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Activities of Polygalacturonase, α-D-Mannosidase, and α-D- and β-D-Galactosidases in Ripening Tomato

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Additional index words. *Lycopersicon esculentum*, glycosidases, enzymes, ripening

Abstract. **The activities of the enzymes polygalacturonase, α-D-mannosidase, and α-D- and β-D-galactosidase in tomato (*Lycopersicon esculentum* Mill) pericarp tissue were measured during ripening of two normal ripening cultivars, Sweet 100 and ACE 55 VF, the slow-ripening *alcobaca* mutant, and their F_1 progeny. The activity of polygalacturonase increased as the fruit ripened from mature green to red stages for all tomato lines ('Sweet 100 > 'Sweet 100' × *alcobaca* > 'ACE 55 VF' > 'Ace 55 VF' × *alcobaca* > *alcobaca*). Of the other enzymes, α-mannosidase showed the greatest quantitative differences between the tomato lines and consistently increased in activity during ripening. There was, however, no association between the activity of α-D-mannosidase and polygalacturonase. The highest β-galactosidase activity occurred in 'Sweet 100', but was generally similar in the other lines. The activity of α-galactosidase varied little between parents and progeny for any stage of ripeness.**

Studies on the activity of glycosidases during the ripening of tomatoes have concentrated on their possible contribution to cell wall metabolism. Wallner and Walker (17) demonstrated high activity of β-D-galactosidase (EC 3.2.1.23) and β-1,3-glucanase (EC xxxx) in cell wall material of ripening tomatoes. Unlike polygalacturonase (PG) (EC 3.2.1.15), an enzyme thought to play a key role in softening of tomatoes (4, 7), the activity of the β-glycosidases was high at all stages of ripeness and did not initiate cell wall breakdown. Nevertheless, it has been suggested that their action may be involved in the cell wall modifications that may not be catalyzed by PG action (5, 7, 17). Recent work by Pressey (16) has indicated the presence of three isoenzymes of β-galactosidase, one of which degraded galactan and increased in activity during ripening. However, the function of most glycosidases remains undefined.

Information on changes of total, rather than only cell wall-associated, activities of glycosidases during fruit ripening also is limited. Activities of α-D-galactosidase (EC 3.2.1.22) and β-galactosidase, glucosidases, mannosidases, and xylosidases have been measured in extracts of tomato mesocarp tissue (14). α-D-Mannosidase (EC 3.2.1.24) and β-galactosidase were highly active at all stages of maturity, although in contrast to the study of Wallner and Walker (17), only α-galactosidase and β-D-glucosidase (EC 3.2.1.21) were associated with the cell wall.

We have used normal and *alcobaca* tomato fruit and their F_1 progeny to investigate if changes in activity of α-D-mannosidase and α-D- and β-D-galactosidases were related to the differences in ripening behavior. The *alcobaca* mutant has a long fruit storage life (9, 10, 12), and the fruit used in this study were part of a study on its genetic characterization (6).

Two tomato cultivars, Sweet 100 (Goldsmith Seeds Co.) and ACE 55 VF (Asgrow), were each crossed with *alcobaca* to provide F_1 progeny. 'Sweet 100' is a fast-growing, early maturing, multifloral plant whose fruit soften rapidly during storage and have a short shelf life, whereas 'ACE 55 VF' has a normal maturation time and storage life (6). The genotypes were observed to reach full red color at different rates, the number of days after breaker being 6, 8, and 12 for 'Sweet 100', 'ACE 55 VF', and *alcobaca*, respectively.

Parent lines and their F_1 progeny were grown under normal greenhouse conditions at Rutgers Univ. Fruit were harvested at the mature green, turning, and firm red stages of maturity. Fruit from each line and maturity class were sliced to provide outer pericarp tissue (3 × 50 g), which was stored at −20°C. Freezing of tomato tissue has been used in other studies on glucosidases (5, 17), but, while our work was in progress, Pressey reported, without providing data, loss of activity of one form of β-D-galactosidase in frozen tissue (16). Possible loss of enzyme activity was not determined in the present study, but use of frozen tissue seems unlikely to have affected the pattern of our results.

