Osmotic Response of Sugar Beet Source Leaves at CO₂ Compensation Point

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ABSTRACT

As sugar beet source leaves lowered the CO₂ concentration to compensation point in a closed atmosphere, leaf thickness and relative water content decreased. Leaf water potential declined rapidly from −0.5 to −1.4 megapascals. At 340 microliters CO₂ per liter, water potential and sucrose, glucose, and fructose contents were steady in photosynthesizing source leaves. Within 90 minutes after leaves were exposed to a CO₂ concentration at the compensation point, leaf sucrose content declined to 60% of the pretreatment level, rapidly in the first 30 minutes and then more slowly. During the subsequent 200 minutes, sucrose content increased to 180% of pretreatment level. Glucose and fructose remained unchanged during the treatment. Degradation of starch was sufficient to account for the additional sucrose that accumulated. Labeled carbon lost from starch appeared in sucrose and several other compounds that likely contributed to the recovery in leaf water content.

Conservation of water during periods of water deficit has survival value for the plant. The role of stomatal conductance in this regard has received considerable attention (2, 9, 10). However, increased stomatal resistance not only lowers efflux of water but also influx of CO₂, thereby limiting productivity (1, 13, 15). On the other hand, reduction of Ψₛ by increasing solute concentration allows continued gas exchange while conserving water under stress conditions. When water stress is induced slowly, solute concentration of leaves increases and the Ψₛ, which induces stomatal closure is lower (14, 20).

A small amount of osmotic adjustment occurs passively as cell volume is reduced by water loss (10). The resulting increase in solute content lowers Ψₛ. Additional osmotic adjustment may occur by metabolically increased solute content of leaves. Commonly, compounds of low mol wt such as sugars, proline, and organic acids accumulate. The onset of increased solute content of leaves implies altered partitioning and utilization of carbohydrates. While studying the effects of low CO₂ levels on the carbohydrate status of sugar beet source leaves (5), it became apparent that water stress induced by lowered atmospheric CO₂ influenced carbohydrate metabolism at Π (14, 21). Under these conditions Ψₛ initially declines rapidly, triggering accumulation of sucrose.

MATERIALS AND METHODS

Plant Material. Sugar beets (Beta vulgaris L., type Klein E, multigerm) were raised in sand-vermiculite-peat moss (1:1) and watered twice daily with nutrient solution (18). Plants were grown in an environmental chamber (14-h day at 24°C, 10-h night at 17°C) with a photon flux density of 340 μmol m⁻² s⁻¹ PAR at leaf blade level.

Labeling and Sampling. Two source leaves were enclosed in individual chambers through which was circulated air containing ¹³CO₂ of constant SR. Concentration of CO₂ was maintained within a narrow range centered at 340 μL L⁻¹ and labeling was carried out as previously described (6, 8). A cold trap in the labeling system lowered RH in the atmosphere around the enclosed leaf to approximately 60%. Leaves were allowed to photosynthesize under steady state conditions for 4 to 6 h to allow pools which turn over slowly to become substantially labeled. Sampling of the source leaves was begun at 0 min, the end of the labeling period and continued at 15 min intervals for 7 h. Four punches, two per leaf, with a combined area of 0.69 cm² were removed at each sampling time and analyzed for total and labeled sucrose, glucose, fructose, and starch (3). The areas sampled were chosen so that they were not isolated from major veins by previous samples. At time 120 min, 2 h after sampling began, the CO₂ concentration was lowered, either to Π by circulating air in a closed system or to 120 μL L⁻¹ by flowing a mixture of air and N₂-gas over the leaves. In the latter case O₂ was lowered to approximately 7%.

Carbohydrate Determinations. Sucrose, glucose, and fructose were assayed using a nonamplified enzymatic assay in which the formation of NADPH was measured with a spectrophotometer (12). Sucrose and fructose were first converted to glucose enzymatically. Labeled sugars were separated by TLC and measured by liquid scintillation counting. Starch was assayed as glucose following incubation with amyloglucosidase (17). Results are expressed on a DW basis to minimize the factor of water-stress-induced tissue shrinkage when reporting carbohydrate concentrations.

Water Status Determinations. Relative thickness of the source leaf was measured with a rotary position transducer (Schaevitz, R30D) and RWC of disks from these leaves was determined. RWC was calculated as RWC = ([FW – DW]/[TW – DW]) × 100. FW was determined immediately upon removal of two punches from the leaf. TW was determined following equilibration of the disks in distilled H₂O for 1 h. DW was determined following oven drying at 80°C to constant weight. RWC was measured at various degrees of water stress, induced either by subjecting the source leaves to Π or by adding PEG-8000 to the rooting medium. To relate RWC and Ψₛ, these quantities were determined for disks from plants stressed by adding PEG to the rooting medium. Ψₛ was measured with a Wescor HR-33T dew point microvoltmeter.

