Abscisic Acid Content and Sugar Metabolism of Peaches Grown under Water Stress

Kenji Kobashi, Hiroshi Gemma, and Shuichi Iwahori
Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba 305-8572, Japan

Abstract. A water-stress treatment was imposed on peach trees [Prunus persica (L.) Batsch ‘Kansuke Hakuto’ (Peach Group)] to elucidate the relationship among sugar accumulation, sugar metabolism, and abscisic acid (ABA) in fruit under water stress. Treatment was carried out on peach trees grown in containers from 8 July 1996 (80 days after full bloom [DAFB]) for 16 days, to achieve a predawn water potential of –0.8 to –1.1 MPa compared to that of –0.4 to –0.6 MPa in control trees. Levels of sorbitol, sucrose, and total sugars, as well as the activity of sorbitol oxidase increased in fruit of water-stressed trees under moderate water stress (–0.8 MPa), whereas under severe water stress (–1.1 MPa), no difference between the water-stressed trees and the controls was observed. Water stress also induced an increase in ABA in the fruit. These initial results indicated that water-stress-induced ABA accelerated sugar accumulation in peaches by activating sorbitol metabolism.

Materials and Methods

Water-stress management. Four-year-old ‘Kansuke Hakuto’ peach trees growing in 45-L plastic containers with loamy soil were used in this study. Two groups of seven trees each were selected. Trees were of similar size and had two to three fruit per tree. In one group, trees were used for the water-stress treatment and those in the other group served as the nontreated controls. The number of leaves per fruit were 72.8 ± 4.2 in the control trees and 75.5 ± 9.1 in the treated trees at the beginning of treatment. From 8 July (80 days after full bloom [DAFB]) to 24 July 1996, corresponding to the third phase of fruit development, soil water potential was regulated at –0.04 to –0.10 MPa in the stress treatment and at or above –0.02 MPa in the controls by regulating irrigation and monitoring soil water status with tensiometers (Terada type-02; Fujiwara Co., Tokyo, Japan). Irrigation was administered uniformly on the whole soil surface in the containers. Trees were maintained in a greenhouse under natural photoperiod and irradiance, in which air temperature was similar to outdoor temperatures due to sufficient ventilation during the experiment. During treatment, leaf water potential, photosynthetic rate, and stomatal conductance were measured. Leaf water potential was measured on one mature leaf per tree with a pressure chamber (DK-7000, Fujiwara Co., Tokyo, Japan) before daybreak.

Photosynthetic rates and stomatal conductance were measured on two mature leaves per tree with a portable closed gas-exchange photosynthesis system (LI-6400; LI-COR, Lincoln, Nebr.) at 1200 hr under a photosynthetic photon flux of =1600 µmol·m–2·s–1. Six trees were selected from the seven trees of both the water-stressed and control trees, and one fruit per tree was harvested from the six trees. A total of six fruit were collected from both the water-stressed and the control trees at each sampling time during treatment.

Analysis of ABA, sugars, and assay of enzyme activities were replicated three times. One replication consisted of a composite sample consisting of two fruit from the six trees.

ABA analysis. Analysis of ABA content was carried out following the method of Uthaibutra and Gemma (1991). Five grams of a frozen fruit sample was ground and extracted twice with 50 mL cold 80% ethanol containing 0.5 g insoluble polyvinylpolypyrrolidone (PVPP). The extract was evaporated in vacuo at 40 °C to water phase, adjusted to pH 2.5 with 0.1 mol·L–1 HCl and extracted three times with 20 mL 100% ethyl acetate. The ethyl acetate phase was evaporated to dryness and the remaining residue was dissolved with 1 mL distilled water containing 50 µL 100% methanol. The extract
was then purified by passing it through a Sep-pak C18 cartridge (WAT051910; Waters Co., Milford, Mass.) and eluting with 1 mL 60% methanol. The eluted sample was centrifuged (3000 g, 2 min) after adding 0.03 g PVPP. The supernatant was filtered through a 0.45-μm nitrocellulose membrane filter (A045A013A; ADVANTEC Co., Tokyo, Japan) and injected into a high-performance liquid chromatograph (HPLC) (8000 series; Toso Co., Tokyo, Japan) equipped with a super ODS column (Super ODS, Toso Co., Tokyo, Japan) and ultra violet detector (254 nm). Procedures of HPLC analysis followed those of Uthaibutra and Gemma (1991).

