Light Regulation of Sink Metabolism in Tomato Fruit

I. Growth and Sugar Accumulation

Han Ping Guan and Harry W. Janes*
Department of Horticulture, Cook College, Rutgers University, New Brunswick, New Jersey 08903

ABSTRACT

Light/dark effects on growth and sugar accumulation in tomato (Lycopersicon esculentum) fruit during early development were studied on intact plants (in vivo) and in tissue culture (in vitro). Through the use of an in vitro culture of tomato fruit, it was possible to investigate the direct effects of light on sink metabolism by eliminating the source tissue. Similar light effects were found in vivo and in vitro. Fruit growth in different sugars indicated that sucrose was the best source of carbon for in vitro fruit growth. Fruit growth increased as sucrose concentration increased up to 8%. Darkening the fruit decreased fruit dry weight about 40% in vivo and in vitro. The differences in the CO₂ exchange rate between light and dark grown fruit indicated that light stimulation of fruit growth was due to mechanisms other than photosynthesis. Supporting this conclusion was the fact that light intensities ranging from 40 to 160 micromoles per square meter per second had no significant influence on fruit growth, and light did not increase growth of fruit cultured with glucose or fructose as a carbon source. However, light stimulated fruit growth significantly when sucrose was used as the carbon source. Light-grown fruit took up 30% more sucrose from the same source and accumulated almost twice as much hexose and starch as dark-grown fruit. A possible expansion of an additional sink for carbon by light stimulation of starch synthesis during early development will be discussed.

As a continuation of our research into the influence of light on tomato production (20), crop photosynthesis (19), and leaf carbon partitioning (16), it was of interest to focus on the development of the fruit. While it has been demonstrated that light can regulate the activity or synthesis of certain enzymes involved in carbon metabolism in source tissues (28, 29), the nonphotosynthetic-related effects of light on sink metabolism have not been as extensively studied. Evans et al. (6) reported that shading wheat heads substantially reduced grain set. In addition, grain set was not affected when photosynthesis was completely inhibited by treatment with DCMU, indicating that the reduction of set was not mediated by photosynthesis. It also has been reported that shading flowers and pods increased abscission and reduced accumulation of photoassimilate in soybean (9).

In tomato fruit, light has been demonstrated to alter protein and pigment synthesis (3, 26). Influence of light quality on tomato color development has been reported in detached tomato fruit (12, 13). Tomato plants grown under high solar radiation produce fruit with higher sugar content (32) and demonstrate a marked decrease in floral abscission (14), but it is not clear whether these effects are totally caused by increasing leaf assimilation or whether a more direct influence of light on sink metabolism also exists.

In this study, light effects on tomato fruit growth and sugar accumulation are studied. This investigation was conducted by growing fruit in tissue culture. Most importantly, through the use of a simplified source-sink system (24, 25), the in vitro culture of tomato fruit allowed us to investigate the direct effects of light on sink metabolism by eliminating the source tissue.

MATERIALS AND METHODS

Tomato Fruit Growth

Tomatoes (Lycopersicon esculentum cv Laura) were grown in a peat-vermiculite based soilless mix in the greenhouse (in vivo) as described previously (20). Five days after anthesis, the first fruit on the first flower cluster was used for all studies unless otherwise stated. To study light/dark effects in vivo, some fruit were covered with aluminum foil and control fruit were tagged.

In Vitro Culture of Tomato Fruit

Fruit were harvested from the greenhouse and sterilized in 0.05% sodium hypochloride for 15 min with slow shaking

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and rinsed in autoclaved distilled water three times. They were stored aseptically at 25°C and 100% relative humidity for 12 h. Fruit were then sterilized again to further reduce contamination. After the second sterilization, the remaining petals and sepals were removed. The pedicel was then cut just above the abscission layer and the small fruit were individually placed into jars containing 40 mL M&S medium (23) with 0.5% agar and 4% sucrose (unless otherwise noted) at pH 5.5 (24). Fruit were grown in the dark or in light (Cool White fluorescent, PPFD 50 μmol m⁻² s⁻¹ unless otherwise noted) in an environmentally controlled chamber (25°C, 16 h light/8 h dark). The fruit dry weight was measured weekly.