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1 Abbreviations: Ψₛ, leaf water potential; Π, CO₂ compensation point; RWC, relative water content; FW, fresh weight; SR, specific radioactivity; TW, turgid weight; NCE, net carbon exchange; DW, dry weight.
RESULTS AND DISCUSSION

Under conditions of constancy of illumination and CO₂ supply, the level of source leaf sucrose was steady during the day in mature sugar beet leaves (4). When switched from an atmosphere of 340 μl CO₂ L⁻¹ to Γ, source leaf sucrose declined to 65% of pretreatment level within 30 min (Fig. 1B). Over the next 60 min, source leaf sucrose slowly declined another 5%. During the remaining 200 min at Γ, source leaf sucrose increased steadily to 180% of pretreatment level. [¹⁴C]Sucrose also increased along with the sucrose. Glucose declined slightly during the treatment, and fructose remained unchanged (Fig. 1A). Depletion of sucrose from the source leaf was consistent with a two-pool model: a pool of sucrose, supporting export directly, and a pool in equilibrium with the former and able to serve as a reserve of sucrose (7). The rapid drop in sucrose when NCE declined to zero is consistent with depletion of sucrose from the mesophyll cytoplasm. The slower decrease likely resulted from export of sucrose entering the cytoplasm from the vacuole compartment.

Relation between the observed accumulation of sucrose in the source leaf and leaf water status was tested by recording changes in relative leaf thickness as a measure of the latter. Examination in this manner was possible because leaf thickness was well correlated with both RWC and Ψᵢ (Fig. 2). In the period prior to reduction of CO₂ to Γ, Ψᵢ, as measured by leaf thickness, oscillated within a narrow range of -0.4 to -0.5 MPa (Fig. 1C). During the first 180 min at Γ, Ψᵢ declined from -0.5 to -1.4 MPa. Approximately 1 h after sucrose began to accumulate after 150 min at Γ, leaf thickness increased gradually, indicating partial recovery of leaf water content. Ψᵢ had reached -1.2 MPa when observation stopped after 5.5 h at Γ. The increase in Ψᵢ coincided with accumulation of sucrose to a level above that before treatment began.

Because Γ-induced water deficit, it was not clear which factor caused accumulation of sucrose. Reducing CO₂ concentration in the atmosphere around the source leaves from 340 to 120 μl L⁻¹ markedly lowered NCE but neither changed sucrose content (Fig. 3B) nor caused water stress. Ψᵢ decreased slightly from -0.45 to -0.62 MPa during the treatment (Fig. 3C). Glucose declined slightly and fructose remained unchanged (Fig. 3A). In leaf disks from sugar beets subjected to water stress by adding PEG to the rooting medium, sucrose content increased with decreasing Ψₑ only when the latter fell below -1.0 MPa (Fig. 4). Above this value sucrose content of the disks did not vary with Ψₑ. Turner et al. (20) observed accumulation of soluble sugars only in response to water deficits below -1.0 MPa. Onset of the response only when Ψₑ fell below a threshold value indicates that accumulation of sucrose at Γ was related to water stress more than to lowered CO₂. Whether the response was triggered by turgor or water potential was not addressed.

At Γ, starch degradation is observed within 30 min and continues throughout the treatment period (Fig. 5 in Fox and Geiger [5]). Starch degradation is sufficient to account for the observed accumulation of sucrose. The loss of [¹⁴C] from starch is more than sufficient to account for the increase in [¹⁴C] in sucrose.

In the present study sucrose accounted for only a fraction of the observed osmotic adjustment. Several compounds in addition to sucrose exhibited a gain in [¹⁴C] content that coincided with the increase in sucrose (data not shown). Turner et al. (20) observed an increase in soluble sugars in sorghum and sunflower leaves. In wheat, glucose is the active osmoticum in expanding
leaves, while sucrose is the primary osmoticum in fully expanded leaves (16). Accumulation of proline is observed in many species (19); ion accumulation may also occur (11). The role of accumulation of small organic molecules is not understood clearly, but they appear to maintain the capacity for metabolic activity under water stress in some manner. Osmotic balance across the tonoplast, achieved by preferential sequestering of ions in the vacuole and organic compounds in the cytoplasm appears to allow accumulation of ions without adverse affects on cytoplasmic enzyme activities or tonoplast integrity.

Although this study focuses on partitioning of additional carbon into sucrose soon after leaf water content decreased, other osmotically active compounds may be equally or more important for osmotic adjustment. Allocation of newly fixed carbon to these compounds needs to be investigated further.

**LITERATURE CITED**