Sugar Analysis. Five grams of frozen sample were ground and extracted twice with 50 mL 80% ethanol. The extract was evaporated to the water phase, and the volume adjusted to 100 mL with distilled water after 5 mL 0.15 M Ba(OH)2 and 5 mL 0.15 M ZnSO4 was added to remove proteins. Then, 1 mL of the supernatant was dried completely after adding 1 mL of 0.1% pentanethiol as an internal standard for gas chromatography (GC) (GC-9AM; Shimadzu, Tokyo, Japan). Trimethylsilylation was accomplished by adding 1 mL 100% pyridine, 0.2 mL 100% HMDS (hexamethyldisilazane), and 0.1 mL 100% TMCS (trimethylchlorosilane) to the dried sample, and heating it at 115°C for 2 min. The samples were then analyzed by gas chromatography equipped with a SE-52 column (GE SE-52; Shimadzu, Tokyo, Japan) and a flame ionization detector.

Enzyme Activity. SS, SPS, SOX, and acid invertase were extracted and their activities were assayed according to the methods of Moriguchi et al. (1990) with some modifications. Enzyme extractions were carried out at 4°C. SS and SPS were extracted by homogenizing 5 to 10 g of frozen sample in 50 mL 0.2 M K-phosphate buffer (pH 7.8), containing 10 mM Na-ascorbate, 5 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol (DTT), and 10% PVPP. After centrifugation at 12,000 g, for 20 min, the supernatant was passed through a Sephadex G-25 column to remove phenolic compounds. The eluate was treated with ammonium sulfate. The proteins in the eluate, which precipitated between 30% and 80% saturation, were redisolved and dialyzed against 0.01 M Tris-HCl buffer (pH 7.2) containing 1 mM DTT, and assayed for enzyme activity. Activity of SS and SPS was assayed at 30°C in 1.2 mL 15 mM Hepes-KOH buffer (pH 8.5 for SS and pH 7.5 for SPS), containing 2 mM uridine 5-diphosphoglucose (UDP), 15 mM fructose (for SS) or fructose-6-phosphate (for SPS), 5 mM MgCl2, 1.3 mM NaF, and 0.2 mL of enzyme extract. The resultant sucrose or sucrose phosphate was determined by the method of Roe (1934).

SOX and acid invertase were extracted by homogenizing 10 to 20 g of frozen sample in 50 mL 0.1 M K-phosphate buffer (pH 7.0), containing 10 mM Na-ascorbate, 1 mM DTT, and 0.3% Triton X-100. After centrifugation at 12,000 g, for 20 min, the pellet was homogenized again with 50 mL 0.1 M K-phosphate buffer (pH 7.0), containing 10 mM Na-ascorbate, 1 mM DTT, and 0.5 mM NaCl, then recentrifuged at 12,000 g, for 20 min. Ammonium sulfate between 40% and 80% saturation was added to the combined supernatant to precipitate the proteins. The precipitate was then dialyzed against 0.01 M Tris-HCl buffer (pH 7.0) containing 1 mM DTT, and assayed for enzyme activity. Activity of SOX and acid invertase were assayed at 30°C in 1.7 mL 59 mM acetate buffer (pH 4.0 for SOX and pH 5.0 for acid invertase), containing 235 mM sorbitol (for SOX) or 70 mM sucrose (for acid invertase), and 0.3 mL enzyme extract. The resultant reducing sugars were determined by the Somogyi-Nelson method (Nelson, 1944). In this study, the activities of only soluble fractions were measured for SOX and acid invertase, because Triton X-100, which was used in the extraction buffer for SOX and acid invertase, can effectively solubilize membrane proteins (Kagawa, 1972). Extraction and assay of SDH were conducted according to the method of Yamaki and Ishikawa (1986). Activities of all the enzymes examined in this experiment were expressed as micromoles of product produced per hour per gram fresh weight (FW) of peaches (μmol product/h/g FW).

Experimental Design and Statistical Analysis. Trees for the control and water-stress treatment were arranged in a completely randomized design on the central part of the greenhouse, which had uniform meteorological conditions. Student’s t test (unpaired) was performed to determine the differences between the control and water-stress treatment.

