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

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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.

It is generally known that fruit of many species of trees tend to contain higher sugar content in years of low rainfall during fruit maturation but the mechanism is still not clear. Concentration of sugars by reduction of water in fruit seems to be mainly responsible for the accumulation of sugars. There are a few cases, however, which can not be explained merely by this effect (Kobashi et al., 1997; Mills et al., 1996; Yakushiji et al., 1998). We reported previously that total soluble solids (TSS) markedly increased in ‘Kansuke Hakuto’ peaches (*Prunus persica*) grown under water stress conditions without a reduction in fruit size. This was paralleled by an increase in ABA content (Kobashi et al., 1997). These results indicate the possibility of the involvement of water-stress-induced ABA in enhancement of sugar accumulation in peaches.

It has been demonstrated that sugar accumulation in sink organs, including fruit, is stimulated by application of some plant hormones including ABA (Brenner et al., 1989; Daie, 1985). We observed previously that an exogenous ABA treatment increased sugar content in peaches, with a simultaneous rise in the activity of sorbitol oxidase (SOX), which metabolizes sorbitol, a translocating sugar, to glucose (Kobashi et al., 1999a). These results indicate that ABA enhances sugar accumulation by activating sorbitol metabolism in peaches. Assuming that the water-stress-induced ABA acts on sugar accumulation, an activation in sorbitol metabolism should be observed also under water-stress conditions.

The objective of the present study was to demonstrate the relationship between the activity of sugar metabolizing enzymes, fructose, glucose, sorbitol, sucrose, and ABA content in peaches subjected to water stress. In peaches, sucrose accumulates predominantly during fruit maturation through the activity of sucrose synthase (SS) rather than sucrose phosphate synthase (SPS) (Kobashi et al., 1999a; Moriguchi et al., 1990). Translocated sorbitol in peaches is catalyzed by SOX, rather than NAD^+ -dependent sorbitol dehydrogenase (SDH). Therefore, we investigated the activities of these four enzymes (SS, SPS, SOX, and SDH), along with acid invertase, which acts on sucrose degradation and is involved in the regulation of sucrose accumulation in sink tissues (Ackerson, 1985; Poovaiah and Veluthambi, 1985). In addition, the photosynthetic rate of individual leaves was measured during stress treatment.

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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. Trees in one group 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 d 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 (DIK-7000, Fujiwara Co.) 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 $\approx 1600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. 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 fruit.

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 polyvinylpyrrolidone (PVPP). The extract was evaporated in vacuo at 40°C to water phase, adjusted to pH 2.5 with $0.1 \text{ mol}\cdot\text{L}^{-1}$ 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

Fig. 1. Effect of water stress treatment on leaf water potential measured just before daybreak. Water stress treatment was conducted from 8 July (80 d after full bloom) to 24 July. Each point represents the mean of seven replications. Vertical bars = SE.

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- μ m 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 Uthairutra 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 % pentaerythritol 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 redissolved 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 (UDPG), 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 M 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 (μ mol 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). ABA content was significantly higher in the water-stressed fruit on both dates (Table 1). Photosynthetic rate and stomatal conductance measured on 18 July were strongly suppressed in the water-stressed trees, being reduced to $\approx$ 36% and 18%, respectively, as compared to the control trees (Table 2).

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 six and three replications for FW and ABA content, respectively, $\pm$ SE.

Date	Treatment	FW (g)	ABA content (ng·g ⁻¹ FW)
16 July	Control	77.2 $\pm$ 6.6	92.8 $\pm$ 16.2
	Treated	80.6 $\pm$ 4.9	232.1 $\pm$ 44.8
		NS	*
24 July	Control	119.6 $\pm$ 7.8	114.6 $\pm$ 8.0
	Treated	100.0 $\pm$ 9.8	266.9 $\pm$ 39.6
		NS	*

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 $\pm$ SE.

Treatment	Photosynthetic rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Stomatal conductance ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
Control	10.02 $\pm$ 0.73	0.28 $\pm$ 0.02
Treated	3.60 $\pm$ 0.58 ***	0.05 $\pm$ 0.01 ***

***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 the control trees on 16 July, although no significant difference was detected on 24 July. SOX activity increased in control fruit from 16 to 24 July to the same extent as induced by water stress. No effect of the stress treatment on acid invertase activity was found in either sampling dates. SDH activity was undetectable irrespective of treatment or sampling time (data not presented).

