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Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach

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In peach (*Prunus persica* [L.] Batsch.), sorbitol and sucrose are the two main forms of photosynthetic and translocated carbon and may have different functions depending on the organ of utilization and its developmental stage. The role and interaction of sorbitol and sucrose metabolism was studied in mature leaves (source) and shoot tips (sinks) of 'Nemaguard' peach under drought stress. Plants were irrigated daily at rates of 100, 67, and 33% of evapotranspiration (ET). The relative elongation rate (RER) of growing shoots was measured daily. In mature leaves, water potential (ψ_w), osmotic potential (ψ_s), sorbitol-6-phosphate dehydrogenase (S6PDH, EC 1.1.1.200), and sucrose-phosphate synthase (SPS, EC 2.4.1.14) activities were measured weekly. Measurements of ψ_s, sorbitol dehydrogenase (SDH, 1.1.1.14), sucrose synthase (SS, EC 2.4.1.13), acid invertase (AI, EC 3.2.1.26), and neutral invertase (NI, EC 3.2.1.27) activities were taken weekly in shoot tips. Drought stress reduced RER and ψ_w of plants in proportion to water supply. Osmotic adjustment was detected by the second week of treatment in mature leaves and by the third week in shoot tips. Both SDH and S6PDH activities were reduced by drought stress within 4 days of treatment and positively correlated with overall ψ_w levels. However, only SDH activity was correlated with ψ_s. Among the sucrose enzymes, only SS was affected by drought, being reduced after 3 weeks. Sorbitol accumulation in both mature leaves and shoot tips of stressed plants was observed starting from the second week of treatment and reached up to 80% of total solutes involved in osmotic adjustment. Sucrose content was up to 8-fold lower than sorbitol content and accumulated only occasionally. We conclude that a loss of SDH activity in sinks leads to osmotic adjustment via sorbitol accumulation in peach. We propose an adaptive role of sorbitol metabolism versus a maintenance role of sucrose metabolism in peach under drought stress.

Introduction

Osmotic adjustment is the active accumulation of solutes within cells, and is typically observed in plants under drought, salt, or temperature stresses (Morgan 1984). Solute accumulation causes a decrease in cell osmotic potential, which in turn allows turgor or cell volume to be maintained or increased relative to non-stressed plants (Morgan 1984). However, it is unclear whether osmotic adjustment is a mitigating response to, or simply a consequence of, dehydrative stress, since in the majority of cases it occurs only after growth has been reduced by stress (Munns 1988). Recent studies with plants genetically altered to favor osmotic adjustment have shown that solute accumulation may result in improved tolerance to some dehydrative stresses (Tarczynski et al. 1993, Pilon-Smits et al. 1995, Karakas et al. 1997).

There is considerable interest in the types of solutes used for osmotic adjustment and where and how their accumulation occurs and is regulated. During drought stress, organic compounds such as polyols, sugars, proline, and glycine-betaine comprise the bulk of solutes used for osmotic adjust-

Abbreviations – AI, acid invertase; BSTFA, N, O-bis(trimethylsilyl)trifluoroacetamide; DMF, dimethylformamide; DTT, dithiothreitol; ET, evapotranspiration; NI, neutral invertase; PVPP, polyvinylpolypyrrolidone; RER, relative elongation rate; SDH, sorbitol dehydrogenase; S6PDH, sorbitol-6-phosphate dehydrogenase; SPS, sucrose-phosphate synthase; SS, sucrose synthase; ψ_s, osmotic potential; ψ_w, water potential.

ment (Wyn Jones 1984). In Rosaceous tree fruits, such as apple (*Malus domestica* [L.] Borkh.) (Wang and Stutte 1992) and cherry (*Prunus cerasus* L. and *P. avium* × *pseudocerasus*) (Ranney et al. 1991), osmotic adjustment during drought stress is facilitated mainly by sorbitol accumulation. Sorbitol or other polyols may function as compatible solutes in transgenic tobacco (Sheveleva et al. 1997), *Plantago* spp. (Gorham et al. 1981, Briens and Larher 1983), *Hedera helix* L. (Moore et al. 1997), and members of the Oleaceae family (Gucci et al. 1997, Guicherd et al. 1997, Peltier et al. 1997). It is important to note that several functions of polyols other than osmotic adjustment, such as translocation and storage of carbon, cryoprotection, prevention of activated oxygen species, boron transport, and energy delivery, have been proposed (Brown and Hu 1996, Escobar-Gutiérrez and Gaudillère 1996).

Our previous work has shown that sorbitol and sucrose may play different roles in the growth and development of peach (Lo Bianco et al. 1999a,b). Specifically, sorbitol dehydrogenase (SDH, 1.1.1.14), the catabolic enzyme in sink tissues, was most active in shoot and root apices, whereas acid invertase (AI, EC 3.2.1.26) and sucrose synthase (SS, EC 2.4.1.13), which cleave sucrose in sinks, were more active than SDH in developing peach fruits. Since sorbitol generally accumulates to a greater extent than sucrose during stress in Rosaceous tree crops (Ranney et al. 1991, Wang and Stutte 1992), it is possible that these enzymes are differentially regulated during stress.

