Changes in Polygalacturonase Isoenzymes and Converter in Tomatoes During Ripening

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Abstract. Tomatoes (Lycopersicon esculentum Mill. 'Tropic') were examined for changes during ripening in fruit firmness, water-soluble pectin, the 2 polygalacturonases (PG I and PG II), and polygalacturonase converter. The loss of fruit firmness and increase in water-soluble pectin at the turning stage of ripeness coincided with the appearance of PG activity. The initial activity was due exclusively to PG I, which continued to increase during ripening. PG I was the major polygalacturonase isoenzyme in extracts of tomatoes at all stages of ripeness, contrary to previous reports. PG II was first detected in pink fruit and increased markedly with ripening. Polygalacturonase converter was present in green tomatoes, but also began to increase at the turning stage. The results indicate that PG I may be formed during extraction of tomatoes from PG II and the converter as they are solubilized.

The polygalacturonase (EC 3.2.1.15) activity in extracts of ripe tomatoes is due to 2 forms of the enzyme (PG I and PG II), which differ markedly in molecular size and heat stability (9). PG I is found first in extracts of ripening tomatoes, and it has been suggested that it initiates cell wall degradation during fruit softening (5). However, the sequential appearance of PG I and PG II may represent a more complex situation than differences in the timing of synthesis of the isoenzymes. Evidence recently was presented that tomatoes contain a glycoprotein (PG converter) that combines with PG II to produce PG I (11). This evidence is consistent with immunological evidence (1, 14) that these isoenzymes contain the same polypeptide. PG converter is present in unripe tomatoes (11), and thus, the early appearance of PG I may reflect the interaction of PG II and the converter when the tissue is homogenized. This study was undertaken to obtain information on the sequential appearance of the 2 enzymes in relation to the level of PG converter in tomatoes during ripening.

'Tropic' tomatoes were grown in a greenhouse. Freshly harvested fruit were separated into the following ripeness stages: mature green (G), breaker (B), turning (T), pink (P), ripe (R), and over-ripe (R1). Fruit firmness was determined with a Magnes-Taylor pressure tester equipped with a 78-mm tip. Two readings were obtained for each fruit after removal of a small area of skin, and 6 fruit were tested at each stage of ripeness. After testing for firmness, the fruit were frozen and stored at −20°C.

The following extraction procedure is based on a previous study (12) that established the optimum conditions for extracting and measuring tomato polygalacturonases. Pericarp tissue (100 g) from each of 6 fruit was homogenized in 100 ml of cold water using Virtis and Polytron homogenizers for 1 min each. The homogenate was adjusted to pH 3.0 with 0.1 N HCl, stirred 15 min, and centrifuged at 8000 × g for 20 min. The pellet was resuspended in 150 ml of cold water by homogenizing 30 sec with the Polytron. The suspension was stirred 15 min and centrifuged. The washed cell wall fraction was then suspended in 150 ml of cold 1.2 M NaCl, and the pH was adjusted to 6.5 by addition of 0.1 N NaOH. The suspension was stirred for 30 min at 3°C while maintaining the pH at 6.5. The supernatant solution obtained by centrifugation was concentrated to 15 ml by ultrafiltration using a PM-10 membrane (Amicon).

PG activity was measured by adding 0.1 ml of ultrafiltrate to 0.2 ml of 0.15 M sodium acetate (pH 4.5) and 0.2 ml of 0.15 M NaCl. Extracts of ripe tomatoes were diluted as necessary. Blanks were prepared by heating duplicate reaction mixtures 5 min at 100°C. The reaction was started by adding 0.5 ml of 1% polygalacturonic acid (8), pH 4.5. After 30 min at 37°C, the solutions were analyzed for reducing groups by the arsenomolybdate method (7). A unit of PG activity was defined as the amount that released 1 μmol of reducing groups per 30 min.

PG I and PG II were assayed after separation by chromatography on a 2.5 × 45 cm column of Sephadex G-100 adjusted to pH 6 and equilibrated with 1.0 M NaCl. Ten milliliters of ultrafiltered extract were applied to the column and 10-ml fractions were collected. The fractions were analyzed for PG and the total activity in each peak was determined. PG I and PG II corresponded to the first and 2nd peaks of activity, respectively.

PG converter was assayed by measuring the conversion of PG II to PG I. A reaction mixture consisting of 0.2 ml of ultrafiltrate, 0.4 ml of 0.1 M acetate (pH 5), and 0.2 ml of 0.15 M NaCl was heated for 5 min at 100°C to inactivate the PG in the extract. Purified PG II (4 units in 0.2 ml of 0.15 M NaCl) was added and the solution was stirred for 15 min. It was then centrifuged and dialyzed against 0.02 M 2-(N-morpholino)ethanesulfonic acid (Mes) (pH 6) containing 0.15 M NaCl for 22 hr and analyzed for PG I using HPLC (10). A unit of PG converter is defined as that amount that converted one unit of PG II to PG I.