Partially thawed pericarp tissue was homogenized for 1 min, using a Cuisinart food processor, in 2 vol (w/v) of 50 mM Na acetate buffer (pH 5.0) containing 1 M NaCl, 0.2% cysteine, and 1% (w/v) soluble PVP-10 (Sigma). The homogenate was filtered through Miracloth (Calbiochem), and the residue again homogenized for 30 sec with another 1 vol of extraction buffer. This homogenate was added to the previously obtained filtrate and stirred slowly for 3 hr. The suspension was filtered through Miracloth and the filtrate centrifuged at 10,000× *g* for 30

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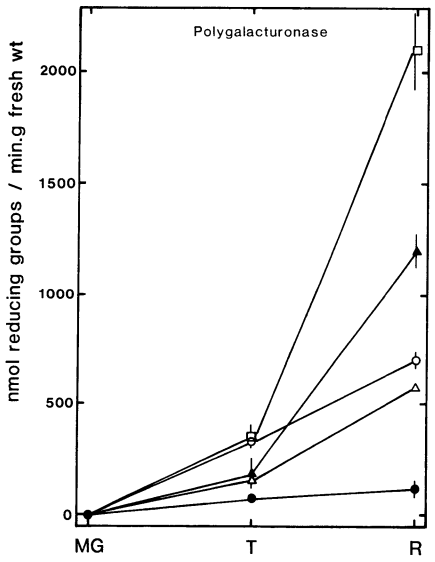

Fig. 1. Polygalacturonase activity at the mature green (MG), turning (T), and red (R) stages of ripeness of Sweet 100 (□), ACE 55 VF (○), *alcobaca* (●) and their F_1 progeny 'Sweet 100' x *alcobaca* (▲) and 'ACE 55 VF' x *alcobaca* (△). Data are accompanied by SE.

min. The supernatant was assayed for glycosidases. A 3-ml aliquot, dialyzed against 3 liters of 0.5 M NaCl for 16 hr to remove reducing sugars, was used for determination of PG activity. All operations were carried out at 4°C or on ice.

The activities of α-D-mannosidase and α-D- and β-D-galactosidases were determined by measuring the release of *p*-nitrophenol from the corresponding *p*-nitrophenyl glycoside (Sigma) at pH 3.8, 4.2, and 5.1, respectively. Enzyme solution (0.1 ml) was added to 0.9 ml of a reaction mixture containing a final concentration of 2.5 mM-*p*-nitrophenyl glycoside in 50 mM Na acetate buffer at the appropriate pH for each glycosidase and incubated at 37°C. The reactions were terminated after 10 and 20 min by the addition of 5 ml of 100 mM Na_2CO_3 to each assay solution, and the absorbance at 405 nm measured to determine the concentration of *p*-nitrophenol. PG activity was estimated by measuring the release of reducing sugars. The PG assay mixture contained 0.1 ml of enzyme solution in 0.2 ml of 1 M NH_4Cl and 0.8 ml of 1% (w/v) of polygalacturonic acid (Sigma grade III washed with 80% ethanol) in 50 mM Na acetate buffer (pH 4.2). Incubation was at 37°C for up to 60 min. Reducing sugars were assayed according to Honda et al. (8) using galacturonic acid as the standard. All enzyme assays were conducted in duplicate.

PG activity increased during the ripening of all parents and progeny (Fig. 1). At the firm red stage of maturity, highest activity of PG (2009 nmol/min per g fresh weight) was measured in tissue, from 'Sweet 100', the fastest ripening cultivar. The PG activity of 'ACE 55VF' (705 nmol/min per g fresh weight) was intermediate between 'Sweet 100' and the mutant *alcobaca* in which PG activity was reached only 114 nmol/min per g fresh weight. The activity of PG in both F_1 progeny was lower than that of the normal parent.

Samples of the cultivars left at room temperature for 14 days also showed the same pattern. For fruit picked at the turning stage, for example, PG activity in the *alcobaca* was 196 nmol/min per g fresh weight, compared with 2563 and 4756 nmol/min per g fresh weight for 'ACE 55 VF' and 'Sweet 100', respectively. 'ACE 55 VF' x *alcobaca* and 'Sweet 100' x *alcobaca* were 1226 and 3404 nmol/min per g fresh weight, respectively.

The increase in PG activity with ripeness supports the concept of PG involvement in cell wall dissolution (7). The relatively low PG activity in the *alcobaca* mutant also agrees with the previous data of Kopeliovitch et al. (9) and Lobo et al. (10).