Little is known about the response of sucrose or sorbitol metabolism during stress at the sink site. In tomato (*Lycopersicon esculentum* [L.] Mill.) fruits, SS activity increased in relation to salinity stress intensity, starch accumulation, and sucrose depletion (Balibrea et al. 1996). In contrast, SS declined dramatically in activity and in content within a few days of withholding water in root nodules of soybean (*Glycine max* L.) (Gonzalez et al. 1995). The activity of some sucrose catabolic enzymes, namely invertase, SS, and pyrophosphate-dependent phosphofructokinase, are sensitive to transplanting or low temperature stresses in sinks (Purvis and Rice 1983, Sung et al. 1993).

The synthesis of sorbitol and sucrose has been studied in source leaves of peach under drought stress (Escobar-Gutiérrez et al. 1998). The activity of sorbitol-6-phosphate dehydrogenase (S6PDH, EC 1.1.1.200), the main enzyme of sorbitol synthesis, increased during drought stress, causing sorbitol accumulation in the phloem, whereas the activity of sucrose-phosphate synthase (SPS, EC 2.4.1.14), which catalyzes sucrose synthesis, was not affected by drought. However, neither sorbitol nor sucrose metabolism was investigated in sinks in this study, thus it remains unclear whether an up-regulation of sorbitol synthesis at the source and/or a down-regulation of sorbitol utilization in the sink result in sorbitol accumulation and osmotic adjustment during drought. There is some evidence of SPS activation in spinach (*Spinacia oleracea* L.) leaves (Quick et al. 1989) and potato (*Solanum tuberosum* L.) tubers (Reimholz et al. 1994) incubated in hyperosmotic solutions of mannitol or sorbitol. Hence, it is possible that sucrose synthesis in leaves can be affected by sorbitol accumulation in Rosaceous tree fruits.

The objectives of this work were to study the influence of drought stress on sorbitol and sucrose metabolism in mature leaves (source) and shoot apices (sink) of peach to determine which enzymatic events lead to their accumulation and contribute to osmotic adjustment under drought conditions.

Materials and methods

Plant material and experimental plan

Fifty-four rooted cuttings of the peach cultivar 'Nemaguard' were grown in a greenhouse in Athens, GA, USA (34°N latitude and 83°40′W longitude), yielding approximately 70% integrated daily solar radiation transmission and temperatures ranging from 22 to 35°C. In October 1998, plants were transferred to 10-l containers filled with Pro-Gro 3P artificial media (70% peat moss, 30% perlite) and watered to run-off every other day until the onset of experiment. Plants were fertilized as needed with a soluble 20N-8P-12K source. Several 100 W incandescent lights, spaced about 2 m apart and 2 m above the greenhouse bench, were used to extend the photoperiod to 14–15 h and prevent premature terminal bud set. On January 6, 1999, after plants had grown for 3 months and were about 50 cm in height, plants were randomly separated into 3 groups of 18, each receiving different amounts of water. Beginning on January 7, 8 plants from the well-watered group were weighed each morning on a Mettler Toledo (Greifensee, Switzerland) 32000 balance to the nearest gram to determine the daily evapotranspiration rate (ET [g plant^{-1} day^{-1}]). Water was supplied daily to the plants in the other two groups at rates of 67% of ET (mild stress) and 33% of ET (severe stress); well-watered plants were irrigated to the point of run-off daily. In mild and severe stress groups, water was supplied to plastic saucers beneath the containers to assure that water reached growing roots. After 4 weeks of treatment, plants from all groups were watered until run-off every day for 1 week. All plants were supplied with soluble fertilizer in the irrigation water twice a week during and following the treatment period.

Two mature leaves at the 5th visible node from the apex and 2–3 shoot tips (apex plus surrounding leaflets < 4 cm long) were collected weekly from 3 plants in each treatment, for a total of 9 plants sampled every week. The 3 plants per treatment sampled were chosen based on having representative leaf water potential within a given treatment. Sampling was conducted always between 10:00 and 11:00 h. Samples were transported quickly from the greenhouse to the laboratory in plastic bags on ice and then rinsed in distilled water and blot dried. One shoot tip per plant was assayed immediately for SDH, since the enzyme is not stable in frozen tissues (Lo Bianco et al. 1998). Half of the remaining samples were frozen at −20°C for subsequent determination of other enzyme activities (temperature stable), whereas the other half were rehydrated with deionized water for 3 h and then stored at −20°C for subsequent determination of osmotic potential and carbohydrate content.

Growth analysis

One shoot per plant was labeled and its actively growing portion (terminal 3–5 internodes) was measured in length daily throughout the experiment. A shoot relative elonga-

tion rate (RER) in mm mm^{-1} day^{-1} was obtained according to the following formula:

$$RER = (L_2 - L_1)/[(T_2 - T_1) \times L_1]$$

where L is length and T is time in days.

Drought measurements

Eight plants of the well-watered group were weighed every day before and after watering to calculate the average daily ET of non-stressed plants (total water lost by plant transpiration and soil evaporation). Leaf water potential (ψ_w) was measured weekly in the same plants sampled for enzyme assays using a pressure chamber; measurements were conducted at 10:00 and 11:00 h eastern time. Osmotic potential at full turgor (ψ_s) was measured on each rehydrated shoot tip and mature leaf using a thermocouple psychrometer (Decagon SCA-10; Decagon Devices, Pullman, WA, USA). NaCl standards covering the range of expected ψ_s were included with each run of samples (usually 6), and a regression developed from these standards was used to calculate the water potentials of the samples. Since the freezing process destroys membranes and eliminates turgor, the water potential equaled ψ_s. It was assumed that the dilution of cell sap by apoplastic water was similar for all treatments.

