

Carbon Use Efficiency and Cell Expansion of NaCl-Adapted Tobacco Cells¹

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ABSTRACT

Carbon use efficiencies (gram cell organic dry weight accumulated per gram sugar assimilated from the medium) of unadapted and NaCl-adapted (428 millimolar) cells of tobacco (*Nicotiana tabacum* L. var Wisconsin 38) were determined to evaluate metabolic costs associated with growth and survival in a saline environment. No net increase in carbon costs was associated with salt adaptation. At low substrate levels, carbon use efficiencies of unadapted and NaCl-adapted cells were not appreciably different (0.495 and 0.422, respectively) and at higher substrate levels carbon use efficiency of NaCl-adapted cells was clearly higher than that of unadapted cells. These results indicate that a homeostasis of metabolic efficiency is established after cells have adapted to NaCl. Altered carbon availability does not cause the reduced cell volume that results from adaptation to NaCl. This does not preclude, however, the possibility that altered intracellular partitioning of carbon affects cell expansion.

Turgor reduction or loss resulting from exposure to water deficits imposed by salinity is the primary cause of initial growth reduction or cessation (20, 21). Despite the fact that turgor and growth are inextricably coupled (30), plant cells are able to respond to alterations in their osmotic environment and regain turgor by osmotic adjustment; however, inhibition of cell expansion still persists (1, 4, 6, 19, 31). The relationship between turgor and cell expansion rate and final cell volume is apparently altered by long-term exposure to osmotic stress (4, 28). Although mechanistically the basis for this alteration remains unknown, we (10-12, 28) have recently presented some hypotheses to explain this reduced growth based on changes in cell wall structure and chemistry of adapted cells.

In addition to inhibitory effects on fresh weight gain, salinity also may reduce dry weight gain of plants due to deleterious effects on cell division (32). However, the principal basis for reduction in plant dry matter accumulation may be a secondary effect of salt exposure mediated by reduced leaf cell expansion (9). Restricted leaf expansion will reduce the photosynthetic area of the plant and thereby limit carbon assimilation and dry weight gain (9, 14). The cumulative effect on total plant biomass production is determined by the extent of inhibition of leaf expansion and the ontological stage at which this inhibition occurs (5, 9).

Under osmotic stress conditions, CO₂ enrichment has been found to partially counteract inhibition of leaf expansion (22), indicating that photosynthate availability may limit growth under these conditions. Growth reduction could simply be the consequence of limited carbon assimilation due to induction of stomatal closure by water deficits (5, 22). However, reduction in carbon assimilation by osmotic stress can also result from effects on nonstomatal processes, including intracellular resistances to CO₂ movement and the light and dark reactions of photosynthesis (5, 14, 27), that might also limit growth.

Survival and growth in saline environments may impose additional energy requirements on plant cells as a result of adaptive processes such as ion transport and compartmentation, osmotic solute synthesis and protein turnover for cellular repair (16, 21, 23, 24). The diversion of carbon into these processes could lead to inadequate availability of carbon for growth. During the initial exposure of plant tissues to osmotic stresses, there was a reduction in carbon use efficiency if the osmotic stress was due to salinity, whereas, there was a small increase in carbon use efficiency if desiccation stress was imposed (17, 18, 26).

Since growth rates remain inhibited even after adaptation to salt stress has occurred it is possible that the reduced growth rate of adapted plants is related to an effect of salinity on carbon balance after adaptation. However, carbon metabolism in whole plants is complex and involves integrating partitioning and utilization of assimilate between tissues and organs. This has made it difficult to assess the energy costs associated with actual growth in an adapted state. To our knowledge there has been no assessment of carbon use efficiency of plants or plant tissues after adaptation to osmotic stress.

Cells growing in suspension culture provide a less complicated system with which to characterize carbon metabolism of plant cells growing after adaptation to salt (29). Cell suspensions can be grown heterotrophically or autotrophically so that the effects of salinity on photosynthesis can be examined separately from effects on carbon catabolism. Cell populations can be isolated which are fully adapted and are capable of tolerating concentrations of NaCl that are lethal to unadapted cells. The physiological and biochemical processes involved in salt tolerance of such cells are analogous to those utilized by cells of salt tolerant plants (1-4, 7, 21). Although cultured cells obviously are not organized into tissues, organs, and whole plant structures, they closely resemble meristematic cells, and may possess growth and metabolic properties similar

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to the heterotrophically growing, meristematic regions of whole plants where cell expansion predominates.