**Carbohydrate Measurement**

Fruit were cultured in M&S medium with 4% sucrose in the dark or in the light. After 3 weeks of culture, the fruit were harvested and frozen in liquid nitrogen. The frozen tissue was ground into a powder in additional liquid nitrogen. Soluble sugars were extracted in 50 mM Hepes-KOH (pH 8.3). The homogenization solution was centrifuged at 20,000 rpm for 15 min. The supernatant was used for sugar assay and the pellet for starch assay. Starch levels were measured using a modification of MacRae’s (17) method as outlined below. One milliliter diluted sample was incubated with 1 mL of the glucose assay reagent (Sigma Chemical Company) containing 1.5 mM NAD, 1.0 mM ATP, 1 unit of hexokinase, 1 unit glucose-6-phosphate dehydrogenase and 2.1 mM MgCl₂, for 30 min. The amount of glucose was measured at 340 nm. Fructose in the sample was transformed into glucose by adding 5 units of phosphoglucose isomerase to the glucose reagent and measured as described in the glucose assay. The total glucose accounted for the hexose concentration. Sucrose was hydrolyzed into glucose and fructose by incubating 0.2 mL diluted sample with 0.2 mL 0.3 M perchloric acid at 90°C for 45 min in a water bath. The solution was neutralized with 0.3 N NaOH and the final volume was brought up to 1 mL with 200 mM Tris-HCl (pH 7.5). The amount of glucose was measured by adding 1 mL glucose reagent as described above. After the pellet was washed with distilled water three times, it was incubated with 50 units of amylglucosidasde at 55°C for 24 h. It was then centrifuged at 15,000 rpm for 15 min. The supernatant was used for glucose assay as described above. The starch level in tomato fruit was expressed as mg glucose/g dry weight.

**Sugar Uptake into Tomato Fruit**

Sugar uptake was measured in vitro by a modification of the method of Dinar and Stevens (5). Five days after anthesis, the first fruit on the first cluster was covered with aluminum foil and control fruit were tagged in the greenhouse. Seven days later, the fruit were taken and put in the M&S agar medium without sucrose. The shaded fruit were kept in the dark and the control fruit were kept under a light intensity of 50 μmol m⁻² s⁻¹ (16 h light/8 h dark). After 24 h all fruit were transferred to 1 mL M&S agar medium with 2% sucrose and 2 μCi [¹⁴C]sucrose. They were maintained in light or dark as before. Ten hours later, whole fruit were cut into small pieces and incubated in 6 mL/g fresh weight tissue solubilizer (Fisher Scientific) at 50°C for 24 h (5). One milliliter aliquots were taken for measurement of radioactivity.

**CO₂ Measurement**

Five days after anthesis, small fruit were harvested from the greenhouse and cultured in M&S medium. Before the measurement, the fruit were recut from the end of the pedicel, then transferred to culture jars (100 × 60 mm) with 40 mL M&S agar medium containing 4% sucrose at pH 5.5. They were kept in an environmentally controlled chamber (25°C, 16 h light/8 h dark, PPFD 50 μmol m⁻² s⁻¹) for 24 h. Two hours after the start of the light period, the culture jars were sealed. Gas samples were taken using a 1 mL syringe. The CO₂ was analyzed by gas chromatography using a QGC-8A Shimadzu gas chromatograph equipped with a Poropaq-Q 100 to 200 mesh column. After the initial samples were taken, half of the jars were kept in the light and the other half in the dark. Four hours later, the second sample was taken. The difference in CO₂ concentration over the 4 h accounted for the rate of CO₂ exchange.