An average water content ratio (WCR) for shoot tips and mature leaves was calculated as follows:

$$WCR = (SW - DW)/SW$$

where SW is the fresh weight at full turgor and DW the dry weight of the same samples. The WCR was then used to convert sugar content (mg g^{-1} rehydrated tissue) to concentration (mol l^{-1}), and ψ_s due to sorbitol or sucrose at 25°C was determined according to the van't Hoff relation:

$$\psi_s\ (MPa) = M \cdot R \cdot T$$

where M is the concentration in mol l^{-1}, T is temperature in K (298), and R is the gas constant (0.00821 l MPa mol^{-1} K^{-1}).

Sorbitol and non-structural carbohydrates

A single shoot tip (0.08–0.14 g of tissue rehydrated to full turgor) or about 0.2 g of mature leaf (rehydrated to full turgor) were ground in a mortar and pestle with respectively 1.5 or 3 ml of 80% (v/v) methanol containing 2.2 mg ml^{-1} of phenyl-β-D-glucopyranose as an internal standard. After homogenization, samples were centrifuged for 3 min at 3900 g_n and 1 ml of the supernatant was stored at 4°C for soluble carbohydrate and sorbitol determination. The pellet was washed with 80% methanol and centrifuged twice. The supernatant was discarded, 3 ml of deionized water was added, and tubes were placed in a water bath at 100°C for 1 h to gelatinize the starch. One ml of deionized water, 1 ml of acetate buffer (pH 4.8), and 0.1 ml of amyloglucosidase enzyme solution (7 U) were added and tubes were placed in water bath at 55°C for 24 h. Tubes were then centrifuged and 1 ml of the supernatant was stored at −20°C for starch determination.

Samples were prepared for sugar quantification by drying 0.1 ml extract in 200-µl GC vials at 40°C and adding 0.07 ml of N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and dimethylformamide (DMF) (1:1, v:v). Vials were then capped, vortexed, and ultrasonicated for 15 min. Derivatization was completed by heating vials at 75°C for 1.5 h. Soluble carbohydrates were quantified with a Hewlett Packard HP 5940 gas chromatograph (Avondale, PA, USA), using a DB5 column (30 m length, 0.3 mm inner diameter, 0.25 mm film thickness).

Starch content was determined by measuring enzymatically the amount of glucose formed (modified from Chaplin 1994). Hexokinase (1 U) and *Leuconostoc* glucose-6-phosphate dehydrogenase (1 U) were used in the presence of ATP and NAD and the total change in optical density was measured on a Spectronic 21-D (Milton Roy, Rochester, NY, USA) spectrophotometer.

Enzyme assays

Enzymes of sorbitol and sucrose synthesis (S6PDH and SPS) where extracted only from source leaves (1 leaf in 6 ml of buffer), whereas enzymes of sorbitol and sucrose catabolism (SDH, SS, AI, and neutral invertase) were extracted only from shoot tips (1 shoot tip in 4 ml of buffer). SDH was extracted and assayed following the protocol of Lo Bianco et al. (1998). Tissues were homogenized in 0.1 *M* Tris buffer (pH 9 at 25°C) containing 8% (v/v) glycerol, with 2-mercaptoethanol (20 m*M*) added immediately prior to each extraction since it was unstable in stored buffer. Tween 20 (0.1%, v/v) and polyvinylpolypyrrolidone (PVPP; 1%, w/v) were added during grinding. Sucrose synthase, SPS, S6PDH, and the soluble fraction of AI and neutral invertase (NI, EC 3.2.1.27) were extracted using 0.2 *M* HEPES/NaOH buffer (pH 7.5 at 25°C) containing 10 m*M* dithiothreitol (DTT), 3 m*M* Mg-acetate, and 6% (v/v) glycerol; 0.1% (v/v) Tween 20 and 1% (w/v) PVPP were added during grinding. In all cases, the tissue was ground in buffer and sand using a pre-cooled (2–4°C) mortar and pestle. The homogenate was filtered and centrifuged at 3000 g_n for 15 min and the supernatant was desalted with Sephadex G-25 (medium) columns at 4°C.

SDH was assayed using 0.1 ml of desalted extract, 0.1 *M* Tris buffer (pH 9.5 at 25°C), 1 m*M* NAD^+, and 300 m*M* sorbitol in 1 ml final volume (Lo Bianco et al. 1998). S6PDH was assayed using 0.05 ml of desalted extract, 0.1 *M* Tris buffer (pH 9 at 25°C), 0.11 m*M* NADPH, and 50 m*M* glucose-6-phosphate in 1 ml final volume (Negm and Loescher 1981). Sucrose synthase, AI, and NI were assayed as described in Xu et al. (1989). Briefly, SS was assayed by measuring the continuous change in optical density at 340 nm at 25°C on a Spectronic 21-D spectrophotometer using 100 m*M* sucrose, 0.5 m*M* UDP, and 1 m*M* PPi as substrates and phosphoglucomutase (1 U), *Leuconostoc* glucose-6-phosphate dehydrogenase (1 U), and endogenous UDP-glucopyrophosphorylase as coupling enzymes. UDP-glucopyrophosphorylase activity was always at least 100 times

greater than SS activity in the same tissues, and thus did not limit the apparent SS activity. AI and NI were assayed with 25 m*M* sucrose at pH 5.0 and 100 m*M* sucrose at pH 7.0, respectively. After a 15-min incubation at 25°C, the reaction was stopped by boiling for 10 min. The AI reaction mixtures were neutralized before boiling. The glucose formed was then measured using hexokinase (1 U) and *Leuconostoc* glucose-6-phosphate dehydrogenase (1 U) in the presence of ATP and NAD.

