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Adaptation of Tobacco Cells to NaCl[1]

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ABSTRACT

Cell lines of tobacco (*Nicotiana tabacum* L. var Wisconsin 38) were obtained which are adapted to grow in media with varying concentrations of NaCl, up to 35 grams per liter (599 millimolar). Salt-adapted cells exhibited enhanced abilities to gain both fresh and dry weight in the presence of NaCl compared to cells which were growing in medium without NaCl (unadapted cells). Tolerance of unadapted cells and cells adapted to 10 grams per liter NaCl was influenced by the stage of growth, with the highest degree of tolerance exhibited by cells in the exponential phase. Cell osmotic potential and turgor varied through the growth cycle of unadapted cells and cells at all levels of adaptation, with maximum turgor occurring at approximately the onset of exponential fresh weight accumulation.

Adaptation to NaCl led to reduced cell expansion and fresh weight gain, while dry weight gain remained unaffected. This reduction in cell expansion was not due to failure of the cells to maintain turgor since cells adapted to NaCl underwent osmotic adjustment in excess of the change in water potential caused by the addition of NaCl to the medium. Tolerance of the adapted cells, as indicated by fresh or dry weight gain, did not increase proportionately with the increase in turgor. Adaptation of these glycophytic cells to NaCl appears to involve mechanisms which result in an altered relationship between turgor and cell expansion.

To survive in a saline environment, plants must cope with water deficits resulting from lowered external water potentials to maintain turgor and grow (11, 21). Besides adjusting to osmotic stress, the plants must be able to alleviate the detrimental effects of high external concentrations of both Na^+ and Cl^- on metabolic processes such as enzyme activity, protein synthesis, nitrogen absorption and assimilation, and photosynthesis (11, 13, 34). Membrane function may be affected not only by high ionic concentrations but also by the proportions of certain ions, particularly Na^+:Ca^{2+} and Na^+:K^+ (6, 10, 22, 46). Finally, problems of dehydration and ion toxicity imposed by salinity must be overcome without severely depleting the metabolic energy available to the plant (34, 45).

Plants apparently rely on several mechanisms by which they adapt to salinity stress (11, 13, 34, 45). Many of these mechanisms utilize numerous cells and tissues in a coordinated series of processes in order to effect salinity tolerance and therefore require the anatomical organization which exists in intact plants. These include transport mechanisms which remove Na^+ and Cl^- from the xylem and redistribute the ions into the phloem for export from the roots preventing the ions from reaching actively growing cells (11, 13). Many halophytes have anatomical structures such as salt glands or bladders, or specialized trichomes which serve as repositories for accumulated salt and thus limit exposure of growing cells to NaCl (11).

Other mechanisms by which plants deal with salinity involve properties intrinsic to individual cells and include such processes as sequestering of ions into the vacuole (11, 13, 45), synthesis and accumulation of organic solutes like proline and glycinebetaine (44, 45), and active exclusion of Na^+ and Cl^- from the cell (1, 13). These cellular mechanisms are especially important to nonhalophytes which lack anatomical structures such as salt glands. Furthermore, such cellular mechanisms of salt tolerance may be more amenable to genetic manipulation than more complex mechanisms involving cell and tissue interactions or unique morphological structures (45).

In studies with plants, it is difficult to separate cellular mechanisms of tolerance from those based on the use of anatomical structures or physiological specialization requiring the cell and tissue organization which exists in the intact plant (43). The use of *in vitro* cultures, such as callus or cell suspensions, offers a means to focus only on those physiological and biochemical processes inherent to the cell which contribute to salinity tolerance. Studies utilizing cell and callus cultures indicate that correlations of salinity tolerance of a plant with that of cultured cells and tissues occur only if the tolerance of the plant is due predominantly to cellular based mechanisms (32, 36, 38, 43).

Cell lines with enhanced tolerance to NaCl have been isolated from many glycophytic species (1, 7, 9, 18, 24, 30, 31, 33, 35, 40, 44) and various physiological processes appear to contribute to the adaptation of cells to salinity. For instance, while salt tolerant cells of tobacco accumulate Na^+ and Cl^- (20, 44), salt tolerant citrus cells accumulate less Na^+ and Cl^- than do salt sensitive cells (1). Although most reports claim stability of the salt tolerance trait through prolonged periods of culture in the absence of salt (1, 9, 30, 31, 44), there are reports where tolerance was only stable in some lines (35) and we have reported a lack of stability of the salt tolerance phenotype of cells isolated in medium with 10 g L^{-1} NaCl (18).

To evaluate cellular mechanisms of salt tolerance in a glycophytic species, we have isolated cell lines of *Nicotiana tabacum* L. var Wisconsin 38 adapted to different concentrations of NaCl. We report here the growth characteristics and NaCl tolerance of these adapted cells. We present evidence that turgor increases as the cells become adapted to increasing concentrations of NaCl. Our results indicate that the relationship between turgor and cell expansion in these cells has been altered in response to NaCl.

MATERIALS AND METHODS

Development and Culture of Cell Lines Adapted to NaCl. In a previous report, we described the procedures for the isolation and maintenance of a cell suspension of *Nicotiana tabacum* L. var Wisconsin 38 as well as the procedure for the isolation of a cell line which is capable of growth in medium with 10 g L^{-1} NaCl (S-10) (18). Additional NaCl adapted tobacco cell lines

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Table I. *Tobacco Cell Lines Growing in Medium without NaCl (S-0) or in Media with 10 (S-10), 14 (S-14), 20 (S-20), or 25 (S-25) g L^{-1} NaCl*

Concentrations of NaCl and water potential values are for media prior to inoculation of cells. Listed also are the number of cell generations in which the respective cell lines have been maintained at that level of NaCl.