Of the three glycosidases, α-D-mannosidase showed the greatest quantitative difference between the tomato lines and was the only one to show consistently increased activity with ripeness (Fig. 2). However, there was no direct association between the activity of α-D-mannosidase and the activity of polygalacturonase; the lowest activity of α-D-mannosidase occurred in 'Sweet 100', which had the highest PG activity. The activity of α-D-mannosidase in *alcobaca* was intermediate between 'Sweet 100' and 'ACE 55 VF'. Both F_1 progeny showed intermediate levels of enzyme activity between those of their parents.

The highest β-D-galactosidase activity was present in 'Sweet 100'. Except for slightly higher activities in 'Sweet 100' × *alcobaca*, the β-D-galactosidase activities were similar in the other lines. The activity of α-D-galactosidase did not vary greatly between parents and progeny for any stage of ripeness.

When the activity of the glycosidases was expressed on a protein basis, again only α-D-mannosidase activity increased during ripening in tomato lines and the patterns were similar to that shown on a fresh-weight basis (data not shown). Specific activities of α-D- and β-D-galactosidase were highest in mature green tissue of 'Sweet 100' and 'Sweet 100' × *alcobaca*, but declined in the turning and red stages of ripening. There was little change in specific activities of both galactosidases in the other tomato lines during ripening.

Pharr et al. (14) found that α-D-mannosidase was the most active glycosidase in fruit of 'Tiny Tim', and that an 11% increase in activity occurred between the mature green stage (136 nmol *p*-nitrophenol released/min per g fresh weight) and the firm red stage (151 nmol/min per g fresh weight) of ripeness, but no relationship between α-D-mannosidase activity and physiological function has been identified as no substrate for this enzyme in vivo has yet been identified. In pear fruit, there was a five-fold increase in α-D-mannosidase activity in the cell wall during ripening, even though no α-mannosidase were found in the wall (1). It has been suggested (1, 14) that the activity of glycosidases based on *p*-nitrophenyl substrates may give an inaccurate picture of in vivo activity. However, marked changes in the activity of this enzyme associated with metabolic changes have been demonstrated in tissues containing reserve proteins (13, 15), and its presence has frequently been used as a vacuolar marker (2, 3). Recently, Murray (11) has proposed a lysosomal location for α-D-mannosidase in *Pisum sativum* seeds, where it may be involved in the turnover of endoplasmic reticulum glycoproteins and glycolipids. It is conceivable that α-D-mannosidase isozymes are involved in the post-translational processing of glycoproteins in fruit. Its possible role in fruit ripening warrants further study.

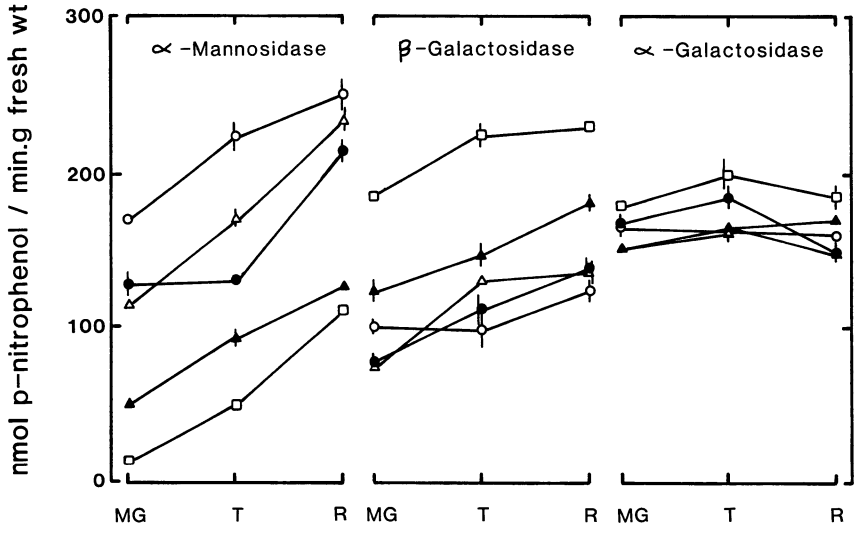

Fig. 2. α-D-Mannosidase, β-D-galactosidase, and α-D-galactosidase activity at the mature green (MG), turning (T), and red (R) stages of ripeness of 'Sweet 100' (□), 'ACE 55 VF' (○), *alcobaca*, (●), and their F_1 progeny 'Sweet 100' x *alcobaca* (▲) and 'ACE 55 VF' x *alcobaca* (△). Data are accompanied by SE.