SPS was assayed with 5 m*M* fructose-6-phosphate, 15 m*M* glucose-6-phosphate, and 10 m*M* UDP-glucose (V_{max} assay) or with 3 m*M* fructose-6-phosphate, 9 m*M* glucose-6-phosphate, 10 m*M* potassium phosphate, and 10 m*M* UDP-glucose (selective assay) at pH 7.5 (Huber and Huber 1991). After a 15-min incubation at 25°C, the reaction was stopped by adding 0.07 ml of 30% KOH and boiling for 10 min. The sucrose formed was measured with the anthrone method (van Handel 1968). Protein content was determined by the method of Bradford (1976). Enzyme specific activity was expressed as nanomoles of NADH, NADP, or sucrose produced per minute per milligram of protein.

Statistical analysis

Sigma Stat procedures (SPSS Inc., Chicago, IL, USA) were used to determine simple correlation coefficients, standard errors, and differences among means of treatments. Tukey's studentized comparison was used to separate means. Correlation coefficients reported in text are all significant at $P \leq 0.05$.

Results

Influence of drought treatments on ψ_w, ψ_s, and RER

Evapotranspiration varied greatly from day to day due to variation in cloudiness and temperature (Fig. 1A). Sharp reductions in ET were often followed 1–2 days later by parallel reductions in RER (Fig. 1B). The average RER was similar for the 3 treatments for the first week until January 14 (Fig. 1B). Starting on January 15, RER of well-watered plants differed significantly from plants in the 33% ET treatment, and from January 22 to February 1, RER was higher in well-watered plants than in plants of either stress treatment. RER of 67% ET plants returned to the same level of control plants 1 day after rewatering, whereas RER of 33% ET plants remained low even after rewatering (Fig. 1B). Due to limited growth of the 33% ET plants after rewatering, only measurements for RER were made for these plants.

Plants watered at 33% ET exhibited significantly lower ψ_w than well-watered plants on all 4 sampling dates during the drought period (Fig. 2A). Alternatively, ψ_w of plants watered at 67% ET was similar to that of well-watered plants during the first 2 weeks of the drought period, diverging only after January 18. Water potentials were similar in well-watered and 67% ET plants following rewatering.

Osmotic adjustment was detected in both mature leaves and shoot tips of stressed plants beginning 2 weeks after drought treatments were imposed (Fig. 2B,C). Mature leaves adjusted osmotically 1 week earlier than shoot tips, and had lower ψ_s than shoot tips throughout the experiment. The maximum difference in ψ_s between well-watered and stressed plants (i.e., the maximum osmotic adjustment) was about 0.5 and 0.6 MPa for mature leaves and shoot tips, respectively. Osmotic potentials for mature leaves and shoot tips were similar between well-watered and 67% ET plants on the final day of the drought period (February 1), perhaps due to the low ET conditions and relatively high ψ_w on this date. However, ψ_s of 33% ET plants remained below those of well-watered plants even on this date.

Metabolism of sorbitol and sucrose

Both S6PDH in mature leaves and SDH in shoot tips were affected by drought stress within 4 days of treatment (Fig. 3). Both stress treatments reduced SDH activity relative to well-watered plants on all sampling dates during the drought period with the exception of the February 1 date for the 67% ET treatment. Alternatively, the activity of S6PDH was reduced by stress on 3 of 4 dates for the 33% ET plants, but only 1 of 4 dates for the 67% ET plants. One week after rewatering, activities of both S6PDH and SDH returned to well-watered levels for the 67% ET treatment.

The activity of both sorbitol enzymes were positively correlated to ψ_w when data from all sampling dates were pooled ($r = 0.493$ and 0.653 for S6PDH and SDH, respectively). However, only SDH activity showed an overall

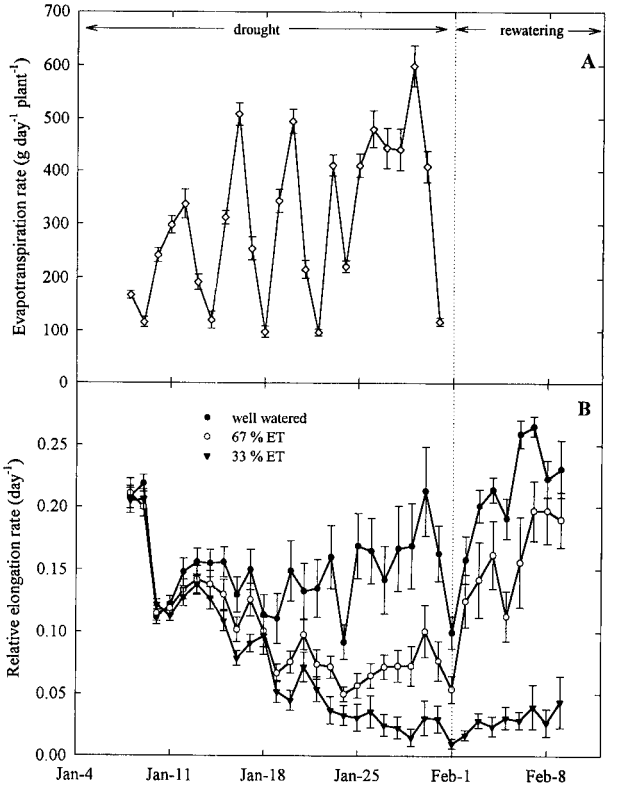

Fig. 1. Average ET rate of well-watered plants (A) and RER of 'Nemagurd' peach shoots (B). Error bars represent standard errors of the means.