Cell Line	NaCl		ψ_w	Cell Generations
	g L^{-1}	*mM*	*bar*	
S-0	0	0	−6	
S-10	10	171	−14	80
S-14	14	240	−17	90
S-20	20	342	−23	75
S-25	25	428	−27	75

were obtained by transferring S-10 cells into medium with 14, 20, and 25 g L^{-1} NaCl in a sequential manner (Table I). A line adapted to 35 g L^{-1} (599 mM) NaCl has been isolated but was not used in the experiments reported in this paper. Cells were maintained in medium with the previous level of NaCl for a minimum of 50 cell generations prior to inoculation into medium containing the next highest level of NaCl. Stocks of the cell lines were maintained routinely in 500- or 1000-ml Erlenmeyer flasks as batch cultures and recultured when the cells had reached the early stationary phase of growth. Cells in the late linear phase of growth were used for experimentation.

Tolerance and Water Relations Determinations. Tolerance and water relations measurements were made using cell samples taken throughout the growth cycle of each cell line. Cells were inoculated into 2000 ml of nutrient medium contained in a 4-L Erlenmeyer flask at a fresh weight density of 0.02 g ml^{-1}. The nutrient medium contained either no NaCl for the S-0 cells or the level of NaCl in which the cells were being maintained routinely as stocks.

Tolerance was evaluated by determining the fresh and dry weight accumulation of cells in media with different levels of NaCl. Cells were removed from the primary culture and concentrated by either removing medium by filtration in a coarse fritted glass funnel or by decanting off excess medium. Care was taken when removing medium by filtration not to remove all of the excess medium from the cells. Cells subjected to harsh filtration have been found to be metabolically impaired (39). Filtered cells were resuspended into fresh medium containing the same level of NaCl as the primary culture. The resuspended cells or the cells which were concentrated by decanting were adjusted to a fresh weight density of 0.25 g ml^{-1} and used as inoculum for determination of tolerance. Cells were inoculated into 25 ml of medium in 125-ml Erlenmeyer flasks containing various concentrations of NaCl at a fresh weight inoculum density of 0.02 g ml^{-1} and harvested after 32 d. Thirty-two days was the time required for the primary culture of the cell line with the slowest growth rate (S-25) to reach stationary phase. Cultures reaching stationary phase prior to 32 d were harvested at that time, because cells remaining in stationary phase for prolonged periods began to senesce.

Osmotic potentials were determined by plasmometry (4, 14) and were based on the concentration of NaCl causing incipient plasmolysis in 50% of the viable cells. Water potentials of the culture media were measured by determination of the freezing point with a Precision Systems, Inc. (Springfield, MA) automatic osmometer or by the dew point method with a Wescor (Logan, UT) thermocouple psychrometer-hygrometer model HR33T, with model C52 sample chambers (5). Calibration was accomplished using NaCl solutions. For the purpose of estimating turgor, cells were assumed to be in water potential equilibrium with the culture medium and cell turgor values were calculated as the difference between the water potentials and osmotic potentials.

Cell Size. Cells from early stationary phase were viewed with a Nikon Optiphot microscope and Nomarski interference-contrast optics.

Growth Measurements. Cells were harvested on Whatman No. 4 filter paper in a Büchner funnel with aspiration. Fresh weights were recorded, and cells were allowed to dry at least 24 h in an oven at 80°C before dry weights were determined.

RESULTS

Comparison of Growth Characteristics of NaCl Adapted and Unadapted Cell Lines. Although the lag phases of adapted and unadapted cells were similar, approximate fresh weight doubling

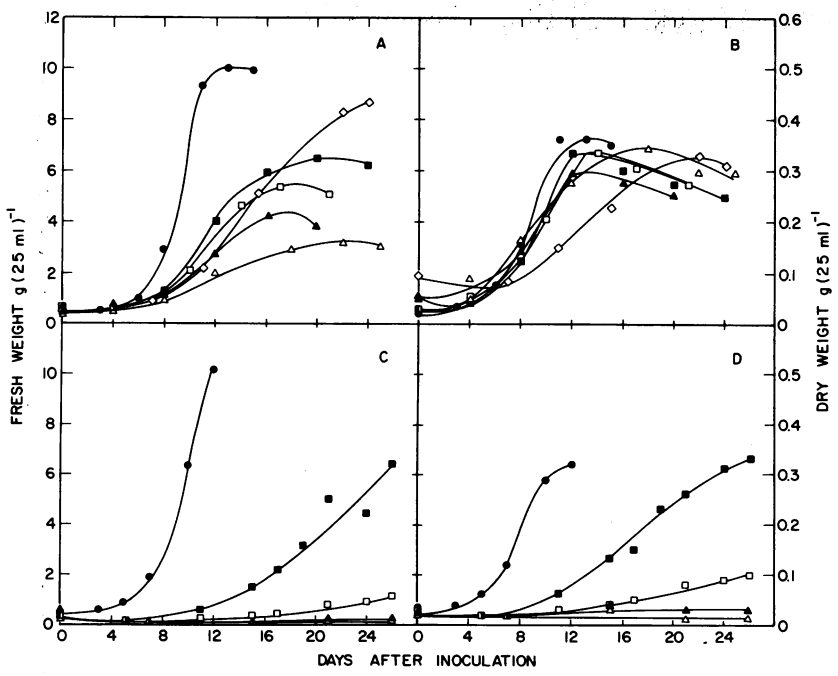

FIG. 1. Fresh (A and C) and dry (B and D) weight accumulation of tobacco cells as a function of time. A and B, S-0 (●), S-10 (■), S-14 (□), S-20 (▲), or S-25 cells (△) in media containing the concentration of NaCl to which the cells are adapted; 0, 10, 14, 20, and 25 g L^{-1}, respectively, and S-25 cells in medium without NaCl (◇). C and D, S-0 cells in media with 0 (●), 10 (■), 14 (□), 20 (▲), 25 (△) g L^{-1} NaCl.