Our results clearly demonstrate that carbon availability *per se* is not the basis for reduced expansion of cells adapted to grow in the presence of NaCl. Further, there is no net increase in carbon costs associated with maintenance of the adapted state and growth in the saline environment.

MATERIALS AND METHODS

Cell Suspensions

The basal medium and procedures for the growth and maintenance of tobacco (*Nicotiana tabacum* L. var Wisconsin 38) batch cultures of unadapted cells and cells that are adapted to 428 mM NaCl have been described previously (1). However, casein hydrolysate was eliminated from the basal medium. NaCl-adapted cells were maintained in 428 mM NaCl for more than 100 subcultures prior to use in experiments. Cell suspensions were grown on a gyratory shaker (110 rpm) in the dark at 26°C.

Fresh, Dry, and Organic Dry Weight Determinations

Media containing 5, 10, 20, 30, 40, or 50 g L⁻¹ sucrose (250 mL of medium in 1 L Erlenmeyer flasks) were inoculated with cells at a density of 20 g fresh weight L⁻¹. Stock cultures in late logarithmic phase of growth were adjusted to a fresh weight density of about 250 g L⁻¹ immediately prior to inoculation of experimental media. For unadapted cells, this adjustment involved addition of fresh medium containing no sugar, while medium was decanted off to adjust the density of salt-adapted cells.

The procedure for harvesting cells for determination of fresh and dry weight densities throughout the growth cycle was essentially as described previously (1). The medium filtrate was collected for measurement of sucrose and reducing sugar contents. The cells were rinsed with three volumes of an isotonic NaCl rinse solution. A portion of the cells was stored at -20°C in a tared aluminum foil envelope, lyophilized, and then used for determination of dry weight. The samples were subsequently ashed in a muffle furnace (6 h at 600°C) in order to determine organic dry weights by subtraction of the inorganic content (after ashing) from the total dry weight. After ashing, the aluminum foil envelopes were sealed and stored in a desiccator to prevent water absorption by the samples. The weight of the aluminum foil envelopes was corrected for oxidation which occurred as a result of ashing.

Cell Number and Volume Determinations

Cell samples of 0.25 g fresh weight were incubated in 0.5 mL chromic acid (15% w/v) at 65°C for 30 min, vortexed vigorously, and then diluted to 10 mL. Cell number was determined using a Levy-Hausser hemacytometer, 0.2 mm chamber depth with a chamber volume of 3.2 μL. The hemacytometer chamber was filled at least twice for each cell number determination. Cell number determinations were continued until at least 90% of the counts fell within two standard deviations of the mean of a normal distribution.

Cell volume was expressed as a function of the cell water content determined by difference between the fresh and dry weights. One g of intracellular water was assumed to be equivalent to 1 mL of cell volume.

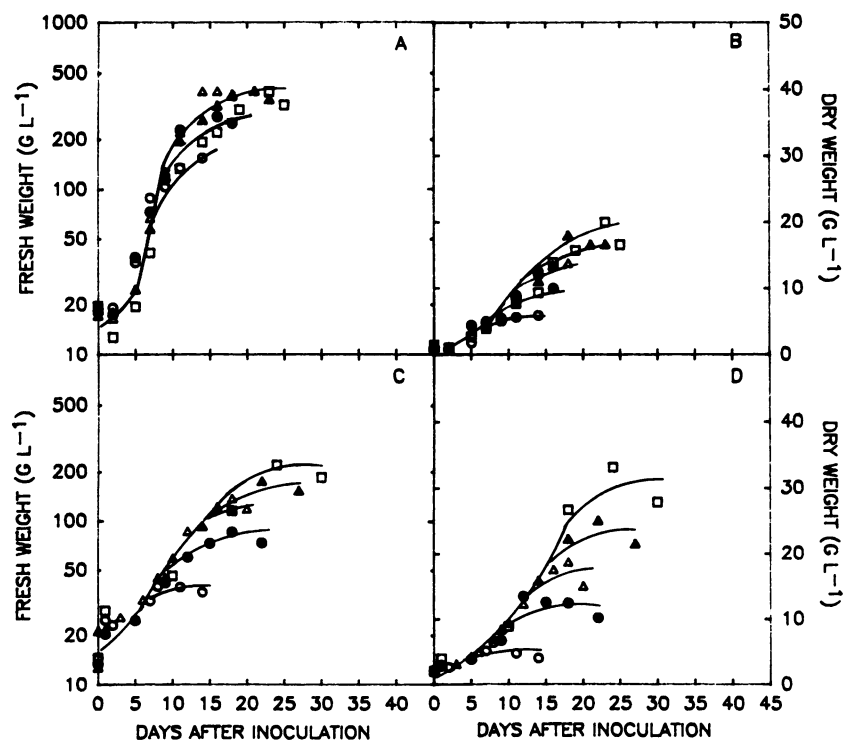


Figure 1. Fresh weight (A and C) and dry weight (B and D) growth kinetics of unadapted (A and B) and NaCl-adapted (25 g L⁻¹, C and D) cells supplied with different initial sucrose levels: 10 (○), 20 (●), 30 (△), 40 (▲), 50 (□) g L⁻¹ sucrose. Each point represents the mean of two replicate samples from a single flask.