Results

The water-stress treatment conducted for 16 d beginning 8 July (80 DAFB) resulted in lower predawn leaf water potential in ‘Kansuke Hakuto’ peach trees. Water potential from 16 to 24 July was −0.8 to −1.2 MPa in water-stressed trees as compared to −0.4 to −0.6 MPa in control trees (Fig. 1). Fruit growth was not affected statistically by the water-stress treatment on either 16 or 24 July, although water-stressed fruit was slightly smaller as compared to the controls on the latter date (Table 1).

Table 1. Effect of water stress treatment on fresh weight (FW) and ABA content in ‘Kansuke Hakuto’ peach fruit. Water stress treatment was conducted from 8 July (80 d after full bloom) to 24 July. Values are means of three and replications for FW and ABA content, respectively, ±SE.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>FW (g)</th>
<th>ABA content (ng g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 July</td>
<td>Control</td>
<td>77.2 ± 6.6</td>
<td>92.8 ± 16.2</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>80.6 ± 4.9</td>
<td>232.1 ± 44.8 *</td>
</tr>
<tr>
<td>24 July</td>
<td>Control</td>
<td>119.6 ± 7.8</td>
<td>114.6 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>100.0 ± 9.8</td>
<td>266.9 ± 39.6 *</td>
</tr>
</tbody>
</table>

* NS nonsignificant or significant at P < 0.05 by Student’s t test (unpaired).
Table 2. Effects of water stress treatment on photosynthetic rate and stomatal conductance of ‘Kansuke Hakuto’ peach leaves on 18 July (10 d of treatment). Values are means of 14 replications ±SE.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Photosynthetic rate (µmol m⁻²·s⁻¹)</th>
<th>Stomatal conductance (mol m⁻²·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.02 ± 0.73</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>Treated</td>
<td>3.60 ± 0.58</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

***Significant at P < 0.001 by Student’s t test (unpaired).

The effect of the water-stress treatment on sugar content in the peaches is presented in Table 3. Sucrose content increased from 16 to 24 July in the fruit of both water-stressed and control trees, whereas the contents of the other sugars showed no conspicuous changes during that period. On 16 July, levels of sucrose, sorbitol, and total sugars were higher in the fruit on the water-stressed trees than those on the control trees. On 24 July, however, no significant difference was found in the contents of any sugars between the water-stressed fruit and the controls. Variability of sucrose content in both the water-stressed fruit and the controls on 24 July was higher than those of the other sugars on both dates.

The effect of the water-stress treatment on activities of sugar metabolizing enzymes in peaches is presented in Table 4. SS activity in the fruit on both the water-stressed trees and the controls rose approximately five fold from 16 to 24 July, and, was not affected by the water-stress treatment. SPS activity was low compared to that of SS, and was not also affected by the stress treatment. SOX activity was higher in the fruit on the water-stressed trees than on those on the control trees. On 24 July, however, no significant difference was found in the contents of any sugars between the water-stressed fruit and the controls on 24 July was higher than those of the other sugars on both dates.

From the results on 16 July, it is suggested that water-stress-induced ABA stimulated sugar accumulation in peaches by activating sorbitol metabolism. The relationship between sugar metabolism and sugar accumulation in sink tissues has been studied in plants that translocate mainly sucrose. In sink tissues of such plants, invertase plays an important role in buildup of sink strength through maintenance of a sucrose concentration gradient between the tissue and phloem (Daie, 1985). The activity of invertase in sink tissues was influenced by water stress (Mukai et al., 1996), as well as by plant hormones such as indoleacetic acid (Poovaiah and Veluthambi, 1999a).

Kobashi et al. (1999a) reported that ABA treatment enhanced total sugar accumulation in peaches, which was accompanied by a rise in SOX activity. These results indicate that ABA is involved in sugar accumulation in peaches. Since the main translocating sugar of peach is sorbitol (Moing et al., 1997), SOX, which converts sorbitol to glucose (Moriguchi et al., 1990), is considered to play an important role in sugar accumulation in peaches (Kobashi et al., 1999a).