**RESULTS**

**Light/Dark Effects on in Vitro Tomato Fruit Growth**

Ten days after anthesis, small tomato fruit were cultured in 4% glucose, 4% fructose, or 4% sucrose. Fruit were grown in the dark or in the light (PPFD 50 μmol m⁻² s⁻¹, 16 h light/8 h dark, 25°C). After ripening (56 d of culture), fruit dry weight was determined. Table I shows that the detached tomato fruit cultured in 4% sucrose grew three times larger than those in 4% glucose or 4% fructose in the light. This indicates that sucrose is a much better source of carbon for in vitro tomato fruit growth, as was also reported for the growth of excised roots and callus from tomato (2). This is consistent with the finding that sucrose is the major sugar translocated into tomato fruit in vivo (11). No significant differences in growth

<table>
<thead>
<tr>
<th>Carbohydrate Source</th>
<th>Treatment</th>
<th>Fresh weight</th>
<th>Dry weight</th>
<th>Size (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Light</td>
<td>1.11 ± 0.072</td>
<td>43 ± 9</td>
<td>1.65 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>0.99 ± 0.072</td>
<td>41 ± 3</td>
<td>1.38 ± 0.29</td>
</tr>
<tr>
<td>Fructose</td>
<td>Light</td>
<td>1.98 ± 0.019</td>
<td>74 ± 7</td>
<td>2.18 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>1.53 ± 0.015</td>
<td>62 ± 6</td>
<td>1.75 ± 0.46</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Light</td>
<td>6.68 ± 0.059</td>
<td>227 ± 20</td>
<td>4.32 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>2.81 ± 0.010</td>
<td>114 ± 4</td>
<td>2.25 ± 0.31</td>
</tr>
</tbody>
</table>

Table I. Effects of Carbohydrate Sources on Tomato Fruit Growth in the Light or in the Dark

Ten days after anthesis, small fruit were harvested from the greenhouse and cultured in M&S medium with 4% glucose, 4% fructose, or 4% sucrose. They were grown in the dark or in the light (PPFD 50 μmol m⁻² s⁻¹, 16 h light/8 h dark, 25°C). After ripening (56 d of culture), fruit fresh weight, dry weight, and fruit size (cross sectional area, cm²) were determined. Data are means ± sd of 10 replications.
were found between light- and dark-grown fruit, when glucose or fructose was used as a carbon source. However, light greatly stimulated fruit growth with sucrose as the carbon source. Fruit growth in different sucrose concentrations (Fig. 1) demonstrates the influence of decreased source supply on tomato fruit growth. The growth of light- and dark-grown fruit increased as sucrose concentration was increased up to 8%. Light-grown fruit, however, grew almost twice as much as dark-grown fruit at each sucrose concentration. This result indicates that light can stimulate fruit growth. Similar light/dark effects on tomato fruit growth (Table II) were observed on intact plants in the greenhouse (in vivo) and under tissue culture conditions (in vitro). Although the fruit in tissue culture were only approximately 1% of the weight of those on the intact plant, relative growth differences between light- and dark-grown fruit were very similar. The in vitro fruit growth time course (Fig. 2) shows an “S” shaped pattern similar to that on the intact plant (12, 27), with the most rapid growth period between days 21 and 28. Dark-grown fruit also display a growth pattern similar to light-grown fruit.

### Light Stimulates Tomato Fruit Growth by Nonphotosynthetic Mechanisms

Light stimulation of tomato fruit growth in vivo and in vitro has been consistently observed. Because young tomato fruit contain Chl, a question arises as to whether the light effects on fruit growth are due to a stimulation of sugar uptake, sugar metabolism and storage, and/or to photosynthesis. Because dark-grown fruit in 8% sucrose were nearly as big as light-grown fruit in 4% sucrose (Fig. 1), a growth difference totally due to photosynthesis would mean that photosynthesis had to contribute 50% of the dry matter under a light intensity of 50 μmol m⁻² s⁻¹.