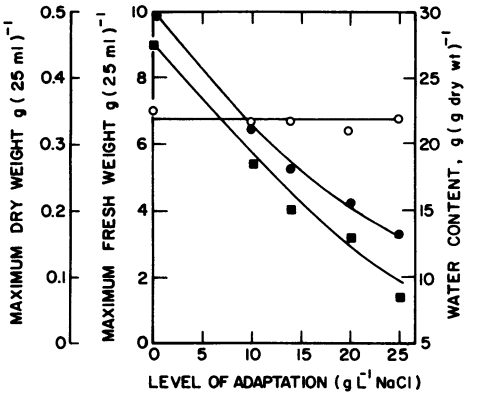

FIG. 2. Water content (■) and fresh (●) and dry (○) weight maxima attained in a culture growth cycle by cells adapted to the level of salt indicated. Water content (■) of the cells at stationary phase was calculated as (fresh weight − dry weight)/dry weight.

times increased from 2 d for S-0 cells to 4 d from S-25 cells (Fig. 1A). Maximum fresh weight declined from 10 to 6.5, 5.3, 4.2, and 3.3 g (25 ml)$^{-1}$, respectively, for the S-0, S-10, S-14, S-20, and S-25 cell lines, whereas the rate of dry weight accumulation and maximum dry weight gain were independent of external NaCl concentration (Figs. 1A, and 2). The differences in fresh weight gain maxima of cells adapted to NaCl resulted in a decreased fresh to dry weight ratio, from 28 for the S-0 cell line to 19, 16, 14, and 10 for the S-10, S-14, S-20, and S-25 cell lines, respectively. The water content of the cells at full expansion (stationary phase) decreased with the level of adaptation (Fig. 2).

The decrease in maximum fresh weight of salt adapted cells was not attributable to a decrease in maximum cell number (data not shown) but was due to a decrease in average cell volume (Fig. 3). The S-25 cells were 4 to 5 times smaller than the S-0 cells at stationary phase. The number of cell doublings during the growth cycle was slightly reduced for the salt adapted cells, however, since there were more cells in the S-25 inoculum the total number of cells present at the end of growth was fairly similar. The cells in the S-0 population exhibited greater variability in cell volume than did cells of the salt adapted populations. This may be due to the fact that salt adapted cells undergo only radial or isodiametric cell expansion immediately following cell division, and fail to undergo extensive directional expansion or elongation as do S-0 cells.

S-0 cells underwent a very extended lag phase compared to adapted cells in media containing 10 g L^{-1} NaCl (Fig. 1, C and D). At higher levels of NaCl, the S-0 cells exhibited little if any growth during the period observed (Fig. 1, C and D). This prolonged lag phase is in part due to a significant loss of cell viability (data not shown), and must represent also an adjustment period during which the cells must cope with large Na^+, Cl^-, and water potential gradients in order to initiate growth. Clearly one adaptive feature of the NaCl lines is the ability to exhibit a typical growth pattern without the necessity of a prolonged lag phase.

After approximately 75 generations in medium with 25 g L^{-1} NaCl, S-25 cells did not lose the ability to grow in medium without NaCl (Fig. 1, A and B). When inoculated into medium without salt, the turgor of S-25 cells continued to decline until stationary phase when these and S-0 cells had approximately the same turgor (data not shown). For several passages after inoculation into medium without NaCl, the S-25 cells continued to exhibit a decreased rate of fresh weight gain (expansive growth).

Tolerance of Cell Lines to NaCl. The ability to grow in media with higher concentrations of NaCl increased with the level of NaCl to which the cells were adapted (Fig. 4). Although the tolerance of the S-0 and S-10 cells varied during the culture growth cycle (Fig. 4, A, B, E, F, I, and J) the average tolerances over the entire growth cycle exhibited by the different cell lines were clearly different (Figs. 4, I–L, 5A, and 6). The relationship between tolerance and the level of adaptation was not proportionately equivalent (Fig. 6). The adapted cells have undergone a physiological adjustment which apparently renders them more tolerant to higher concentrations of salt and allows them to be more able to resist dehydration when exposed to NaCl (Fig. 5B).

Although the ability of cells to grow in higher levels of NaCl increased with the level of salt adaptation, S-25 cells were unable to gain as much dry weight at equivalent increments of NaCl