Sugar Determinations

The concentrations of sucrose and reducing sugars in media and cell extracts were determined before and after treatment with invertase (2). Intracellular concentrations of sucrose and reducing sugars were determined after extracting lyophilized cell samples (0.1 g of dry weight) with three aliquots (5 mL) of ethanol (2). Glucose and sucrose (after treatment with invertase) were used as standards.

RESULTS

Cell Growth

The rate of fresh weight gain (post-lag phase) was markedly lower for cells adapted to 428 mM NaCl than for unadapted cells (Fig. 1, A and C) as a result of the reduced cell expansion rate exhibited by salt-adapted cells (2, 4, 11). Altering carbon substrate availability did not enhance the maximum rate of fresh weight gain, indicating that the cells were not under carbon limited growth conditions at the external concentrations of sucrose used in this research. Salt adaptation apparently has not resulted in a substantial increase in absolute carbon substrate requirement for maximal rate of cell expansion, although it is possible that carbon would limit cell expansion rate at substrate concentrations below 10 g L^{-1} sucrose (the lowest concentration of sucrose used in this study). Maximal rates of dry weight gain were equivalent for unadapted and NaCl-adapted cells (Fig. 1, B and D). Attainment of maximum dry weight coincided with depletion of reducing sugars from the medium. Both unadapted and NaCl-adapted cells rapidly inverted sucrose to fructose and glucose (data not shown), and it is probable that these reducing sugars were transported into the cells.

Fresh and dry weight maxima of unadapted and NaCl-adapted cells were dependent on the level of sugar supplied except that no further increase in fresh weight accumulation of unadapted cells occurred in medium supplemented with greater than 30 g L^{-1} sucrose (Fig. 2A). However, increased substrate availability had no effect on maximal cell volume (Fig. 3A), indicating that absolute carbon substrate supply does not limit cell size after adaptation to salinity. This does not preclude the possibility that restricted cell expansion after salt adaptation could be attributable to changes in metabolic partitioning of carbon.

Increased substrate supply enhanced total dry matter accumulation of NaCl-adapted cells to a greater extent than unadapted cells (Fig. 2B), indicating a greater efficiency for total dry matter accumulation for NaCl-adapted cells. Based on the similarities in the dry weight growth rates of unadapted and NaCl-adapted cells during the period of exponential growth (Fig. 1, B and D), cell division rates appeared unaffected by sugar availability. However, total numbers of cell divisions during a culture passage was enhanced by increasing carbon substrate supply (Fig. 3B).

Carbon Use Efficiency

Adaptation to salinity did not result in increased net carbon requirements (Fig. 4A). The difference between net carbon uptake into cells and increase in organic dry weight represents