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**Fig. 1.** Changes in fruit firmness and water-soluble pectin in tomatoes during ripening. G (mature green fruit), B (breaker), T (turning), P (pink), R (ripe), R1 (over-ripe). Data for firmness are means of 12 readings (SE = 1.7) obtained with a Magnes-Taylor pressure tester. Data for the water-soluble pectin are means of 2 experiments (SE = 0.13) and are expressed on a fresh weight basis.
Water-soluble pectin was determined by homogenizing tomato tissue and washing twice with cold water at pH 3 as described above. The washed cell wall fraction then was suspended in 100 ml of cold water, adjusted to pH 6 with 0.1 N NaOH, and stirred 30 min at 3°C. The suspension was centrifuged and the supernatant solution was analyzed for uronic acid by the hydroxydiphenyl method (2).

Changes in fruit firmness and water-soluble pectin during ripening of 'Tropic' tomatoes are presented in Fig. 1. The level of soluble pectin found in ripe tomatoes is considerably higher than that reported by other workers (3, 13). Sawamura et al. (3) extracted 0.29 mg pectin per gram of fresh weight of tomato by homogenizing the tissue in water at the endogenous pH. Brady et al. (13) found only 0.12 mg of water-soluble pectin per gram of fresh weight of tomato by extracting with dilute Tris buffer (pH 7.4) followed by partial purification of the pectin on DEAE-Sephadex A-50. The increased amount of soluble pectin extracted in the present study can be attributed to the preliminary washing of the cell wall fraction with water at pH 3, which removes salts from the tissue. The solubility of pectin is increased in the absence of salts when the washed sediment is extracted with water at pH 6. This effect is analogous to the results described by Whittenberger and Nutting (15) on the effects of salts on the viscosity of tomato juice. They observed that when the juice was centrifuged and the sediment was washed repeatedly with distilled water, the viscosity of the suspension increased to a value far exceeding that of the original juice. It appears that the pectin in tomato cell walls swells to form essentially a gel in the absence of salts at low pH. Some of this pectin is then solubilized when the pH of the suspension is raised to 6. There may be other explanations for the high yields of soluble pectin obtained by this method. However, it should be noted that autolysis of the cell walls by PG would not be important during the washing of the cell walls at pH 3 and the extraction with water at pH 6 at a low temperature. PG does not hydrolyze large substrates at these pH values, and the enzyme is effective only in the presence of relatively high concentrations of salts even at the optimum pH of 4.5 (8).

The changes in PG I, PG II, and PG converter during tomato ripening are presented in Fig. 2. PG activity was first detected in extracts of fruit at the turning stage, in agreement with other reports (3, 14). The initial detectable activity was due exclusively to PG, which increased sharply during ripening. PG I activity remained predominant at all stages of tomato ripeness. In contrast, Tucker et al. (14) reported that the level of PG I remained very low in tomatoes during ripening, and Mosheifi and Luh (6) found that this isoenzyme was absent in ripe tomatoes. The low recovery of PG I in both of these studies may be attributed to considerable loss of activity due to precipitation during dialysis of crude extracts against 0.15 M NaCl (12).

PG II was found in extracts of fruits at the pink stage and it also increased on further ripening of tomatoes (Fig. 2). In contrast to the appearance of the enzymes after ripening began, PG converter was present in the green fruit. The level of PG converter remained constant through the breaker stage and then increased with ripening. However, the increase of PG converter was much smaller than the increase of PG activity. The amount of converter available to combine with PG II is exhausted as the fruit ripens, and the result is the appearance and increase of free PG II. It should be noted that the amount of PG converter was lower than that of PG I in ripe tomatoes. This difference is probably due to the loss of some of the converter during the heating step that releases it from PG I (11).

The first PG isoenzyme that is detected in extracts of ripening tomatoes is PG I (3, 4, 14). The appearance of this enzyme coincides with the loss of fruit firmness and release of soluble pectin (Figs. 1 and 2). It has been suggested that PG I is involved in the initiation of cell wall degradation (5). PG II is detected in extracts of ripe fruit and has been implicated in later stages of degradation (5). However, the discovery of PG converter in tomatoes may invalidate this apparent sequence of PG isoenzyme synthesis and their specific roles in cell wall hydrolysis. Rather than actually occurring as an isoenzyme in pericarp tissue, PG I may be formed during extraction of the tissue from PG II and the converter, which are solubilized. Evidence supporting this possibility was obtained by differential extraction of the PG isoenzymes and converter by varying the pH and ionic strength of the extraction solution (12). The changes of PG converter and the PG isoenzymes in tomatoes during ripening described in the present study are consistent with this mechanism of PG I formation.