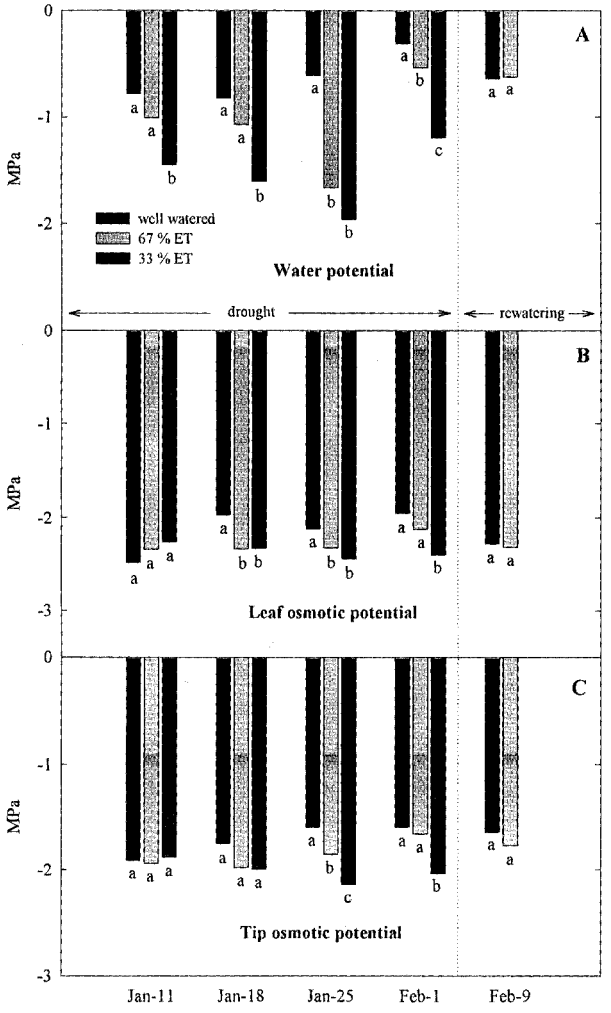

Fig. 2. Leaf water potentials (A), leaf osmotic potentials (B), and shoot tip osmotic potentials (C) of 'Nemaguard' peach under 3 different drought treatments. Different letters on top of bars indicate significant differences among treatments for each sampling date ($P \leq 0.05$).

positive correlation with ψ_s ($r = 0.627$), suggesting that SDH activity decreased as solutes accumulated.

Shoot tip SS activity responded to drought stress after 3 weeks of treatment (Fig. 4A). However, SS activity and ψ_w were positively correlated when data from all dates were pooled together ($r = 0.647$). The activity of AI, NI, and SPS did not show any significant overall correlation with ψ_w, and showed only occasional weekly differences among drought treatments (Figs. 4B,C and 5). Activities of all sucrose enzymes in 67% ET plants were similar to well-watered plants after rewatering, with the exception of higher activities for SPS under limiting substrate conditions for previously stressed plants (Figs. 4 and 5).

Sugar content

Sorbitol accumulation in both mature leaves and shoot tips of stressed plants was observed starting from the second week of drought treatment (Figs. 6A and 7A). Sorbitol content also exhibited an overall inverse correlation with ψ_w and ψ_s in shoot tips ($r = -0.795$ and -0.677, respectively) and mature leaves ($r = -0.849$ and -0.665, respectively). The contribution of sorbitol to osmotic adjustment generally ranged from 63 to 80% in mature leaves and from 32 to 76% in shoot tips, except on January 18 for the 67% ET plants, where it was only 23–27%. Sorbitol content was also inversely related to SDH activity in shoot tips ($r = -0.475$).

Sucrose content was 4–8 times lower than sorbitol in mature leaves and 1–7 times lower than sorbitol in shoot tips (Figs. 6A,B and 7A,B). Sucrose accumulated in stressed plants only occasionally: at the second sampling date in shoot tips and at the end of the drought period in mature leaves (Figs. 6B and 7B). The contribution of sucrose to osmotic adjustment was never greater than 12% in either mature leaves or shoot tips. Starch content varied greatly, and only on January 25 accumulated in shoot tips of 33% ET plants (Fig. 7C). Starch content of shoot tips also correlated positively with SS activity ($r = 0.537$). Fructose and glucose content did not show any specific response to drought stress (data not shown).

