Effects of Maleic Hydrazide Treatment on the Size and Number of Cells and Sugar Accumulation in the Fruit of Melons (Cucumis melo L.)

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Abstract. The relationships between the size and the number of cells and sugar accumulation in melon fruit have been examined. Maleic hydrazide (MH) was used to investigate the relationships. Although cell size was markedly larger in MH-treated fruit than in untreated fruit in the early stages of fruit development, the number of cells in MH-treated fruit was less than in untreated fruit in latter fruit development. Sucrose, glucose, and fructose content were higher in MH-treated fruit than in untreated fruit. It is therefore suggested that sucrose accumulation in fruit subjected to MH treatment is accelerated as a result of early cell enlargement and that sucrose content increases further as a result of the decrease in the number of cells in the fruit during late development.

Sucrose is the most important factor determining the sweetness of melon fruit. The rate of sucrose accumulation in melon fruit increases during the latter half of the fruit development as the result of cell enlargement (Kano, 2002). In addition, sucrose accumulation has been demonstrated to occur in response to cellular enlargement if cell size is increased by auxin treatment during early fruit development (Kano, 2002) as well as in response to heating fruits (Kano, 2006). Conversely, sucrose accumulation in melon fruit can be suppressed by mechanical restriction resulting from relative decreases in the number of large cells associated with such treatment (Kano, 2004a) or by treatment with plant growth inhibitors (Kano, 2004b). Sucrose accumulation is suppressed in Japanese pears as a result of an increase in the number of small cells that result from 2-chloro-4-pyrdyl-N-phenylurea treatment (Kano, 2003). Thus, it is considered that increased sucrose accumulation is associated with a higher number of large cells relative to small cells in the fruit. Most of the imported sugars accumulate in the vacuole of sink-tissue storage cells (Leigh et al., 1979; Yamaki and Ino, 1992). Cell size during the latter stage of fruit development is mostly equal to that of the vacuole because vacuoles in peaches force the cytoplasm to the outside of the mesocarp cells in the middle stages of fruit development (Ishida et al., 1973). Taken together, these findings suggest that the occurrence of lots of large cells is associated with increased sucrose content. The treatment of seeds with maleic hydrazine (MH) was found to increase cell size and vacuolation in wheat seedlings (Mendhulkar, 2000), and low concentrations of MH have been shown to increase cell length in algae (Gupta and Kumar, 1970). Furthermore, the inhibition of sprouting in onion has been demonstrated by MH treatment (Benkeblia et al., 2004; Benkeblia et al., 2002; El-Otmani et al., 2003) as a result of its effects on inhibiting cell division (Marcano et al., 2004; Zilkah et al., 1981). Thus, MH treatment has the effect of both accelerating cell enlargement as well as inhibiting cell division. Consequently, I hypothesized that sucrose content increases in melon fruit with early cell enlargement and with a cessation in cell division after an inflexion point.

I therefore treated melon fruit with MH to clarify the relationship between the size and the number of cells and sugar accumulation in the fruit.

Materials and Methods

Plant materials. Earl’s Knight Natsukei No.2 (Cucumis melo L.) melon seeds were planted in a seedbed on 20 Mar. 2004 with nursery plants spaced at 40-cm intervals in a plastic film greenhouse on 20 Apr. 2004. The flowers that opened on ≈14 June were used in this experiment. Only one fruit was borne on about the 12th node of each plant and the main stems were pinched at the 20th node. Three fruits were used in each treatment. The fruits on the 30th day after anthesis (DAA), considered to be the time when sucrose accumulation is initiated, were thoroughly sprayed with an MH solution at 2000 mg L⁻¹. The three fruits that were collected and weighed at 40 and 50 DAA for the control and MH treatments, respectively, were used for cell size and sugar analyses.

Measurements of cell number and size in the fruit. Two ≈10-mm thick disks were cut from each of the three fruit from the control and MH-treated plants; one disk was excised from the fruit at the maximum transverse diameter toward the calyx end for cell analysis, whereas another was excised from the maximum transverse diameter toward the peduncle end for sugar analysis. Using a sharp table knife, a sample measuring ≈10 mm × 10 mm was removed from the disk with 5 mm straddling the maximum diameter line across each disk (Fig. 1). Rectangular parallelepipeds (hereafter RP), each measuring 7 mm, were serially sampled across the diameter of the disk using the same sharp table knife. Except for the RP containing seeds and the RPs at both ends containing the epidermis, all of the RPs along the 7-mm-long strips through the diameter of the fruit body were 8 and 9 and 12 and 12 at 40 and 50 DAA, respectively. All of the RPs obtained from fruit from each treatment were dehydrated by treatment using a series of alcohol concentrations (70%, 80%, 90%, and 100%) before being embedded in paraffin. Seven 10-μm-thick sections were prepared from these paraffin blocks, and the clearest section from each RP treatment was examined under a microscope. As shown in Figure 2, the maximum diameters of individual cells along the maximum transverse diameters of the RPs were measured. Cell size was calculated by dividing the total cell diameter measured in each RP from the three fruits or all RPs of three fruits by the number of cells of each RP of three fruits or the number of cells of all RPs of three fruits.

