contrast to its negative effect on lycopene accumulation (*26*). Regarding BG and BITC levels, while they cannot be correlated to the myrosinase activity levels detected in fruit tissues, changes in activity were well-correlated to transcript levels in seeds. A possible explanation for some of the observed discrepancies would be differences in the permeability of the pulp and peel to the volatile BITC and the fact that seeds are the most likely source of volatile BITC. To the best of our knowledge, this is the first time the amount of volatile BITC in the internal cavity of papaya fruit has been quantified. Volatile BITC originating from the seeds would provide at least part of the total BITC that could protect the fruit from a fruit fly infestation, and this source of BITC may be independent of the BITC produced by the pulp or peel. The fact that BG levels in the pulp did not decrease during ripening and that no thermal treatment is needed before consumption of papaya fruit reinforces the idea of utilizing this fruit as an interesting dietary source of benzyl glucosinolate and isothiocyanates.

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