Water Permeability during Tomato Fruit Development in Normal and rin Nonripening Mutant

B. W. Poovaiah, Yosef Mizrahi, Herbert C. Dostal, Joe H. Cherry, and A. Carl Leopold
Department of Horticulture, Purdue University, West Lafayette, Indiana 47907

Received for publication March 4, 1975 and in revised form August 14, 1975

ABSTRACT

This work tested one aspect of the relations between membrane permeability and fruit ripening. Membrane permeability was measured as [3H]water efflux rate from preloaded fruit pericarp disks. Different stages of fruit development were compared between two tomato (Lycopersicon esculentum Mill) strains: the normal Rutgers and the isogenic nonripening rin strain. The first significant increase in permeability was measured in Rutgers tissue at 110% of development, after fruit ripening had already begun as indicated by ethylene and CO2 evolution and lycopene synthesis. The rin did not show any increase in tissue permeability during fruit development or maturation.

Our results do not support the idea that the first event of the ripening process is an increase in membrane permeability. Nevertheless, the nonripening mutant fails to show the normal increase in permeability.

Sacher (9) has proposed that the ripening of fruits may be caused by changes in the permeability properties of cell membranes. However Burg et al. (2) have measured the rate of leakage from banana slices into both hypo- and hypertonic sugar or glycerol solutions and concluded that membrane permeability remains constant during banana ripening. Brady et al. (1) found that bananas ripened in exogenous C2H4 showed an increased leakage of solutes after the initiation of the climacteric. Vickery and Bruinsma (10) concluded that there were no changes in permeability of the plasmalemma and tonoplast to potassium during ripening. They noted an increase in efflux of solutes from fruit tissues during ripening which they considered to be due to the increasing activities of ions in the cytoplasm.

Recently, an abnormally ripening mutant, rin, has been suggested as a tool to approach the problem of how ripening is regulated in the tomato fruit (3, 4, 7). We observed that during tissue drying for dry weight measurements, tissue of the rin mutant lost water at a slower rate than that of the normal Rutgers. One of the explanations for this observation might be a difference in membrane permeability to water. Since Poovaiah and Leopold (8) have demonstrated that leaf senescence is associated with an increase in hydraulic permeability of tissue, we felt that a similar increase might occur during fruit ripening, a suggested form of senescence (9). It is possible that senescence may be a consequence of the deterioration of cell membranes.

This research was done to compare changes in hydraulic permeability in tomato fruits of a ripening strain and the nonripening rin strain.

MATERIALS AND METHODS

Tomato plants (Lycopersicon esculentum Mill) of both Rutgers and rin were grown in soil in the greenhouse. The nonripening mutant, rin, was partially isogenic to Rutgers (fourth backcross). The plants were trained to one stem and only one or two flowers of each cluster were pollinated by hand and tagged. All other flowers were removed. The physiologic stage of development of the fruits was calculated as the number of days after pollination (5). One hundred per cent of development is taken as that day when the fruit reaches its maximal size. In normal Rutgers, this is approximately the time when the first color change can be observed. The time required to reach 100% of development was determined on a population of 10 fruits of 10 different plants.

C2H4 and CO2 were measured as already described (6). Hydraulic permeability was measured with fruit pericarp disks (2.5 g). For each treatment six disks were taken with an 11-mm diameter stainless steel cork borer. Two disks were taken from each of three different fruits from three different plants. The initial weight of the disks was taken and the disks were incubated in 10 ml of [3H]water (1 sCi/ml) in a closed tube for 120 min. The disks were shaken gently during incubation. At the end of the incubation period fruit disks were blotted on a paper towel and transferred to 10 ml of tritium-free water. At appropriate intervals 0.1-ml aliquots of the ambient water were removed and radioactivity was determined by liquid scintillation counting. The half time to reach equilibrium with the external medium was used as a measure of tissue permeability.

Six disks were removed from the same fruits, using the same techniques of each physiological stage and frozen at −20 C. The disks were homogenized with a hand glass homogenizer in 5 ml of hexane-acetone mixture (5:4:v/v). Two more aliquots of 5 ml were used to rinse the homogenizer and were combined with the original sample. After centrifugation for 10 min at 5000 rpm the upper layer was analyzed in a Beckman DU spectrophotometer. Lycopene was measured as absorbance at 505 nm while the Chl values were obtained at 665 nm.

RESULTS

In the first experiment we measured the hydraulic permeability of disks of rin and Rutgers fruits at different stages of development. Half time values for equilibrium are presented as a parameter of the hydraulic permeability in Rutgers fruits (Fig. 1). Fruit tissue at 70% and 110% of development gave values of 10 and 6

1 Research was supported in part by a contract from General Foods Corporation, Tarrytown, N. Y. Journal paper No. 5823. Agricultural Experiment Station, Purdue University, West Lafayette, Ind. 47907.
2 Permanent address: Department of Horticulture, Washington State University, Pullman, Wash. 99163.
3 On Leave from Ben-Gurion University, Beer-Sheva, Israel.

---

*Plant Physiol. (1975) 56, 813-815*
min, respectively. When the values for half time to equilibrium are plotted against per cent of fruit development (Fig. 2) it is obvious that, in the rin strain, no significant increase in permeability occurred during fruit development. Rutgers fruits show substantially greater permeability after 100% of development.