Sugar analysis. The RPs from the disk taken from the maximum transverse diameter toward the peduncle end were used for sugar analysis. With exception of those RPs containing the seeds and both epidermal layers, all of the RPs were wrapped in cheesecloth and squeezed using forceps to extract the juice. The juice was diluted 10 times with distilled water before being centrifuged at 8000 × g for 15 min and filtered through a 0.45-μm filter. Then, 20 μL of filtrate was analyzed by high-performance liquid chromatography (LXC-10ADvp; Shimadzu, Kyoto, Japan) fitted with a refractive index detector (RID-10A; Shimadzu) equipped with Shime-pack SCR-101C (Shimadzu) at 0.8 mL·min⁻¹ at 80 °C. Standard solutions of sucrose, glucose, and fructose at 20,000 mg·L⁻¹ were injected into the high-performance liquid chromatograph before injection of the filtrates. Mean sucrose, glucose, and fructose content in each RP was calculated by dividing the total sucrose, glucose, and fructose content of each RP from three fruits by three. Mean sucrose, glucose, and fructose content in whole fruits was then estimated by dividing the total sucrose, glucose, and fructose content of all RPs from three fruits by the total number of PRs from the three fruit.
The number of RPs with more than 25 cells was six in MH-treated fruit compared with 10 in the untreated fruit (Table 2). The mean cell volume of strawberry fruit increases slowly during active cell division, but rises rapidly and linearly for 10 d after cell division stops (Guiven and Breen, 1992) with sucrose content in the vacuoles of strawberry fruit increasing from 25 to 35 DAA (Oofusu-Animo and Yamaki, 1994). Sucrose accumulates rapidly in the larger cells of melon fruit (Kano, 2002) because cell enlargement during early melon development results in dramatically increased sucrose content as a result of the increased duration of sucrose accumulation before harvesting (Kano, 2005). The treatment of various crops with MH has also been demonstrated to increase leaf carbohydrate content (Currier et al., 1951; Derridj et al., 1986), especially Gossypium herbaceum (McIlrath, 1950), Zea mays (Naylor, 1951) and Nicotiana tabacum (Seltmann and Nichols, 1984). Given these results, it is reasonable to assume that the sucrose accumulation in the cells of melon fruits treated with MH increases as a result of the early enlargement of vacuoles by rapid cell enlargement.

A fewer number of cells were found in MH-treated fruit at 50 DAA compared with the untreated fruit. After reaching the inflection point, before which fruit growth is primarily the result of cell division, any increase in melon size is the result of cell enlargement only. In the fruits of Cucurbits, this usually occurs when the fruit diameter is ~20 mm (Masuda, 1970; Sinnott, 1939). Cell division in the tissues near the exocarp in the fruit of Lagernaria vulgaris continues until the middle stages of fruit growth (Simmott, 1939) with cellular multiplication continuing until harvest in the fruit of the avocado (Schroeder, 1953). In this study, the total...
the mid to latter development stages in melon fruit. Therefore, the gradual decrease in cell number response to MH treatment only becomes apparent during the mid to latter stages of fruit development when some time has elapsed. Moreover, sucrose content was considerably higher in MH-treated fruit than in the untreated fruit 50 DAA. No cell division was observed during the latter stages of fruit development as a result of MH treatment, resulting in the production of fewer large cells and thus, smaller sucrose sink for the whole fruit. Consequently, the constant and active sucrose accumulation in large cells, which are fewer in number in MH-treated fruit, has the effect of further increasing sucrose content.

However, a clear relationship between cell size and sucrose content was not observed in RPs from untreated fruit and MH-treated fruit at 40 and 50 DAA. Although numerous vascular bundles develop in the mesocarp and endocarp of melon fruit, they change in number and diameter as a result of differential growth in the fruit (Kanahama and Saito, 1987). This is likely to be the reason for the apparent absence of any relationship between cell size and sucrose content of the RPs examined.

The following conclusion can be drawn from the results observed in MH-treated fruits: sucrose accumulation is promoted as a result of early cell enlargement associated with MH treatment and the concomitant decrease in the number of cells in the fruit during late development, which results in a further increase in sucrose content.

**Literature Cited**


Table 3. Effects of maleic hydrazide (MH) treatment on sugar content (g L⁻¹) in each rectangular parallelepiped (RP) and mean content in all the RPs.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>40 DAA</th>
<th>50 DAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>MH-treated</td>
</tr>
<tr>
<td></td>
<td>RP</td>
<td>RP</td>
</tr>
<tr>
<td>40 DAA RP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>9.5 ± 9.4</td>
<td>15.4 ± 10.2</td>
</tr>
<tr>
<td>L2</td>
<td>34.9 ± 7.1</td>
<td>45.5 ± 5.0</td>
</tr>
<tr>
<td>L3</td>
<td>32.1 ± 3.4</td>
<td>41.2 ± 5.6</td>
</tr>
<tr>
<td>L4</td>
<td>45.3 ± 9.0</td>
<td>45.2 ± 3.1</td>
</tr>
<tr>
<td>R1</td>
<td>39.1 ± 5.9</td>
<td>56.3 ± 6.7</td>
</tr>
<tr>
<td>R2</td>
<td>35.7 ± 10.5</td>
<td>41.0 ± 1.7</td>
</tr>
<tr>
<td>R3</td>
<td>23.8 ± 11.9</td>
<td>38.6 ± 0.3</td>
</tr>
<tr>
<td>R4</td>
<td>0.4 ± 0.7</td>
<td>42.3 ± 8.8</td>
</tr>
<tr>
<td>R5</td>
<td>1.0 ± 0.7</td>
<td>38.0 ± 13.2</td>
</tr>
<tr>
<td>Mean</td>
<td>27.6 ± 16.6</td>
<td>38.0 ± 13.2</td>
</tr>
<tr>
<td>Statistical significance</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

Data are means ± SD of three replications in each RP.

The values of mean in the table are means ± SD of 24(3 × 8), 27(3 × 9), and 30(3 × 10) replications in the control fruit 40 DAA, MH-treated fruit 40 DAA, the control fruit 50 DAA and MH-treated 50 DAA, respectively.

**Significant at P < 0.01 by test.

Refer to Figure 1 for RPs in the table.

DAA = days after anthesis.