Discussion

As expected, relatively long-term drought stress reduced stem elongation and elicited osmotic adjustment in 'Nemaguard' peach, in agreement with previous observations

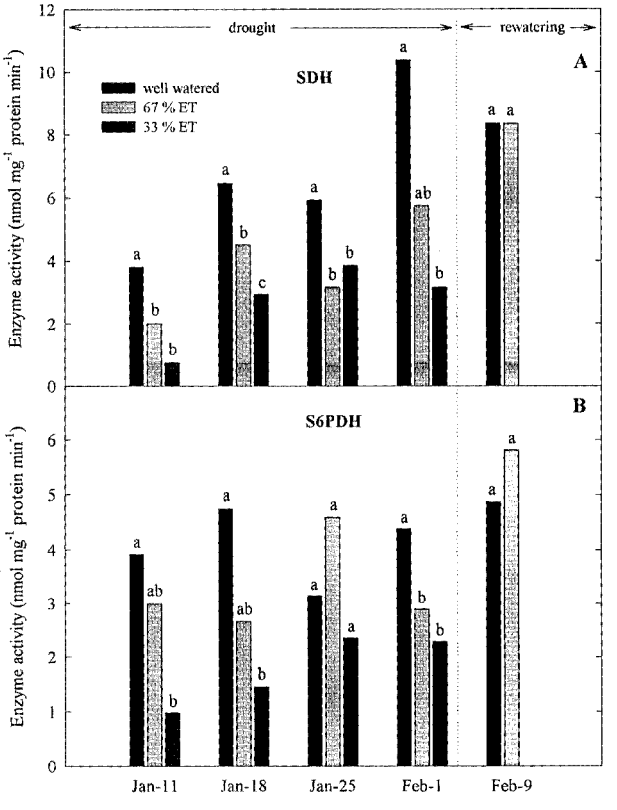

Fig. 3. Activity of SDH in shoot tips (A) and S6PDH in mature leaves (B) of 'Nemaguard' peach under 3 different drought treatments. Different letters on top of bars indicate significant differences among treatments for each sampling date ($P \leq 0.05$).

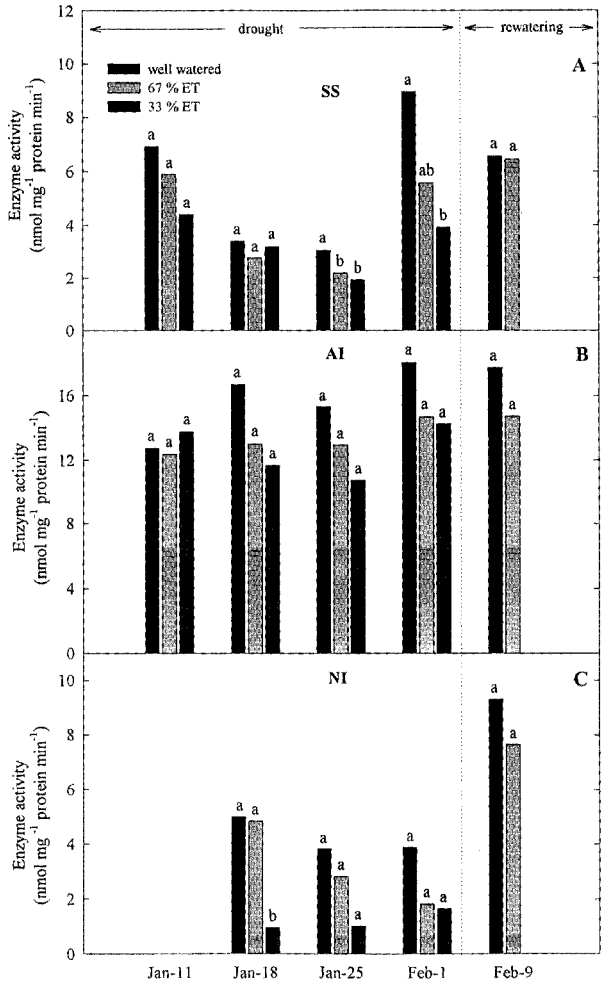

Fig. 4. Activity of SS (A), AI (B), and NI (C) in shoot tips of 'Nemaguard' peach under 3 different drought treatments. Different letters on top of bars indicate significant differences among treatments for each sampling date ($P \leq 0.05$).

(Rieger 1992). Osmotic adjustment occurred in both 67% ET and 33% ET treatments, but only plants receiving water at 67% ET were able to resume growth after drought release (Fig. 1). The 33% ET plants that had experienced more severe stress may have undergone developmental changes resulting in the onset of dormancy (Seeley 1990), or alternatively, 1 week of rehydration may have been insufficient to restore active growth to these plants.

Drought stress affected the in vitro activity of SDH and S6PDH and sorbitol contents in both sinks and source leaves (Figs. 3, 6 and 7). In plants of both stress treatments, decreases in SDH activities were observed (by January 11) before sorbitol accumulation became significant (by January 18 for 33% ET plants and by January 25 for 67% ET plants). Hence, it is likely that the accumulation of sorbitol in the shoot tips resulted from reduced sorbitol utilization relative to import. This reduction in utilization and consequent accumulation of sorbitol in sinks would eventually lead to a reduced export from (and subsequent accumulation in) mature leaves. Furthermore, since sorbitol accumulation in source leaves was accompanied (67% ET plants) or preceded (33% ET plants) by reduction in S6PDH activity, it is possible that sorbitol export from the source was impeded to a relatively greater extent than sorbitol synthesis. The fact that a decrease in SDH activity preceded a significant decrease in growth reinforces the results of our previous works – that SDH activity can be used as a measure of sink strength in vegetative sinks of peach (Lo Bianco et al. 1999a,b).

