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Communication

Xylanase and Xylosidase Activities in Avocado Fruit[1,2]

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ABSTRACT

The activities of xylanase and xylosidase were demonstrated in mature avocado (*Persea americana* Mill.) fruits from different cultivars. When monitored on the day of harvest during the season at 1-month intervals, xylanase activity decreased and xylosidase activity increased between January and February and then remained stable until May. When monitored during the ripening process (January harvest), xylanase activity was constant, and xylosidase activity reached a peak at the climax of ethylene evolution and cellulase activity. Xylanase, which originated from *Trichoderma viride* and was added to the medium in which avocado discs were incubated, induced ethylene evolution.

The interest in avocado fruit softening as one aspect of the ripening syndrome led us (12) and others (2) to study the relations among cellulase activity in the fruit, cell wall structure, and softening. The important role of ethylene in avocado fruit ripening has been reviewed by Biale and Young (4). Xyloglucans, heteroxylans, and xylans are structural components of primary and secondary cell walls of dicotyledons and Gramineae and of secondary walls of Angiosperms (3), and the release of xylose residues during enzymatic degradation of avocado fruit cell walls has been reported (9). Endoxylanase activity was described in several fruits and higher plants (1, 10, 11, 16), and more recently it was reported that an ethylene-inducing protein, produced by *Trichoderma viride,* is an endoxylanase (7). All the above suggest that xylan-hydrolyzing enzymes may be involved also in avocado fruit softening. In this paper we report on the presence of endoxylanase and xylosidase activities in mature and ripening avocado fruits, and on the induction of ethylene synthesis in discs of avocado fruit by *Trichoderma* xylanase.

MATERIALS AND METHODS

Plant Material

Avocado fruits (*Persea americana* Mill. cv "Hass") were harvested monthly during the season. On the day of harvest, determinations were carried out on five fruits separately for firmness, ethylene evolution, and cellulase, endoxylanase, and β-xylosidase activities. At the January harvest, fruit was also stored at 22°C for ripening and periodically five fruits were sampled and the physiological and enzymic parameters determined. In addition, fruits of cvs "Ardit" and "Green Gold" were analyzed for xylanase and xylosidase activities. Fruit pulp discs (12 mm diameter and 4 mm thick), including the peel, were prepared 1 d after harvest from fruits of cvs "Fuerte," "Ardit," and "Green Gold" for ethylene induction experiments.

Acetone powders were prepared by grinding avocado fruit pulp in an Osterizer blender with cold (−20°C) acetone, as described previously (12).

Enzymatic Activity Assays

Endoxylanase

Forty milligrams of acetone powder were mixed in a test tube with 1 mL of acetate buffer (50 mM, pH 5.5) and 1 mL of RBB-xylan[3] (Sigma) solution that was prepared according to Biely *et al.* (5). The test tubes were stoppered and incubated in a water bath at 37°C for 22 h. The assay was terminated by the addition of 4 mL of absolute ethanol. The test tubes were vortexed, left at room temperature for 15 min, and then centrifuged at 2000*g* for 7 min. The absorbance of the supernatant was recorded at 595 nm. The results are expressed as activity units per g fruit (fresh weight). One unit of xylanase activity was determined as the activity liberating 1 nmol of RBB, to the supernatant, during 60 min.

In another method for xylanase activity assay, 400 mg acetone powder were extracted with 5 mL of 50 mM sodium acetate buffer (pH 5.5) overnight at 4°C. After the addition of 2 mL more of buffer, the extract was centrifuged for 15 min at 10,000*g*, and the supernatant was passed through a 0.45 μm Millipore HAWP filter. Aliquots of 200 μL of this filtrate were used for the determination of xylanase activity. Boiled and nonboiled aliquots were incubated for 16 h at 37°C with birch wood β-D-xylan (Roth) and then released reducing sugars were assayed by a modified (6) bicinchonic acid assay (15). The difference between the absorbance of nonboiled and

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[2] This paper is dedicated to the memory of Jacob B. Biale, a great scientist and teacher and a dear friend.

[3] Abbreviations: RBB-xylan, Remazol Brilliant Blue R-D-xylan (4-*O*-methyl-D-glucurono-D-xylan-Remazol Brilliant Blue R); EIX, ethylene-inducing xylanase.