This assumption is refuted by the following observations. When small tomato fruit were grown in the dark or in light of different intensities, the dry weight of dark-grown fruit was about 60% that of light-grown fruit, but no significant differences were found among different light intensities ranging from 40 to 160 μmol m⁻² s⁻¹ (Fig. 3). The CO₂ exchange rate between light- and dark-grown fruit was also measured. While no net CO₂ was taken up in the light or dark (as found also by Laval-Martin et al. [15]), a decrease in the CO₂ production rate was seen in the light when compared to the dark. This may be due to some fixation of respired carbon or a decrease in respiration in the light, or both. Based on the difference in the CO₂ exchange rate between light and dark, an increase in carbohydrate (calculated as CH₂O) fixed in the light was

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**Table II. Light/Dark Effects on Tomato Fruit Growth In Vivo and In Vitro**

<table>
<thead>
<tr>
<th></th>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>g dry wt/fruit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in vivo</td>
<td>4.900 ± 0.140</td>
<td>2.740 ± 0.160</td>
</tr>
<tr>
<td>in vitro</td>
<td>0.049 ± 0.002</td>
<td>0.026 ± 0.005</td>
</tr>
</tbody>
</table>

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**Figure 1.** Effect of sucrose concentration on in vitro tomato fruit growth in the light or dark. Seven days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in M&S medium with different sucrose concentrations (2, 4, 6, or 8%). The osmotic potential in the medium was held constant by the addition of sorbitol at the lower sucrose concentrations. They were grown in the dark or in the light (PPFD 50 μmol m⁻² s⁻¹, 16 h light/8 h dark, 25°C). After 42 d of culture, fruit dry weight was determined.

**Figure 2.** The growth pattern of tomato fruit in vitro. Five days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in M&S medium with 4% sucrose in the dark or in the light (PPFD 50 μmol m⁻² s⁻¹, 16 h light/8 h dark, 25°C). Fruit dry weight was determined weekly.
Lightdark effects on tomato fruit growth in vitro.

Five days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in M&S medium with 4% sucrose in the dark or in different light intensities (PPFD 40, 80, 120, or 160 μmol m⁻² s⁻¹, 16 h light/8 h dark, 25°C). After 21 d of culture, fruit dry weight was determined.

shown (Fig. 4). The overall rate of carbon fixation decreased during fruit development, as did the difference between fixation in light- and dark-grown fruit (Fig. 4). By comparing the increase in carbohydrate fixed (using the curve in Fig. 4) to the actual growth difference between light- and dark-grown fruit, less than 5% of the differences in dry weight can be accounted for by differences in the CO₂ exchange rate. These results support the suggestion made by Mor and Halevy (21) and Mor et al. (22) that light enhanced growth may be due to stimulating the importation of sucrose into macromolecules, or possibly to an enhanced unloading process, rather than to increasing source supply through photosynthesis.

Light/Dark Effects on Sugar Uptake and Sugar Accumulation

Because a similarity has been found in the uptake rate of [¹⁴C]sucrose into detached tomato fruit and the rate of carbon import by attached fruit (5, 10), sucrose uptake into tomato fruit was measured in the detached fruit. Table III indicates that sucrose uptake in dark-grown fruit was only 70% of that in light-grown fruit. Since the rate of carbon import decreases as the fruit size increases, it has been suggested that sink strength in tomato fruit is determined mainly by sink activity (30). Therefore, differences in sugar accumulation are also expected between light- and dark-grown fruit. Table IV shows no differences in sucrose concentration between light- and dark-grown fruit. However, light-grown fruit accumulated almost twice as much starch and hexose as did dark-grown fruit, suggesting that light effects on fruit growth and sink strength may be due to an expansion of a sink for carbon.