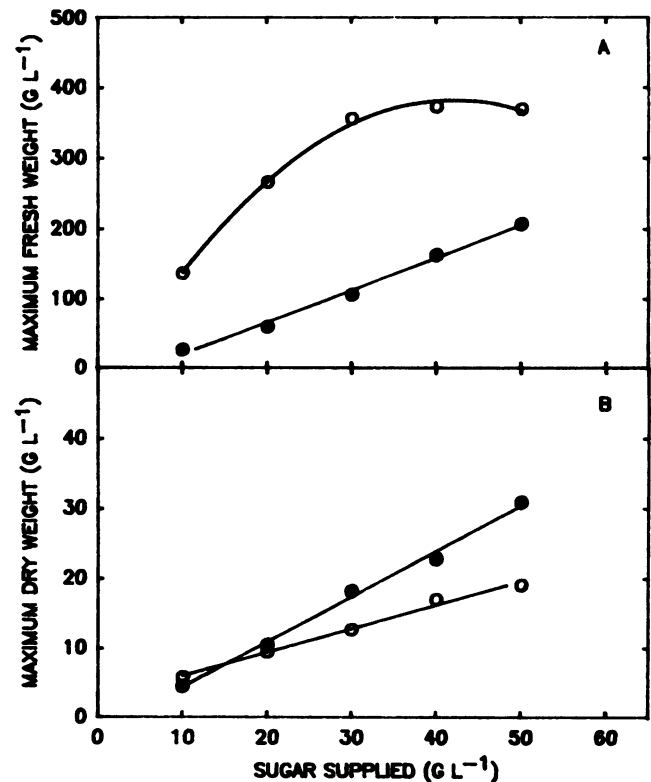


Figure 2. Maximum fresh (A) and dry (B) weight accumulation, during a culture growth cycle, of unadapted (O) and NaCl-adapted (25 g L^{-1} , ●) cells supplied with different initial sucrose levels. Each point represents the mean of two replicate samples from a single flask.

the respiration necessary to achieve biomass accumulation. At higher substrate concentrations, carbon use efficiency (organic dry weight gain/[sucrose + reducing sugars] uptake from the medium) of NaCl-adapted cells was considerably higher than that of unadapted cells. The higher carbon use efficiency exhibited by NaCl-adapted cells may be related to the reduced cell expansion since the onset of expansion in leaves has been correlated with increased respiration rates (13).

Relative to biomass accumulation, only small and comparable amounts of organic compounds were released into the medium by either unadapted or NaCl-adapted cells (10). The higher carbon use efficiency of NaCl-adapted cells relative to unadapted cells and the increased carbon use efficiency of these cells with greater substrate supply was not the result of accumulation of simple unmetabolized sugars (Fig. 4B). Approximately 3.5 and 4.0% of the maximum organic dry weight accumulated by unadapted and NaCl-adapted cells, respectively, when supplied with 40 g L^{-1} sucrose, were attributable to sucrose and reducing sugars. Intracellular levels of these sugars declined as the cells remained in the stationary growth phase, presumably being consumed to maintain cell viability.

DISCUSSION

Comparison of carbon use efficiencies of unadapted and NaCl-adapted cells does not support the hypothesis (21, 24) that there are additional metabolic costs associated with sur-

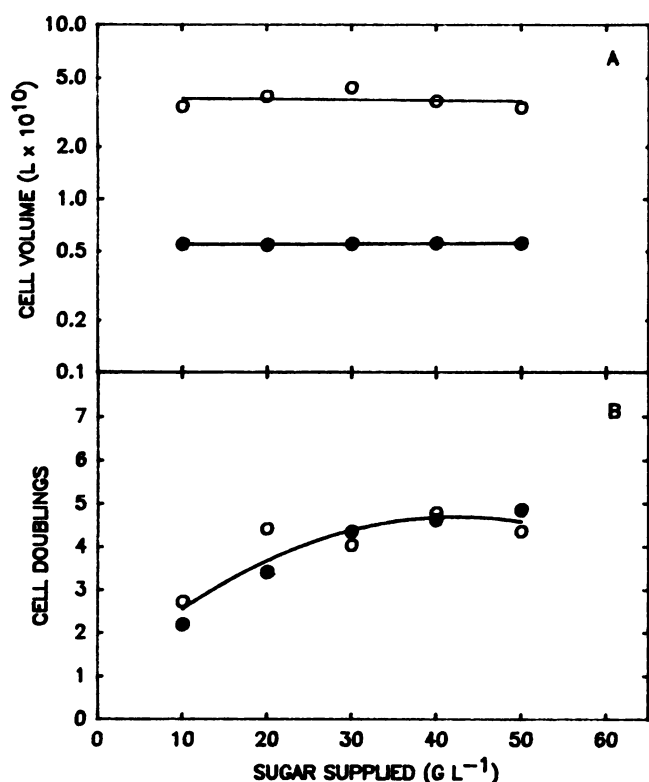


Figure 3. Maximum cell volume (A) and total number of cell doublings (B), during a culture growth cycle, of unadapted (○) and NaCl-adapted (25 g L⁻¹, ●) cells supplied with different initial sucrose levels. Cell volume was calculated as (fresh weight - dry weight)/cell number. Number of doublings was calculated as cell number doublings = $(1/\ln 2) (\ln X_{\text{final}} - \ln X_{\text{initial}})$ where X = cell number.

vival and growth in a saline environment. Carbon flux into 'metabolically expensive' cell wall components, particularly cellulose, is significantly reduced in cells adapted to osmotic stress and this has been attributed to the accumulation of more 'metabolically inexpensive' solutes necessary for osmotic adjustment (2, 8, 11). However, the enhanced carbon use efficiency of NaCl-adapted cells at higher levels of carbon input cannot be attributed solely to a reduction in cellular metabolism and thereby an accumulation of assimilated but largely unmetabolized carbon (Fig. 4). The increased carbon use efficiency of NaCl-adapted cells indicates that adaptation has led to metabolic alterations that result in increased biosynthetic efficiency under conditions of high availability of reduced carbon. Perhaps salt adaptation reduces or eliminates wasteful respiratory processes which occur during growth in nonsaline environments (15).