Literature Cited

Early Shoot and Root Quality Effects on Nursery and Field Development of Tissue-cultured Loblolly Pine

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Abstract. Height, number of roots, root length, and shoot quality rating on loblolly pine (Pinus taeda L.) tissue culture plantlets were measured in the laboratory and related to subsequent nursery and field performance. Shoot quality rating was the most important characteristic and was most consistently correlated with nursery and field traits. These results imply that only plantlets with the best shoot quality rating should be used so that growth rates will be similar to seedlings.

Advances have been made with tissue culture propagation of loblolly pine, and more than 3000 plantlets have been produced from seeds from 25 different half-sib families and established in various greenhouse, nursery, and field experiments (1, 3, 5–7). These experiments indicate that the growth patterns of the plantlets have been different than seedlings grown under similar conditions. The plantlets initially had a slower growth rate when compared to seedlings (5, 7, 10). The lag period occurred while the plantlets acclimated to the greenhouse, nursery, or field environment. Once the plantlets became acclimated, their growth rate was similar to that of seedlings (1, 5, 6, 10).

Although plantlets now can be produced routinely, extreme differences in the growth of some plantlets occur. Kelly (2) found that plantlets with high shoot and root quality had a lower mortality rate than plantlets with poor-quality shoots, and that the probability of survival increased as root and shoot development and quality improved. This trend also has been observed with seedlings in various grading studies. For example, South and Mexal (9) found that seedlings of a superior grade had increased survival and growth, which increased volume yield.

It is likely that a plantlet’s subsequent growth and survival is correlated with early traits seen in the laboratory. Therefore, a method of early selection for subsequent plantlet survival and growth would be useful. This experiment was initiated to determine a) the influence of early shoot and root quality traits measured in the laboratory on subsequent growth of loblolly pine tissue culture plantlets, and b) whether these traits affected the growth of plantlets relative to seedlings.

Tissue culture plantlets were produced from adventitious buds on the cotyledons of excised embryos from 5 half-sib families of loblolly pine (8). Once the shoots were 1 to 2 cm long and the roots were about 5 mm long, the plantlets were transplanted to the greenhouse. Of a total of 309 plantlets transplanted to the greenhouse, only 28 plantlets were used in this study, since this experiment was part of a larger one.

Early shoot and root characteristics of the plantlets (height, number of roots, length of each root, and shoot quality rating) were measured from Mar. through Apr. 1982, when the plantlets were transplanted from the laboratory to the greenhouse. The shoot quality rating ranged from 1 to 5 and was determined as follows: 1 = dark green color, healthy needles, vigorous apical growth; 2 = green color, slight chlorosis of the needles, good apical growth; 3 = green-yellow color, significant chlorosis of the needles, slight apical growth; 4 = yellow-green color, extensive chlorosis and slight necrosis of the needles, little apical growth; and 5 = yellow color, extensive necrosis of the needles, no apparent apical growth.

Once transplanted to the greenhouse, the plantlets were grown in 164-ml (10-cubic inch) RL Super Cells using methods of Amerson et al. (1). Seeds were cold-stratified and sown in the greenhouse mist bench one to 3 weeks after the plantlets were transferred from the lab. The seedlings germinated and grew in the RL Super Cells.

After about 6 weeks in the greenhouse, survival was tallied, and the plantlets and seedlings were planted in a randomized complete-block design at Federal Paper Board’s nursery in Lumberton, N.C. The 2 plant types were randomized in 4 blocks with 7 plants per type per block for a total of 56 trees. All ratings were not represented equally, so there was a range of 5 to 8 plantlets in each of the 5 rating categories. Plantlets from rating categories 4 and 5 survived poorly and were not transferred to the field.

The trees were planted bare-root at 7.6 × 15.2 cm spacings. Conventional nursery practices (9) were used to grow the trees through the nursery growing season.

After the plants had been in the nursery for about 3 months, the plantlet and seedling roots were box-pruned. To obtain the box-pruned effect, a sharp, thin blade mounted

Table 1. Correlation of laboratory measurements with nursery and field measurements.

<table>
<thead>
<tr>
<th>Laboratory characteristic</th>
<th>Nursery—1982</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ht</td>
<td>Caliper</td>
</tr>
<tr>
<td>Shoot quality rating</td>
<td>−0.64*</td>
<td>−0.55*</td>
</tr>
<tr>
<td>Height</td>
<td>0.33</td>
<td>0.37</td>
</tr>
<tr>
<td>Number of roots</td>
<td>−0.24</td>
<td>−0.20</td>
</tr>
<tr>
<td>Total root length</td>
<td>−0.14</td>
<td>−0.079</td>
</tr>
</tbody>
</table>

*Significantly greater than zero at $p < 0.05$