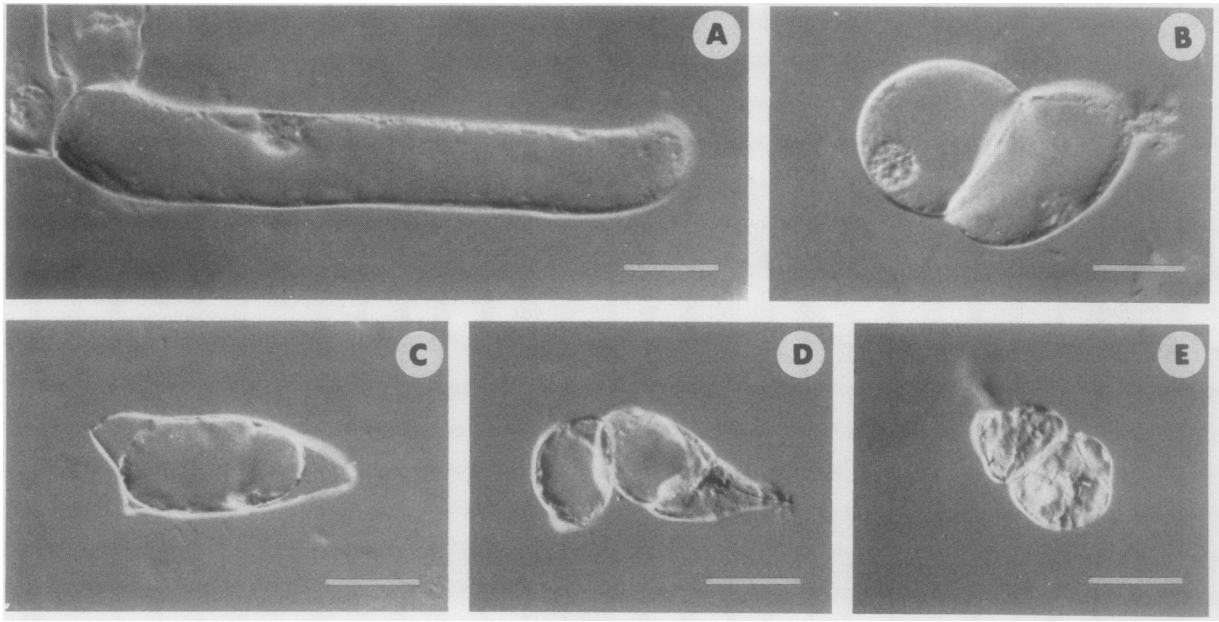

FIG. 3. Cells from stationary phase cultures showing decreased cell size with adaptation to increasing concentrations of NaCl. Nomarski interference-contrast optics were used to view S-0 (A,B), S-10 (C), S-20 (D), and S-25 (E) cells; bars represent 50 μm. The S-0 cells are predominantly of the form shown in A.