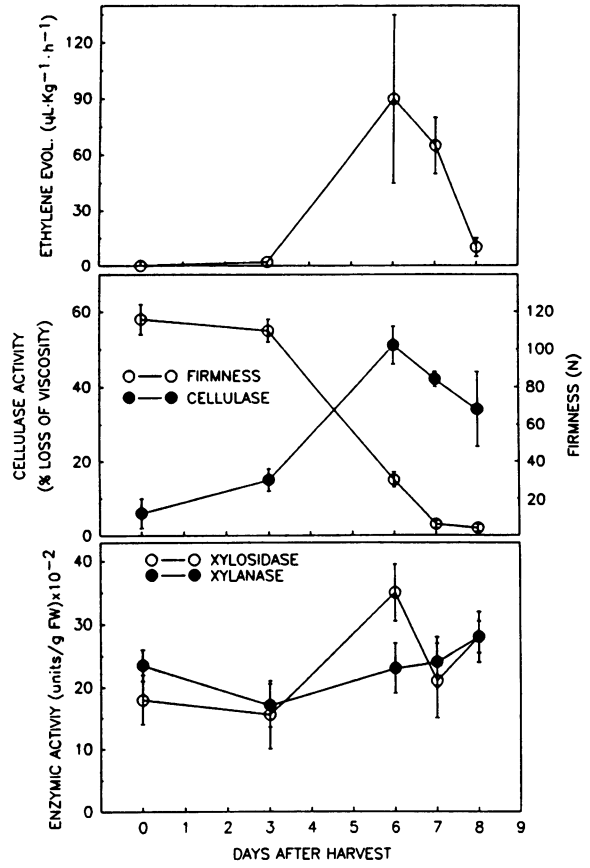

Figure 1. Xylanase, xylosidase, and cellulase activities, ethylene evolution, and firmness during the ripening process of avocado fruits cv "Hass" (January harvest). Data are averages of five replicates; bars represent standard deviation.

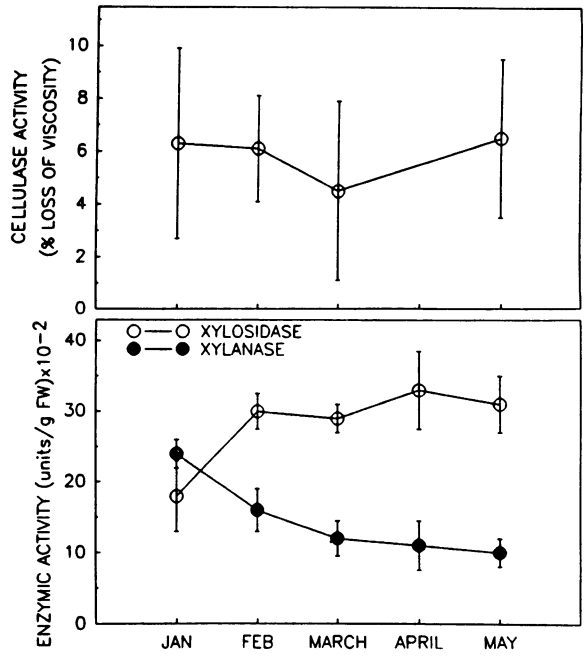

Figure 2. Xylanase, xylosidase, and cellulase activities in avocado fruits cv "Hass" at harvest day. The fruits were picked throughout the season from January to May 1989 at 1-month intervals. Data are averages of five replicates: bars represent standard deviation.

boiled aliquots of each fraction was calculated and regarded as the enzymic activity.

Xylosidase

Three hundred milligrams of acetone powder were stirred for 1 h in 8 mL of 50 mM phosphate buffer (pH 7.0), at 4°C. Then the mixture was centrifuged for 10 min at 6500*g*, and the supernatant was filtered (Millipore, 0.45 μm pore). Xylosidase activity was assayed when using *p*-nitrophenyl β-D-xylopyranoside (Sigma) as substrate (13). A 6 mM solution of *p*-nitrophenyl β-D-xylopyranoside was prepared in a 50 mM phosphate buffer (pH 7.0). For the reaction, 250 μL of substrate solution were added to 250 μL of filtrate in a 1.5 mL microtube which was shaken for 2 h at 37°C on a Vortex shaker. The reaction was interrupted by addition of 0.5 mL 1 M Na_2CO_3. The content of the microtubes was diluted in 2 mL of water and read at 405 nm in a Spectronic spectrophotometer. The results are expressed as units of activity per g fruit (fresh weight). One unit was defined as the activity liberating 1 nmol *p*-nitrophenyl in 60 min.

Cellulase

Cellulase was determined using 1% carboxymethyl cellulose as substrate with a viscometer as described previously for avocado fruit (12).

Ethylene Induction by Xylanase

Five fruit discs (2.8 ± 0.1 g), with the peel side up, were placed in 25 mL Erlenmeyer flasks containing 1 mL of assay medium plus antibiotics (7) to which 10 mg per mL *Trichoderma* xylanase (Fluka) were added or not. In other flasks, 10 μg per mL pure EIX (6) were added instead of the Fluka xylanase. The discs were covered up to two-thirds of their height by the incubating medium before they were infiltrated at 150 mm Hg for 2 min. Then, the flasks were sealed with vaccine caps and incubated at 20°C in the dark for 24 h. The atmospheres of the flasks (six per treatment) were sampled and assayed for ethylene after 4 and 24 h.