**DISCUSSION**

Lightdark effects on tomato fruit growth and sugar accumulation show great similarity between greenhouse and tissue culture conditions. Fruit growth in vitro also displays a similar growth pattern to that on intact plants (27). The in vitro cultured fruit is limited in size; however, ripening, color, and flavor are normal (24), indicating that the in vitro cultured fruit may serve as a simplified source-sink system useful as a model to study tomato sink activity.

In this study, the light-dependent growth of tomato fruit grown in vitro is compared to growth of fruit on the intact plant. Darkening the fruit decreased growth about 40% (Table II). Light-grown fruit can take up more sucrose (Table III) from the same source and accumulate almost twice as much.

**Table III. Light/Dark Effects on Sink Strength of Tomato Fruit**

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>Sink Strength (μmol sucrose/g dry wt/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>4.82 ± 0.21</td>
</tr>
<tr>
<td>Dark</td>
<td>3.32 ± 0.08</td>
</tr>
</tbody>
</table>
starch (Table IV) as that in dark-grown fruit. No effect of light was found on growth of fruit cultured with glucose or fructose as the carbon source (Table I), indicating that photosynthesis does not contribute to the growth. If light influences photosynthesis, differences in growth in different light intensities would be expected. The data (Fig. 3), however, indicate that light probably influences tomato fruit growth primarily through mechanisms other than photosynthesis. Additionally, the carbon exchange rate (Fig. 4) indicates that, while differences exist between light- and dark-grown fruit, they do not significantly contribute to the differences in dry weight between the two treatments. Similar results were found in cherry tomatoes (15). Although CO$_2$ (as NaH$_2$CO$_3$) fixation by tomato fruit is enhanced by light, CO$_2$ exchange in the fruit remains a net evolution at the saturating light intensity of 2000 $\mu$mol m$^{-2}$ s$^{-1}$ (15). Probably the most significant contribution of the light and dark fixation in fruit is to preserve the carbon released from respiration for protein and pigment synthesis during ripening (26).

A positive correlation between peak levels of starch in early fruit development and the final levels of soluble sugars has been reported (4). Walker and Ho (31) also reported that sink strength of tomato fruit was primarily determined by sink activity, and sugar accumulation in tomato fruit may be driven by subsequent intracellular metabolism. While darkening the fruit only during the process of sugar uptake (differences between 0 and 48 h of darkening) had no effect on carbon import (31), fruit grown in the dark for 1 week had a lower rate of sucrose uptake and lower levels of hexose and starch (Table IV) than light-grown fruit. The data appear to indicate that light does not directly influence the sugar uptake process, but may stimulate sugar uptake by modulating subsequent metabolism in tomato fruit.

Therefore, our work suggests that, similar to source tissue (28, 29) and young shoots (21, 22), light may have a nonphotosynthetic influence on carbon partitioning in sink organs. However, the control points and enzymes affected may be different from source tissues. Our research further suggests that light effects on fruit growth may be due to an expansion of an additional sink for carbon. Light effects on the key enzymes of sugar metabolism in tomato fruit are reported in the subsequent paper.

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>Carbon Concentration</th>
<th>mg glucose/g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>Sucrose</td>
<td>21.37 ± 2.09</td>
</tr>
<tr>
<td></td>
<td>Hexose</td>
<td>109.26 ± 1.36</td>
</tr>
<tr>
<td></td>
<td>Starch</td>
<td>27.50 ± 0.38</td>
</tr>
<tr>
<td>Dark</td>
<td>Sucrose</td>
<td>22.22 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>Hexose</td>
<td>67.15 ± 1.38</td>
</tr>
<tr>
<td></td>
<td>Starch</td>
<td>11.16 ± 0.11</td>
</tr>
</tbody>
</table>

LITERATURE CITED

11. Ho LC (1979) Regulation of assimilate translocation between leaves and fruits in tomato. Ann Bot 43: 437-448
17. MacRae JC (1971) Quantitative measurement of starch in very small amount of leaf tissue. Planta 96: 101-108