When mature green Rutgers fruits were harvested at 100% development a very small decrease in equilibrium half time value was measured (from 10 to 9 min). Even though this decrease is not statistically significant it may still be valid and it may be construed to mean that an increase in permeability occurred concomitantly with the onset of ripening. To test this possibility we compared fruits at a stage just before the onset of ripening and fruits just at the beginning of the climacteric rise to other stages of development. We were able to discriminate between these precise stages of development by harvesting Rutgers fruits 8 days before the expected rise in C2H4 and/or CO2. By daily measurements of C2H4 and CO2 evolution rates (Fig. 3) we were able to distinguish between three groups of fruits: (a) fruits that had not yet begun the ripening process; (b) fruits in the first day of ripening; and (c) fruits that had advanced 1 to 2 days into the climacteric rise, at which stage the first lycopene can be measured (Table I). The half time values of these three groups were the same (Table I). The first increase in permeability occurred only in Rutgers fruits at 110% of development, i.e., after beginning of the ripening process as evident by lycopene synthesis (Table I).

![Figure 1: Time course of elution of tritiated water from pericarp disks of Rutgers fruits at 70 and 110% of development.](image1)

**Fig. 1.** Time course of elution of tritiated water from pericarp disks of Rutgers fruits at 70 and 110% of development. Six disks were incubated in [3H]water, blotted, and transferred to tritium-free water. At the indicated intervals aliquots were counted. Half-time to equilibrium was defined as the time required to reach 50% of the counts that were counted at the plateau.

![Figure 2: Half-time to equilibrium for tritiated water out of pericarp disks of rin and Rutgers tomato strains as a function of fruit development.](image2)

**Fig. 2.** Half-time to equilibrium for tritiated water out of pericarp disks of rin and Rutgers tomato strains as a function of fruit development. Half time values were obtained from [3H]water efflux experiments as described in Fig. 1.

![Figure 3: Time course of C2H4 and CO2 evolution rates of Rutgers fruits pollinated at the same day (March 20, 1974). The measurements were made 8 days before the expected 100% of development (May 6, 1974). Fifteen fruits were used for this experiment; at day 8 the measurements showed that four fruits were at stage 1, eight fruits at stage 2, and three fruits at stage 3. Three fruits from each group were chosen for the hydraulic permeability experiments and pigment extraction.](image3)

**Table I. Chlorophyll and Lycopene Content of rin and Rutgers Strains at Different Stages of Fruit Development**

<table>
<thead>
<tr>
<th>Stage of Fruit Development</th>
<th>Chlorophyll Absorbance</th>
<th>Lycopene Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rin</td>
<td>Rutgers</td>
</tr>
<tr>
<td>%</td>
<td>0.95</td>
<td>0.90</td>
</tr>
<tr>
<td>100 green (1)</td>
<td>0.95</td>
<td>0.90</td>
</tr>
<tr>
<td>100 green (2)</td>
<td>0.95</td>
<td>0.90</td>
</tr>
<tr>
<td>100 first color (3)</td>
<td>0.95</td>
<td>0.90</td>
</tr>
<tr>
<td>105</td>
<td>0.45</td>
<td>2.22</td>
</tr>
<tr>
<td>110</td>
<td>0.93</td>
<td>0.90</td>
</tr>
<tr>
<td>140</td>
<td>0.99</td>
<td>0.90</td>
</tr>
</tbody>
</table>

1 (1), (2), and (3) relate to stages of ripening mentioned in Figure 3. The numbers are absorbance per g fresh weight. Chlorophyll and lycopene were measured in a hexane-acetone (5/4:v/v) mixture extracted from disks taken from the fruits which served for the hydraulic permeability measurements (Figs. 1 and 2, Table II).
Table II. Hydraulic Permeability of Rutgers Pericarp Disks at Different Stages of Fruit Development

<table>
<thead>
<tr>
<th>Stage of Development</th>
<th>Half Time to Equilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>min</td>
</tr>
<tr>
<td>40</td>
<td>9.5</td>
</tr>
<tr>
<td>70</td>
<td>10.5</td>
</tr>
<tr>
<td>Preripening (1)</td>
<td>9</td>
</tr>
<tr>
<td>First day of ripening (2)</td>
<td>10</td>
</tr>
<tr>
<td>Early ripening (3)</td>
<td>10</td>
</tr>
<tr>
<td>105</td>
<td>10.2</td>
</tr>
<tr>
<td>110</td>
<td>4.5</td>
</tr>
</tbody>
</table>

1 (1), (2), and (3) relate to stages of ripening mentioned in Figure 3. Half time values were obtained from [3H]water efflux experiments as described in Figure 1.

DISCUSSION

The results illustrate three important points. (a) The nonripening mutant strain, rin, did not show any change in water permeability as measured by the half time to equilibrium. (b) After the ripening process had been initiated in the normal strain, there was an increase in the hydraulic permeability of the cell membrane. (c) This change occurred after other parameters of ripening had already been altered. The idea that a drastic alteration in membrane permeability is associated with the ripening process is supported by the fact that this parameter did not change in the isogenic nonripening strain rin even at 140% of development when membrane integrity of the normal strain at this stage was totally disintegrated.

These findings support the hypothesis of Brady et al. (1) that increases in permeability are not causative of the onset of fruit ripening, but rather may be involved in the progressive changes involved in ripening. One can find increases in C₂H₄ and CO₂ evolution rates as well as color development before any changes in water permeability can be observed. This indicates that the observed changes in membrane permeability are a secondary event in the ripening process. Our measures of hydraulic permeability in tomatoes are in total agreement with the results of Brady et al. (1) who measured solute leakage in banana fruits, and found an increase in leakage of solutes only after the beginning of the climacteric when the ripening process was induced by exogenous C₂H₄.

Acknowledgment—We express our appreciation to Dr. E. C. Tischelscala, Purdue University, who generously supplied seeds of rin and Rutgers.

LITERATURE CITED