In the present study, levels of sorbitol, sucrose, and the activity of SOX were significantly higher in water-stressed fruit than in those of the controls after an 8-d water-stress treatment (16 July). After 16 d of water stress (24 July), sugar content and the enzyme activities were unaffected when compared to controls (Tables 3 and 4). Furthermore, fruit FW was not significantly different in the stressed and nonstressed trees on either sampling date (Table 1). ABA content in the fruit of water-stressed trees was significantly higher compared to fruit in control trees on both sampling dates (Table 1). Results on 16 July were consistent with those observed with ABA treatment of peaches (Kobashi et al., 1999a) in that sugar contents increased in the fruit, accompanied by an increase in SOX activity and an increase in ABA content. These results suggest involvement of endogenous ABA in sugar accumulation under water-stress conditions.

Discussion

Kobashi et al. (1999a) reported that ABA treatment enhanced total sugar accumulation in peaches, which was accompanied by a rise in SOX activity. These results indicate that ABA is involved in sugar accumulation in peaches. Since the main translocating sugar of peach is sorbitol (Moing et al., 1997), SOX, which converts sorbitol to glucose (Moriguchi et al., 1990), is considered to play an important role in sugar accumulation in peaches (Kobashi et al., 1999a).

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Partitioning of 13C-photoassimilates to fruit was increased by modifications that negatively affected ABA-induced sugar accumulation. Under water-stress conditions, many physiological changes occur in plant organs other than fruit due to water stress. Decline in sink activity in vegetative organs is considered one of the factors that can contribute to sugar accumulation in fruit under water stress (Mills et al., 1996). Therefore, the increase in translocation to fruit through changes in sink activity of vegetative organs under water stress might be responsible for the increase in sorbitol.

Some explanation is needed regarding the results of 24 July. Under water-stress conditions, many physiological changes occur in plant organs depending on the severity of water stress (Hsiao, 1973). In the present study, water stress in peach trees was in the moderate level (−0.8 MPa) on 16 July, then the stress in the same trees became severe (−1.1 MPa) on 18 and 24 July (Fig. 1). The more severe water stress on 24 July might have given rise to physiological changes that negatively affected ABA-induced sugar accumulation. Partitioning of 13C-photoassimilates to fruit was increased by moderate water stress (Kobashi et al., 1999b; Yakushiji et al., 1998), but not affected by severe water stress in satsuma mandarin (Citrus unshiu Marc.) (Yakushiji et al., 1998) and was decreased in peaches (Kobashi et al., 1999b).

Results herein indicate that sink activity in peaches was increased by moderate water stress but not by a severe stress. Although the mechanism is still not clear, it is possible that lower cell turgor in fruit tissue counteracted the effect of ABA on sugar accumulation in peaches under severe water-stress conditions. A tendency for growth of water-stressed fruit to decline on 24 July may imply a decline in cell turgor in fruit tissue, because cell turgor is an important factor responsible for fruit growth (Coombe, 1976). It is known that sink activity is affected directly by turgor of sink cells (Ho, 1988). Pomper and Breen (1996) showed the rate of 14C-sucrose uptake by strawberry (Fragaria xananassa Duchesne) fruit discs from an incubation solution, in which osmolarity was regulated with mannitol concentration, decreased as cell turgor decreased.

In the present study, it was observed that photosynthetic rate was inhibited markedly under severe water stress (Table 2), potentially leading to a decrease in total production of photoassimilates. This may have also masked the effect of ABA on sugar accumulation under severe water stress on 24 July. Moreover, it should be also considered that moderate and severe water stress was imposed on the same trees continuously at different times in this study. That is, the water stress in fruit on 24 July had been imposed for 8 d longer as compared to fruit sampled on 16 July, and was also 8 d older than fruit on 16 July. The difference in stress duration and fruit growth stage may also have been attributed to a difference in the response of sugar metabolism and sugar accumulation to water stress.

In summary, we suggest that under moderate water stress, water-stress-induced ABA was involved in sugar accumulation in peaches by activating sorbitol metabolism. Under more severe water stress, however, the effect of ABA on sugar accumulation in fruit was counteracted by water-stress-related physiological changes, possibly such as a decline in photosynthesis or changes in cell turgor. From a practical viewpoint, our results may be useful for designing irrigation programs where dry conditions during fruit maturation stage are recommended empirically for producing high quality fruit, especially in peach. Further studies are warranted with respect to the effects of duration and timing, as well as the severity of water stress, on sugar accumulation in fruit and whole plant carbon partitioning in peach trees.

Literature Cited