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Compositional Changes in Yam Bean During Storage

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Additional index words. weight loss, respiration, starch, soluble solids, sugars, total phenols, titratable acidity, *Pachyrrhizus erosis*

Abstract. **Composition of yam bean (*Pachyrrhizus erosis* L. Urb) tubers stored at 22° and 12.5°C was monitored monthly for up to 5 months. A continual loss of moisture occurred in tubers at both storage temperatures—after 4 months tubers stored at 22° lost 14.5% of their original weight; those stored at 12.5° lost 9.6%. A higher respiration rate of 15 to 28 ml·kg^{-1}·hr^{-1} occurred during the first 2 months of storage at 12.5°. This was nearly double the respiration rate of tubers stored at 22°. Ethylene was not detected at any time. There are a continual breakdown of starch in tubers. After 3 months, tubers stored at 12.5° had one-sixth the harvest content of starch. At 22°, starch declined to two-thirds the harvest content. The decline in starch content at 12.5° was related to an increase in total sugars in the tuber, particularly sucrose. The sucrose content of the tuber stored at 12.5° tripled over a 3-month period. Glucose and fructose levels declined over the same period irrespective of storage temperature. The results suggest a chilling response that led to a sweeter tuber. Titratable acidity was very low, as was total phenols, and both did not change after harvest.**

Yam bean is one of a few leguminous root crops. It is native to Mexico and northern Central America, but is now widely cultivated in Southeast Asia (6). The light brownish turnip-like tubers have a white, crisp, and succulent flesh, with a sweet, pleasant flavor. On a dry-weight basis, the roots contain three to five times the protein of other root crops, e.g. cassava (*Manihot esculenta* Crantz), potato (*Solanum tuberosum* L.), sweet potato (*Ipomoea batatas* Lam), and taro [*Colocasia esculenta* (L). Schott] (7). However, an unusually high moisture content (near 90%) decreases the nutritive value (2, 12). The tubers are eaten raw or lightly cooked (8, 16). Yam bean has been suggested as having potential for wider cultivation (12).

Postharvest problems include dehydration, mold growth, and sprouting (1, 16). Storage temperatures in the range of 0° to 5°C are reported to extend storage life (1, 2, 16). This work was undertaken to determine the compositional changes in the root during storage and to provide data to assist in making storage recommendations.

Yam bean seeds of a local low growth, squat, tuberous-rooted unnamed cultivar (13), selected from previous cultivar comparison tests, were planted on 3 Apr. and 1 Oct. at the Waimanalo Experiment Station, Oahu, Hawaii. Field procedures have been described previously (13). Tuberous roots were harvested 10 months after the first planting and 6 months after the second planting. Roots weighing between 300 and 700 g were stored in the dark at 125°C (85% RH) and 22° (70% RH). At regular intervals, 12 roots were removed for weighing and analyses. The 12 roots were divided into four lots, with three roots in each lot. The data were the means of four independent samples analyzed in duplicate or triplicate.

Dry weight was estimated after drying a sample for 48 hr at 60°C. A subsample (5 g) of fresh root tissue minus skin was homogenized in 10 ml of deionized water and the pH determined. The homogenate was used to determine titratable acidity as described by Paull et al. (14). The results were expressed as μeq acid/g of fresh weight of tissue. Total soluble solids of 100 μl of expressed juice was determined by refractometry. Fresh root tissue (2 g) was homogenized in 18 ml of 90% (v/v) ethanol. An aliquot of the cleared supernatant was used for analysis for total phenols and sugars. The ethanol precipitate was used for starch, sugar, and organic acid analyses (14). Starch was determined by the iodimetric method (11).

Respiration rate was determined by sealing individual roots into a jar for 1 hr at 12.5° and 22°C and removing aliquots for CO_2 analysis. Carbon dioxide was determined using a infrared gas analyzer (Infrared Industries, Santa Barbara, Calif., Model IR-703). Respiration rate was expressed as mg CO_2/kg per hr. Ethylene was detected using a gas chromatography fitted with a photoionization detector.

Tubers stored at 22° and 12.5°C continually lost weight during storage (Fig. 1A). The water loss from yam bean tubers during the first month of storage was 0.12% of their initial weight per day when stored at 12.5° and 0.21% per day at 22°. The higher rate of water loss at 22° (70% RH) than 12.5° (85% RH) was due to differences in relative

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