Firmness

Fruit firmness (Newtons required to penetrate the fruit) was determined using a Chatillon pressure tester as described previously (12).

Ethylene

Fruits were sealed periodically for 1 h and the headspace was sampled and determined by gas chromatography (12).

RESULTS

Enzymic Activity of Avocado Extracts

Activity of β-1,4 endoxylanase and β-1,4 xylosidase was found in fruits of cv "Hass" (Figs. 1 and 2). Both assay methods yielded similar results. Therefore, in the experiments described in Figures 1 and 2, only the RBB-xylan method was used. Xylanase activity in Hass fruits was practically constant throughout the ripening process (softening, ethylene evolution, and cellulase activity) except for the level measured 3 d after harvest, which was lower than the others (Fig. 1). The activity of xylosidase reached a peak at the climax of ethylene evolution and cellulase activity. When the activities of xylanase and xylosidase were determined in "Hass" fruits on the day of harvest during the season, it appeared that the former decreased and the latter increased between January and February, and then stayed stable (Fig. 2). Cellulase activity measured in the same samples (April data missing) was also quite stable during the season (Fig. 2). Xylanase and xylosidase activities were demonstrated also in fruits of cvs "Ardit" and "Green Gold" (data are not shown).

Ethylene Induction by Xylanase

The amounts of ethylene accumulated, for 24 h, in flasks containing xylanase (Fluka), were significantly (Duncan's multiple range test) higher than in the control flasks, with discs of cvs "Green Gold" and "Fuerte" but not of "Ardit" (Fig. 3). A very low concentration of the pure xylanase (EIX) also induced significant production of ethylene in cvs "Ardit" and "Fuerte."

DISCUSSION

We demonstrated here the presence of the activity of β-1,4-xylanase and β-1,4-xylosidase during avocado fruit ripening (Fig. 1) and the relations of the activities to other fruit-ripening parameters. The release of xylose residues from avocado cell walls hydrolyzed by cellulase has been described (9). The presence of cellulase activity in avocado fruit on the day of harvest (Fig. 1) was described previously in fruits which were harvested late in the season (8). The activity of xylanase and xylosidase was reported in microorganisms and higher plants (1, 6, 10, 11, 13, 14, 16). Between January and May, β-1,4-xylosidase activity increased and β-1,4-xylanase activity decreased (Fig. 2). The ratio between the activities of these two enzymes changed from approximately 1:1 (xylanase to xylosidase) in January to approximately 1:3 in May. It has been shown (14) that the hydrolysis rate of hemicellulose by *Penicillium funiculosum* xylanases and the nature of the products detected were affected by the ratio of xylanase to xylosidase activities.

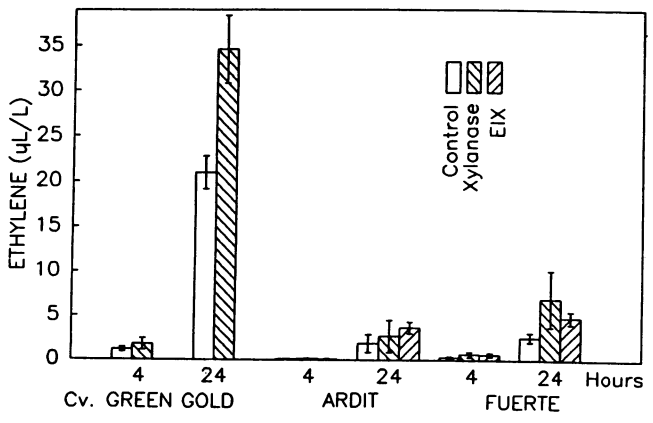

Figure 3. Ethylene concentration in the headspace of the 25 mL Erlenmeyer flasks containing five discs of fruit pulp. Each value is the average of six replicates: bars at the tops of the histograms represent standard deviation.

The nature of the substrates used in our assays, RBB-xylan and *p*-nitrophenyl-D-xylopyranoside, suggests that we are really dealing with β-1,4-D-endoxylanase and β-1,4-D-exoxylosidase. The fact that avocado fruit tissue is induced to synthesize ethylene by *Trichoderma* xylanase (Fig. 3) suggests that avocado xylanase might play a role in pathogenesis and ripening of the fruit. The differences between the cultivars might be due also to differences in the maturity of the fruits. The reports describing the participation of xylanase in plant cell wall hydrolysis (14) lead us to the supposition that fungi which are known to infect avocado fruit might include xylanases among the cell wall-hydrolyzing enzymes they secrete during fruit infection. Our findings about xylanase and xylosidase in avocado fruits open up additional avenues for research on the possible role of these enzymes in fruit ripening, particularly in ethylene induction and cell wall hydrolysis processes.

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