Drought stressed sorghum plants (*Sorghum bicolor* [L.] Moench) have reduced respiratory costs. This has been attributed to decreased biosynthesis and increased storage of assimilates linked with reduced cell expansion (11). However, carbon use efficiency decreased when sorghum plants were exposed to salt stress (26). This apparent difference from the results obtained with NaCl-adapted cells could reflect differences in the physiological and biochemical status of the genotypes analyzed. In the case of sorghum plants, carbon

use efficiency was determined over a time interval that included the sum total of the metabolic processes necessary for the plant to respond to the stress and survive, to achieve stress adaptation and to grow after adaptation. In contrast, respiratory costs of tobacco cells were determined for growth after adaptation to salinity was completed.

Our results indicate that the process of cell division and the resulting total cell number is dependent on the availability of carbon (Fig. 3B). However, the maximum cell size (Fig. 3A) and even the rate of expansion are not affected by carbon availability, but rather are dependent on water availability or the osmotic environment of the cell (4, 11). We have documented previously (1, 4) the possible reasons for a coupling between cell expansion and exposure to desiccating or saline environments. It is clear from the results presented here that carbon use efficiency or energy costs *per se* are not responsible for decreased cell expansion associated with adaptation.

Restricted cell expansion that occurs after osmotic stress adaptation may be, however, related to altered metabolic partitioning of carbon (2, 10, 11, 25). Salt adaptation results in substantial differences in the protein and carbohydrate composition of the cell walls and the turnover of certain wall components and these alterations could be directly related to reduced cell expansion (10-12).

Despite the fact that carbon use efficiency is not decreased as a consequence of salt adaptation, the reduced cell expansion

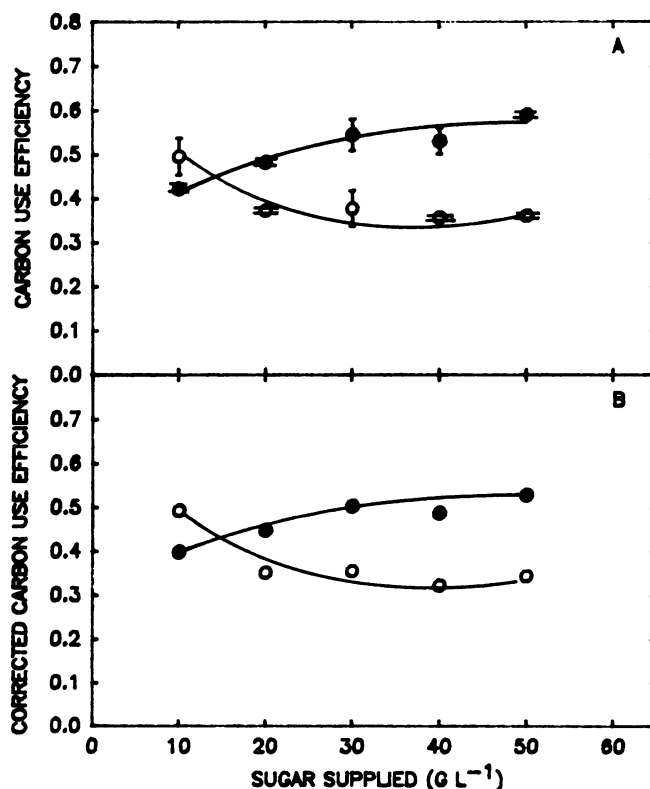


Figure 4. Carbon use efficiencies (organic dry weight gain per g sugar uptake from medium) of unadapted (○) and NaCl-adapted (25 g L⁻¹, ●) cells before \pm SE (A) and after (B) correction for intracellular sucrose + reducing sugars concentrations. Sucrose and glucose were used as standards.

that results from adaptation will limit the photosynthetic capacity of the plant and thereby limit biomass production (5, 9, 14). Therefore, it appears that reduced crop productivity resulting from exposure to stress is not directly related to carbon use efficiency or energy costs, but most likely involves the effects of altered metabolism on cell expansion.

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