As a result of reduced utilization and translocation, sorbitol accumulated and contributed to osmotic adjustment of both sources and sinks. However, in mature leaves, a significant decrease in ψ_s was observed 1 week earlier than in shoot tips. This could be due to the availability of osmotica other than sorbitol in sources prior to sinks. Osmotic adjustment was detected in shoot tips of 33% ET plants by January 25 (Fig. 2C), some days after RER was reduced by drought stress (January 15, Fig. 1B). This is consistent with the idea that osmotic adjustment is a consequence of reduced growth during stress (Munns 1988). However, due to the weekly interval of osmotic potential measurements, we are not able to establish which event, osmotic adjustment (between January 18 and 25) or decline in RER (January 22), occurred first in shoot tips of 67% ET plants.

In a related study, Escobar-Gutiérrez et al. (1998) did not detect osmotic adjustment in leaves of GF 305 peach seedlings under drought stress. They also observed a linear

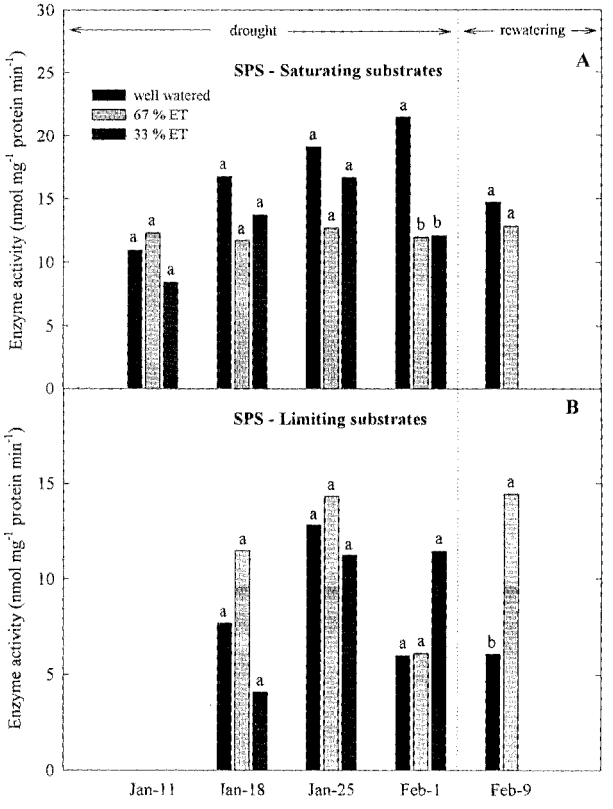

Fig. 5. Activity of SPS assayed with saturating substrates (A) and SPS assayed with limiting substrates (B) in mature leaves of 'Nemaguard' peach under 3 different drought treatments. Different letters on top of bars indicate significant differences among treatments for each sampling date ($P \leq 0.05$).

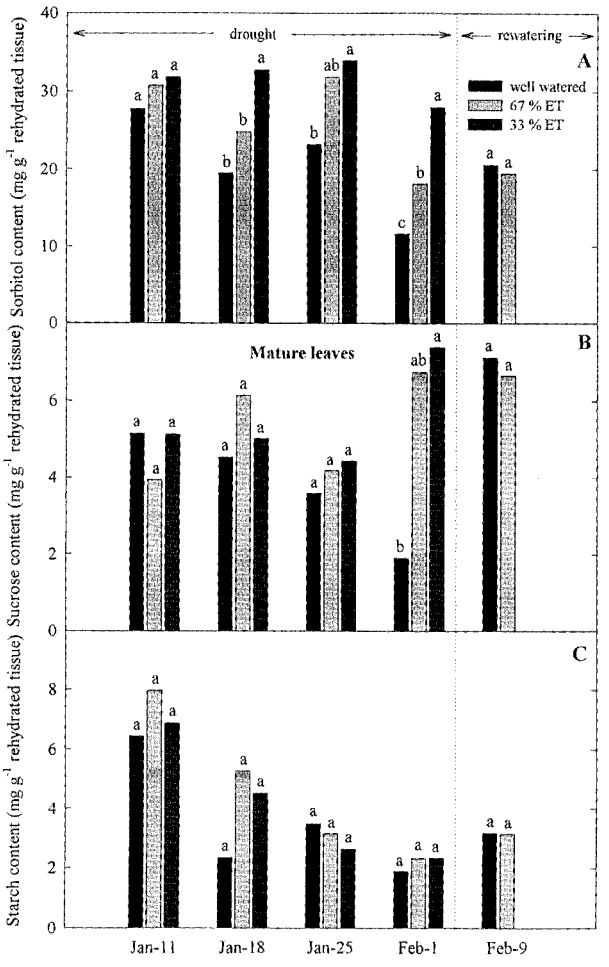

Fig. 6. Sorbitol (A), sucrose (B), and starch (C) content in mature leaves of 'Nemaguard' peach under 3 different drought treatments. Different letters on top of bars indicate significant differences among treatments for each sampling date ($P \leq 0.05$).

increase in S6PDH activity with drought stress and a significant accumulation of sorbitol in phloem sap of severely stressed plants. They concluded that an up-regulation in sorbitol synthesis allowed sorbitol to accumulate in the phloem. Our study, in fact, shows the opposite – osmotic adjustment occurs in both source leaves and sinks, with sorbitol accumulation accounting for the majority of solutes involved. This results primarily from a down-regulation of sorbitol utilization, not up-regulation of its synthesis, as indicated by decreases in both SDH and S6PDH activities. However, several aspects differed between their experiment and ours, including the length of the drought treatment (8 days versus 4 weeks), the method of drought imposition (complete withholding of water versus irrigation proportional to ET), and the light conditions (maximum intensity of 250 μmol m^{-2} s^{-1} versus > 1500 μmol m^{-2} s^{-1}). These factors might have been responsible for the conflicting results between the two studies.