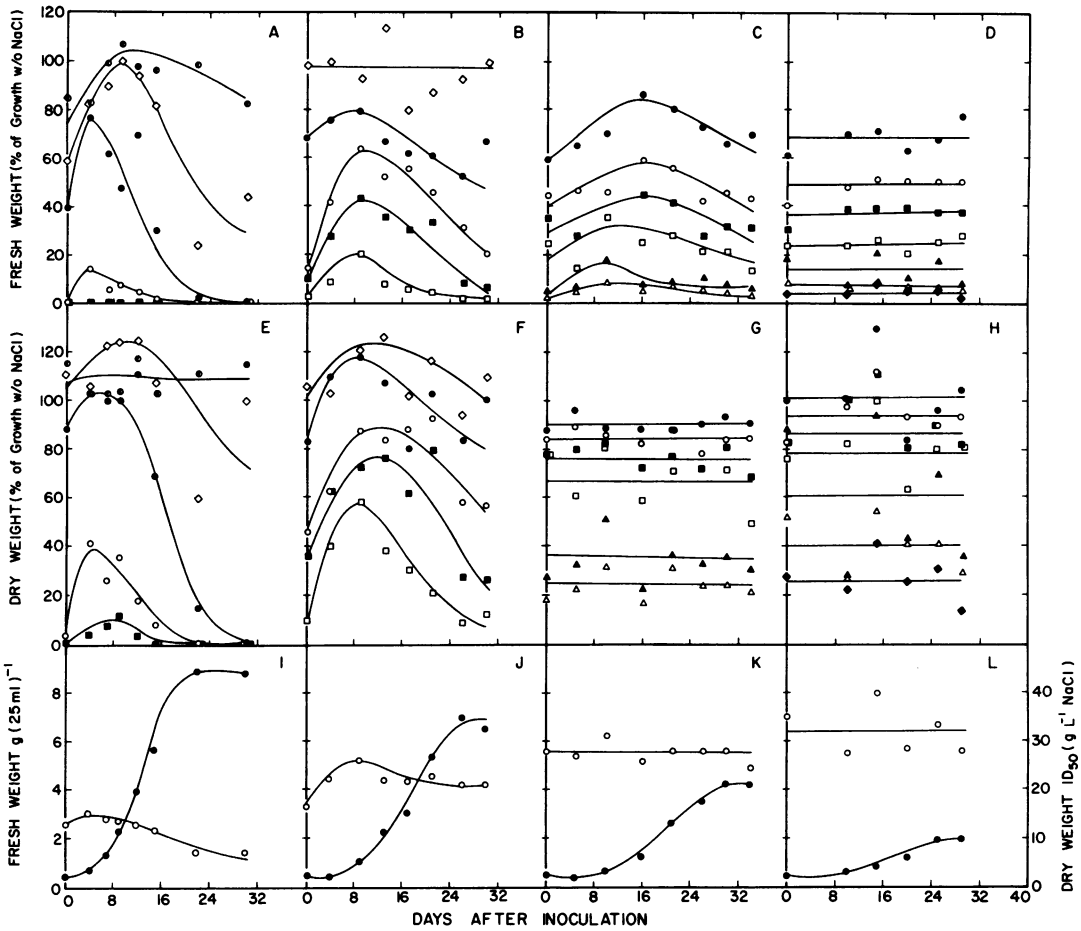

FIG. 4. Growth cycle dependence of tolerance to NaCl for S-0 (A,E,I), S-10 (B,F,J), S-20 (C,G,K), and S-25 (D,H,L) cells. Relative fresh (A–D) and dry (E–H) weight gain in media with various NaCl concentrations; 2 (◐), 5 (◇), 10 (●), 15 (○), 20 (■), 25 (□), 30 (▲), 35 (△) or 40 (◆) g L^{-1} NaCl. Shown in I through L are the NaCl concentrations which inhibited the maximum dry weight gained by 50% (ID_{50}) (○) as a function of growth cycle stage. Also shown in I through L are the fresh weight growth curves (●) for the cultures from which inoculum was taken for dose response evaluations of S-0, S-10, S-20, and S-25 cells. The fresh and dry weights gained, g (25 ml)$^{-1}$, of S-0 cells (A,E) in medium without NaCl were (from d 0): 9.05, 0.26; 9.15, 0.31; 9.57, 0.26; 8.97, 0.25; 9.20, 0.27; 9.06, 0.26; 8.82, 0.27; and 9.07, 0.26. Of S-10 cells (B,F) in medium without NaCl: 7.86, 0.30; 7.66, 0.30; 8.60, 0.33; 7.28, 0.26; 9.81, 0.34; 9.07, 0.29; 8.60, 0.33; and 7.93, 0.30. Of S-20 cells (C,G) in medium without NaCl: 10.83, 0.33; 9.51, 0.30; 9.88, 0.37; 7.85, 0.34; 9.11, 0.35; 8.94, 0.33, 9.88, 0.33; and 9.40, 0.33. Of S-25 cells (D,H) in medium without NaCl: 10.14, 0.28; 9.32, 0.30; 9.22, 0.22; 10.15, 0.33; 9.22, 0.30; and 7.89, 0.26. All values represent the average of two cultures.

above the adaptation level as did S-0 cells (Fig. 7). Thus, maintenance of cells in medium with NaCl adapted them to cope with that level of salinity, but this did not necessarily make them more fit to adjust to higher increments of NaCl. However, increases in the level of NaCl above 25 g L^{-1} may not represent the same degree of stress as an equivalent increase above 0 g L^{-1}. Such evidence suggests that cells of glycophytic species may have an inherent capacity to adapt to saline conditions, but that exposure to salinity does not further enhance this capacity.

As noted earlier for PEG (4) and pathotoxin tolerance (16), a significant influence of the culture growth cycle on salinity tolerance was observed (Fig. 4). The tolerances of S-0 and S-10 cells were most influenced by the stage of the growth cycle. With these cell lines, a several-fold difference in growth in NaCl could be observed depending on the stage of the growth cycle. Such significant influences of the stage of growth on salinity tolerance coupled with the great difficulty of obtaining cultures with synchronized growth cycles make accurate single point determinations of the relative NaCl tolerance of a cell line rather difficult. The influence of growth cycle stage on NaCl tolerance became less pronounced in cell lines adapted to higher levels of NaCl.

The salt adapted cells also exhibited enhanced tolerance to increased osmotic stress (Fig. 8) induced by PEG, mol wt 8000 (5). At this mol wt, PEG will lower the water activity but cannot be used as a solute for osmotic adjustment (14). S-0 cells exhibited slightly greater fresh weight accumulation in media with NaCl than PEG at equivalent external water potentials, particularly at water potentials greater than −14 bar (Fig. 8A). Virtually no difference in fresh weight gain was observed for S-25 cells in media with NaCl or PEG (Fig. 8C), however, S-0 and S-25 cells accumulated considerably more dry weight in media with PEG (Fig. 8, B and D). Residual PEG was not the reason for the significant increases observed in the dry weights of cells grown in PEG *versus* NaCl. Rather, the disparity in dry weight gain in PEG and salt may reflect differences in the mol wt of the principal solutes used for osmotic adjustment in the two instances, particularly if accumulation and compartmentation of Na^+ and Cl^- is a primary process of osmotic adjustment for cells growing in NaCl.

Water Relations Characteristics of the Cell Lines and Their Relationship to Salinity Tolerance. Analysis of the water relations of the cells indicated that they had undergone considerable osmotic adjustment in response to NaCl stress, as evidenced by the higher turgor as the level of adaptation increases (Figs. 9 and

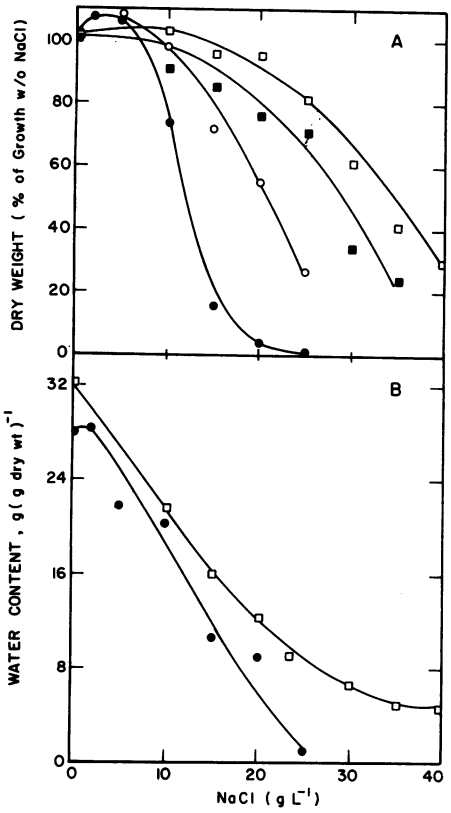

FIG. 5. Average through the growth cycle of relative dry weight gain (A) and water content (B) of S-0 (●), S-10 (○), S-20 (■), or S-25 (□) tobacco cells at various concentrations of NaCl. Values represent averages over the growth cycle of the data in Figure 4. Water content was calculated as (fresh weight − dry weight)/dry weight. The average dry weight, (925 ml)$^{-1}$, in medium without NaCl for S-0, S-10, S-20, and S-25 cells were 0.27, 0.31, 0.33, and 0.28, respectively.

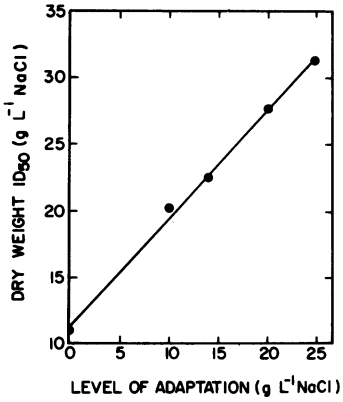

FIG. 6. Tolerance to NaCl of the S-0 cells and cells adapted to varying concentrations of NaCl shown as a function of the level of NaCl to which the cells are adapted. Dry weight ID$_{50}$ values are averages over the growth cycle of the data given in Fig 4 (I–L).