Table 3. Effects of water-stress treatment on sugar content in 'Kansuke Hakuto' peaches. Water-stress treatment was conducted from 8 July (80 d after full bloom) to 24 July. Values are means of three replications $\pm$ SE.

Date	Treatment	Sugar content ($\text{mg}\cdot\text{g}^{-1}$ fresh wt)				
		Fructose	Glucose	Sorbitol	Sucrose	Total
16 July	Control	14.1 $\pm$ 1.5	18.2 $\pm$ 2.8	12.4 $\pm$ 0.7	32.1 $\pm$ 2.8	76.7 $\pm$ 4.9
	Treated	15.8 $\pm$ 0.9 NS	16.4 $\pm$ 1.7 NS	17.7 $\pm$ 0.5 *	58.7 $\pm$ 6.5 *	108.6 $\pm$ 5.8 *
24 July	Control	13.6 $\pm$ 1.9	14.8 $\pm$ 2.9	9.7 $\pm$ 1.6	63.5 $\pm$ 13.2	101.6 $\pm$ 18.2
	Treated	15.5 $\pm$ 1.3 NS	19.7 $\pm$ 2.2 NS	12.6 $\pm$ 1.5 NS	73.2 $\pm$ 15.4 NS	120.6 $\pm$ 13.4 NS

NS,*Nonsignificant or significant at $P < 0.05$ by Student's t test (unpaired).

Table 4. Effects of water stress treatment on activities of sugar metabolizing enzymes 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 replications $\pm$ SE.

Date	Treatment	Enzyme activities ($\mu\text{mol product/h/g}$ fresh wt)			
		SS ²	SPS	SOX	INV
16 July	Control	0.990 $\pm$ 0.558	0.297 $\pm$ 0.049	0.095 $\pm$ 0.007	0.255 $\pm$ 0.041
	Treated	0.982 $\pm$ 0.151 NS	0.335 $\pm$ 0.053 NS	0.131 $\pm$ 0.004 *	0.239 $\pm$ 0.015 NS
24 July	Control	5.455 $\pm$ 2.635	0.783 $\pm$ 0.125	0.144 $\pm$ 0.021	0.207 $\pm$ 0.005
	Treated	4.450 $\pm$ 0.603 NS	0.820 $\pm$ 0.297 NS	0.130 $\pm$ 0.004 NS	0.210 $\pm$ 0.002 NS

²SS = sucrose synthase, SPS = sucrose phosphate synthase, SOX = sorbitol oxidase, and INV = acid invertase.

NS,*Nonsignificant or significant at $P < 0.05$ by Student's t test (unpaired).

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).

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 a rise in SOX activity and an increase in ABA content. These results suggest involvement of endogenous ABA in sugar accumulation under water-stress conditions.

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,

1985) and ABA (Ackerson. 1985). In plants including peach, which translocate mainly sorbitol, little is known about the relationship between sugar metabolism, sugar accumulation, plant hormones, and water stress. Results of the present and previous studies (Kobashi et al., 1999a) indicate that in peaches, the activity of SOX is stimulated by water-stress-induced ABA, perhaps causing an enhancement in sugar accumulation through the maintenance of a sorbitol concentration gradient in the same manner as invertase acts in sucrose translocation.

For the results of 16 July, however, not glucose but rather sucrose and sorbitol increased, despite the rise in only SOX activity, which converts sorbitol to glucose. It is probable that glucose, which could have been produced at a higher rate under water stress, was promptly catabolized to sucrose, since activity of SS, which synthesizes sucrose from glucose and fructose, was very high compared to those of the other sugar metabolizing enzymes in the water-stressed and control fruit. This may be the reason why sucrose rather than glucose increased in the water-stressed fruit. On the other hand, with regard to sorbitol, a sorbitol-synthesizing pathway has not been found in peaches. Therefore, the sorbitol increase in the water-stressed fruit suggests that the sorbitol influx into fruit as a sink was enhanced more than sorbitol metabolism in fruit, probably through physiological changes in organs other than fruit under water stress. Decline of 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 ^{13}C -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 ^{14}C -sucrose uptake by strawberry (*Fragaria × ananassa* 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 also have 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.

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