In contrast to sorbitol, the metabolism of sucrose was only partially affected by drought. In source leaves, SPS activity under saturated assay conditions decreased only at the end of the drought treatment (Fig. 5). Previous studies have reported SPS activation in tissues incubated with hyperosmotic solutions of sorbitol (Quick et al. 1989, Reimholz et al. 1994). However, in the present study, no evidence of SPS activation under drought was found, even when the enzyme was assayed with limiting substrates. This could be due to a different compartmentation of sorbitol and SPS in vivo. In the sink, SS activity decreased late in the drought period in stressed plants. Soluble invertases instead seemed to maintain their level of activity even under severe stress conditions (Fig. 4). As a consequence, sucrose accumulated only at the end of the drought period in leaves and on only one occasion in shoot tips of 33% ET plants (Figs. 6 and 7). Relative to sorbitol, sucrose appears to play a minor role in osmotic adjustment during drought stress.

The positive correlation between SS activity and starch content in sinks of stressed plants has been reported previously. In tomato fruits under salinity stress, SS activity was positively correlated with starch content, implying some sort of involvement of SS in storage metabolism (Balibrea et al. 1996). However, SS activity increased with intensity of salt stress in those organs, unlike our study where SS activity

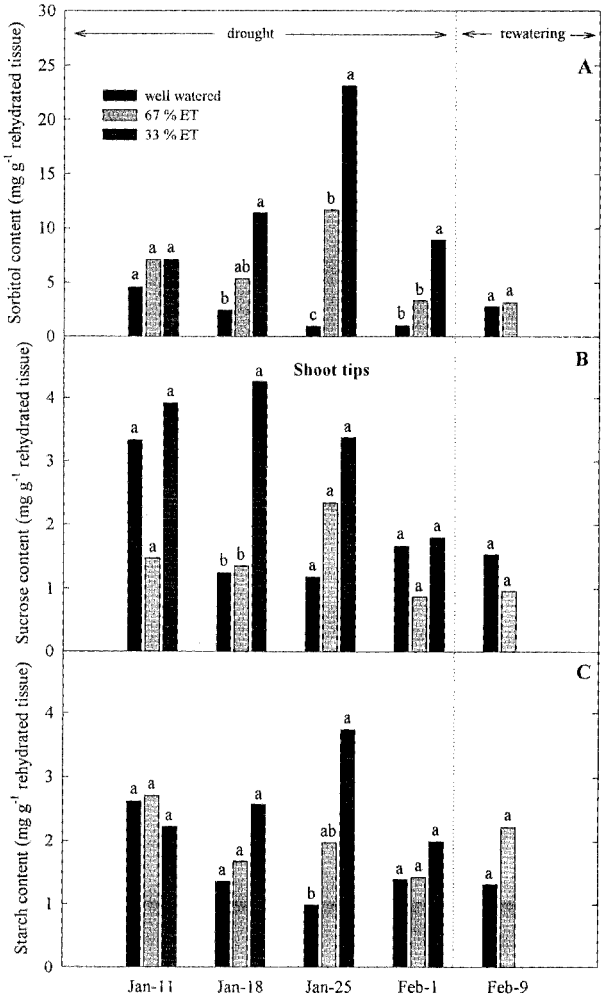

Fig. 7. Sorbitol (A), sucrose (B), and starch (C) content in shoot tips of 'Nemaguard' peach under 3 different drought treatments. Different letters on top of bars indicate significant differences among treatments for each sampling date ($P \leq 0.05$).

was reduced by stress. Also, SS activity has been associated with temporary accumulation of starch in apple fruit (Berüter et al. 1997) and in tomato fruits (Wang et al. 1993).

We suggest that in species like peach, which produce and translocate sorbitol as well as sucrose, the two forms of photosynthetic carbon may accomplish different functions under drought stress. Under normal conditions, both sorbitol and sucrose seem to participate in different ways to growth metabolism and apparently only sucrose to maintenance metabolism (Lo Bianco et al. 1999a,b). Under drought stress, sorbitol utilization in sinks, as indicated by SDH activity, drops sharply (to 25–35% of well-watered plants in this study), and sorbitol accumulates in sinks and sources. The accumulation of sorbitol results in osmotic adjustment, with sorbitol accounting for up to 80% of solutes involved. In contrast, sucrose metabolism is only marginally reduced, and may therefore support maintenance activities and some growth during drought.

We conclude that metabolism of sorbitol is more sensitive to drought stress than that of sucrose, and the loss of SDH activity in sinks during drought, not increased S6PDH synthesis in the source leaves, leads to osmotic adjustment via sorbitol accumulation.

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