10). Upon inoculation into fresh medium, the cells began to adjust osmotically, presumably through the influx of numerous solutes from the nutrient medium, which resulted in an increase in turgor (Fig. 9). The changes in turgor exhibited by cells during a culture growth cycle were considerably greater for salt-adapted (approximately 20 bar for S-25 cells) than for S-0 (4 bar) cells. Although it is difficult to make a precise conclusion from our data, it appears as if maximum turgor occurred at the onset of exponential fresh weight gain when cell division and isodiametric

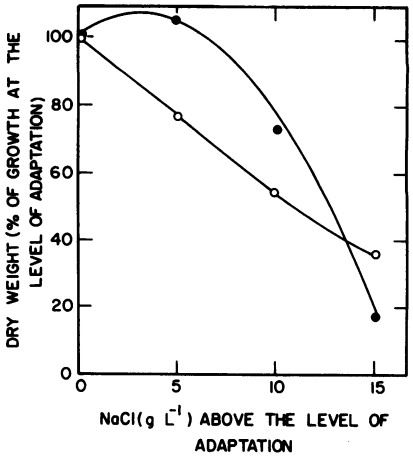

FIG. 7. Relative dry weight gain of S-0 (●) and S-25 (○) cells at varying NaCl concentrations above the level to which the cells are adapted. Values are averages over the growth cycle as presented in Figure 4. The average dry weight, g (25 ml)$^{-1}$ of S-0 cells in medium without NaCl was 0.27 and the average dry weight of S-25 cells in medium with 25 g L^{-1} NaCl was 0.22.

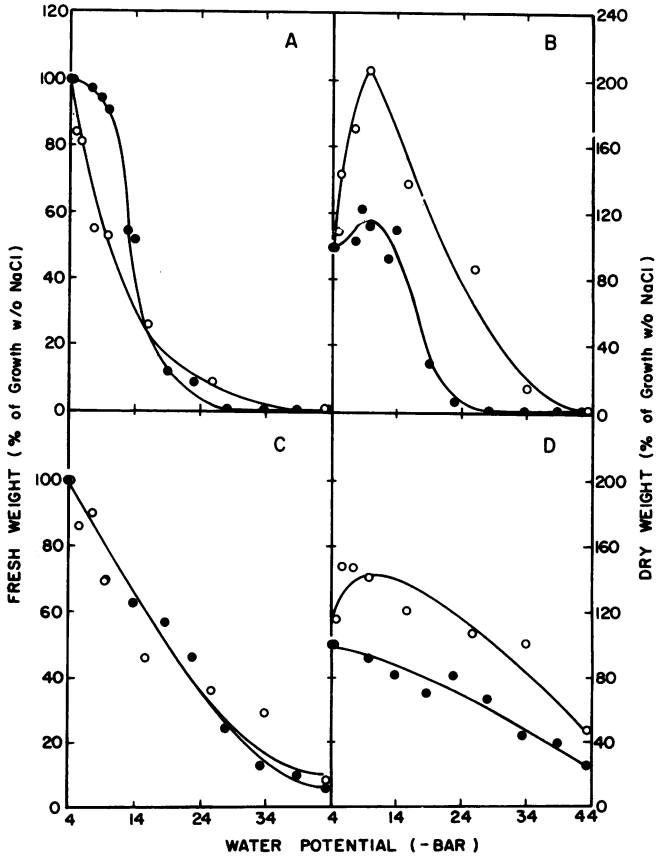

FIG. 8. Growth of S-0 (A and B) and S-25 (C and D) cells in media with NaCl (●) and PEG (○). Relative fresh (A and C) and dry (B and D) weights are plotted as a function of the water potentials of the media containing various concentrations of PEG or NaCl. The fresh and dry weights, g (25 ml)$^{-1}$, for S-0 and S-25 cells in medium without NaCl was 11.10, 0.31, and 6.08, 0.35, respectively. All values represent the average of two cultures.

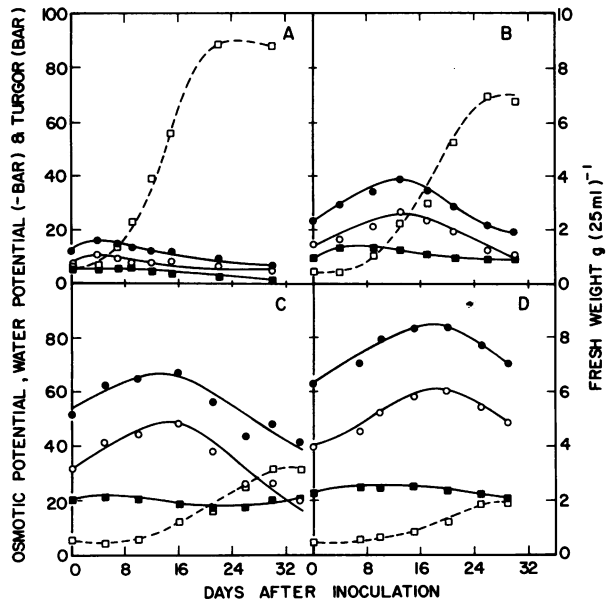

FIG. 9. Water relations of S-0 (A), S-10 (B), S-20 (C), and S-25 (D) cells throughout a culture growth cycle. Osmotic (●) and water potentials (■) and turgor (○) were determined at the stages of the growth cycle shown by each fresh weight growth curve (□).

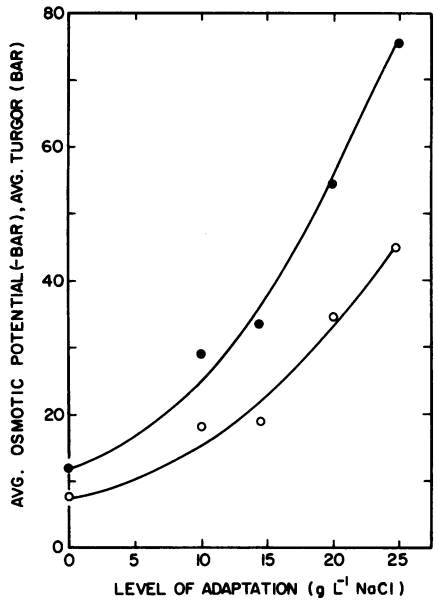

FIG. 10. Osmotic adjustment of S-0 cells and cells adapted to varying concentrations of NaCl. Values for turgor (○) and osmotic potential (●) represent averages of the values taken over a growth cycle (as shown in Fig. 9) for each cell line shown by the level of NaCl to which they are adapted.

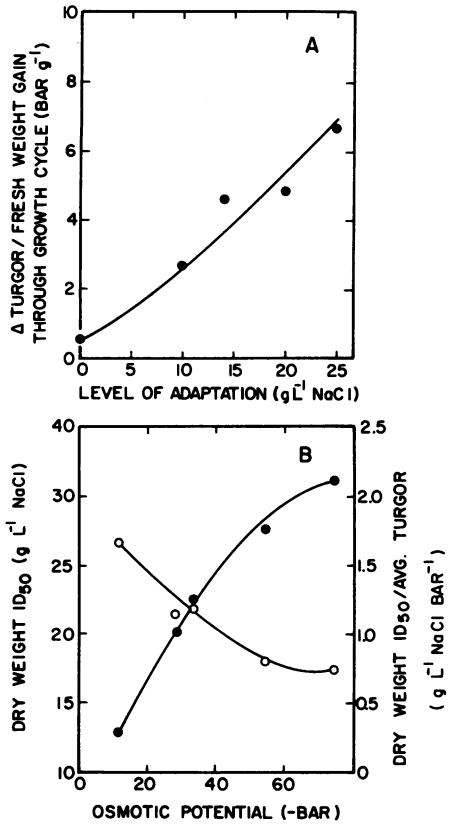

FIG. 11. A, Ratio of the change in turgor to fresh weight gain; (maximum turgor during growth cycle minus minimum turgor during growth cycle)/total fresh weight gain as a function of the level of NaCl to which the cells are adapted. B, Dry weight ID_{50} (●) and the ratio of dry weight ID_{50} to average (over the growth cycle) turgor (○) as a function of the average (over the growth cycle) cell osmotic potential of the tobacco cell lines.

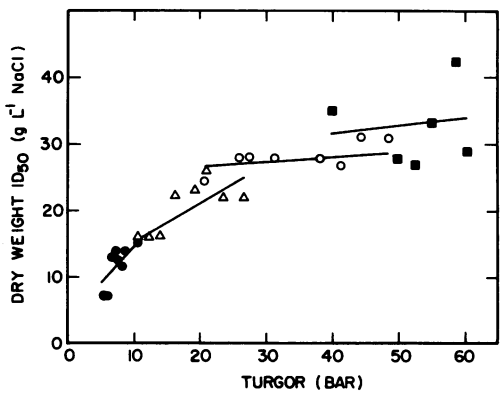

FIG. 12. Relationship between tolerance to NaCl of cells taken from different growth cycle stages and the initial turgor of the same cells. Values for dry weight ID_{50} (taken from Fig. 4, I–L) were plotted as a function of the turgor of the same cells prior to inoculation into dose response media (Fig. 9). Lines represent LS best fit of available points from S-0 (●), S-10 (△), S-20 (○), and S-25 (■) cells.

expansion are the primary processes contributing to growth, and volume of the cell is at its smallest (19). At this time, osmotic adjustment appears to be maximal in preparation for cell expansion which occurs primarily during the phase of exponential fresh weight gain. It is during this phase that the turgor increase is dissipated as a result of cell expansion perhaps through regulation by some turgor sensory mechanism as described by Zimmermann (47). Turgor changes during the growth cycle were not well correlated with growth during the phase of rapid cell expansion as the cells exhibited significant decreases in turgor during periods when the growth rate remained constant (Fig. 9). This is not unexpected as the period over which growth was measured was sufficiently long to allow other factors to impinge upon the expansive properties of the cell (21). However, the adapted cells accomplished less expansion (fresh weight gain) from a given amount of turgor increase which occurred over the course of the growth cycle (Fig. 11A).

The extent of the osmotic adjustment (averaged over the

growth cycle) exhibited by the different salt-adapted cell lines was greater than the change in external water potential to which the cells were exposed (Fig. 10). This high degree of osmotic adjustment occurs during the first passage in the new environment and the cells retain this high level of adjustment after the initial passage, although the absolute levels of adjustment may vary after prolonged periods in the presence of a given level of NaCl. As a consequence of the osmotic adjustment made by the cells and failure to dissipate the turgor by cell expansion, cell turgor (averaged over the growth cycle) increased. The decrease in the amount of growth resulting from a given level of turgor suggests a change in the turgor threshold necessary for growth or in the extensibility properties of the wall (Fig. 11A). However, several factors are known to influence the expansion of cells other than turgor and changes in any of these may also be involved in adaptation (21, 37).

The magnitude of the osmotic adjustment that the salt-adapted cells underwent in response to NaCl followed a curvilinear type relationship such that the most marked increases in turgor occurred in response to very high concentrations of NaCl (Fig. 10). It is possible that the large absolute values are due in part to the limitations of the plasmometric technique for the determination of osmotic potentials (15).

The increased osmotic adjustment made by the salt adapted cells was reflected in their increased ability to grow in media with higher levels of NaCl (Fig. 11B). However, just as with growth in NaCl levels above that to which the cells are adapted (Fig. 7), our data illustrate that the amount of tolerance per amount of turgor also decreased as the cells adapt and osmotically adjust (Fig. 11B), indicating that turgor probably is not the factor restricting the salt-adapted cells from exhibiting enhanced tolerance to NaCl above the level of adaptation (Fig. 7). The loss of the effect of increased turgor on the tolerance after adaptation can be seen also by observing the effect of turgor during the growth cycle on tolerance during the growth cycle (Fig. 12). The effect of turgor adjustment during a growth cycle on tolerance of the cells decreased significantly from S-0 to S-10 cells, and above the level of adaptation of 10 g L^{-1} there appeared to be little if any effect of turgor changes (during a culture growth cycle) on the tolerance to NaCl.

DISCUSSION

Maintenance of turgor by osmotic adjustment is not the primary factor which restricts the limits and rates of cell growth of salt-adapted tobacco cells. It is clear that these cells have undergone considerable osmotic adjustment leading to substantial increases in turgor even after considering possible discrepancies in osmotic potential estimation associated with plasmometry (15). Similar observations of decreased growth despite turgor maintenance have been made with tissue subjected to reduced external water potentials (8, 21, 26, 28). From a biophysical perspective, the salt-adapted cells should have sufficient turgor to elicit cell expansion as observed with the unadapted cells. Growth restriction could be due to changes in hydraulic conductivity; however, it is unlikely that the cultured cells are not in water potential equilibrium with the medium, and estimates of cell water potentials by direct measurement with dew point psychometry confirm this. Consequently, it is reasonable to speculate that the reduced expansion of salt-adapted cells is at least in part a consequence of factors which either directly or indirectly influence the properties of the cell wall (21, 37). Changes in the composition and/or physical characteristics of the wall would have profound effects on cell expansion either through changes in the turgor threshold or alterations in extensibility properties (21, 37). Decreased extensibility properties of cell walls have been implicated in plants adjusted to desiccation conditions (23) and decreased elasticity also resulted from exposure of *Alternanthera philoxeroides* to saline conditions (3). Blaschek and Franz (2) reported a change in the cell wall composition of cultured tobacco cells in response to increased osmolarity of the culture medium. Changes in the wall-loosening characteristics of the cells could prevent expansion and also allow turgor increases. Van Volkenburgh and Boyer (41) have proposed that reduced growth in tissue exposed to lowered water potentials could be due to reduced acidification of the cell wall space, resulting in an inhibition of wall-loosening mechanisms.

Under saline conditions, growth may be limited also by metabolic limitations which might be caused by ion toxicity due to the accumulation of Na^+ and Cl^- used for osmotic adjustment (13, 45). In addition, it is possible that metabolic constraints result from adaptive changes necessary under saline conditions. The cells may be energy limited due to the diversion of metabolic carbon into processes which are required for maintenance of salinity tolerance (17, 34). As cells become tolerant to NaCl, carbon flux is altered to accomodate the biosynthesis of osmotic solutes and the generation of energy necessary for this biosynthesis and other processes (*i.e.* ion transport) important to osmotic adjustment. Such a situation may result in a substantial partitioning of carbon away from growth processes.

An advantage of such a high degree of osmotic adjustment is the ability to resist dehydration as the external water potential is being lowered even to the point of zero turgor (29). However, since the elastic modulus is typically higher for cells under high hydrostatic pressures, there will be a tendency for water to move out of the cell more rapidly, that is, the half time for water loss will be shorter (47). In contrast, cells with a high elastic modulus will lose a smaller proportion of total cell water as turgor is lost (47). Restricted cell expansion may be closely associated with osmotic adjustment under water stress, and may counteract reduced rates of solute uptake (27, 41).

Decreased growth under desiccation stress may be a survival mechanism which allows water conservation and therefore is important to many plants. Reduced growth may be advantageous under water deficits even when osmotic adjustment has produced a water potential gradient favorable for growth, since under natural desiccation conditions only a limited amount of water is available, and lowered cell water potentials will not be able to provide water to sustain growth indefinitely. This is a classic 'water saving' mechanism which is a well-recognized adaptive response to desiccation stress (25). However, this should not necessarily be the case under saline stress where water availability for growth is usually unlimited as long as the water potential gradient favors water uptake. Plants may have evolved mechanisms to restrict their growth under desication stress even when osmotic adjustment would allow water uptake, presumably to conserve water. However, under saline stress such growth restriction would seem unnecessary, and indeed many halophytic plants exhibit little restriction of their fresh weight growth under saline conditions (12, 42). Perhaps the cultured tobacco cells, being glycophytic, activate a growth restriction mechanism in the presence of both desiccation and saline stress which is designed to resist primarily desiccation stress. If this is true, many glycophytic crop plants may exhibit restricted growth under saline irrigation which is not a natural constraint of exposure to salt but may represent the inability of these plants to discriminate between desiccation and saline stress.

Our results establish that cells arising from a salt-sensitive plant can adapt to survive and grow under conditions of extreme salinity, and that adaptation involves considerable osmotic adjustment with increased turgor but reduced cell expansion. These results indicate that cells of glycophytes have the genetic potential for physiological and biochemical processes necessary to adapt to conditions of extreme salinity. These cell lines thus provide a useful system in which to investigate carefully the cellular proc-

esses which are involved in salt tolerance. They also provide an opportunity to study further some of the fundamental questions concerning the regulation of turgor and growth.

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