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Cell growth and water relations of the halophyte, *Atriplex nummularia* L., in response to NaCl

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Summary. Growth reduction or cessation is an initial response of *Atriplex nummularia* L. cells to NaCl. However, *A. nummularia* L. cells that are adapted to 342 and 428 mM NaCl are capable of sustained growth in the presence of salt. Cells that are adapted to NaCl exhibit a reduced rate of division compared to unadapted cells. Unlike salt adapted cells of the glycophyte *Nicotiana tabacum* L., *A. nummularia* L. cells do not exhibit reduced rate of cell expansion after adaptation. However, the cell expansion rate of unadapted *A. nummularia* L. cells is considerably slower than that of unadapted glycophyte cells and this normally low rate of cell expansion may contribute to the enhanced capacity of the halophyte to tolerate salt. Turgor of NaCl adapted cells was equivalent to unadapted cells indicating that the cells of the halophyte do not respond to salt by osmotic "over adjustment" as reported for the glycophyte tobacco (Binzel *et al.* 1985, Plant Physiol. 79:118-125).

Key words: Atriplex - Cell suspension - Salinity - Growth

Introduction

When glycophytes are exposed to salinity or drought, there is a reduction in growth that has been generally associated with a decrease in cell enlargement inferred to be due to the inability of the cells to maintain turgor (ψ_p) (Hsiao et al. 1976). However, it has become increasingly clear that after the initial ψ_p reduction, glycophytes and mesophytes are able to adapt to moderate salt or water stress and maintain ψ_p (Binzel *et al.* 1985, 1987; Bressan *et al.* 1982; Matsuda and Riazi 1981; Michelena and Boyer 1982; Munns and Termaat 1986; Termaat *et al.* 1985). At moderate levels of salinity, ψ_p of glycophyte cells actually increases as a function of the level of adaptation (Binzel *et al.* 1985). These salt-adapted cells continue to exhibit reduced cell expansion rates and it has been suggested that reduced cell expansion has resulted from altered cell wall properties that affect extensibility (Bressan *et al.* 1990; Iraki *et al.* 1989a; Singh *et al.* 1989).

We do not know if reduced cell enlargement that is associated with adaptation to salt is only a response of glycophytes to salt or is an important component of salt tolerance and is therefore inherent to halophytes. Ostensibly, halophytes differ from glycophytes in that high salinity does not result in growth inhibition, and in some instances growth is stimulated at moderate levels of salt (Flowers *et al.* 1986). However, the effects of salinity on cell expansion of halophytes have not been characterized. Since limited cell expansion can result in a substantial reduction in crop productivity (see Bressan *et al.* 1990), such information could offer insight into the effects of salt adaptation on crop yields.

Here we report that adaptation to salinity of *A. nummularia* L. cells results in a growth reduction that is not attributable to reduced ψ_p or a reduction in cell enlargement but rather to reduced cell division.

Materials and methods

Cell cultures. Cell suspensions of *A. nummularia* L. were initiated from callus in the absence of NaCl. NaCl adapted cells, capable of growth in medium with 342 or 428 mM NaCl, were obtained by transferring the unadapted cells sequentially into media containing higher levels of salt (85.5 mM increments beginning at 171 mM). The NaCl adapted cells were maintained in medium with the respective level of NaCl for at least 100 generations prior to the initiation of experiments.

Cell growth and volume measurements. For growth and water relations experiments, cells in the early stationary phase of growth were inoculated into 300 ml of medium in a 1 L Erlenmeyer flask at a fresh weight density of 0.02 g ml^{-1} (Binzel *et al.* 1985). For unadapted cells, the medium contained 0, 342 or 428 mM NaCl and for NaCl adapted cells contained the level of NaCl to which the cells were adapted, 342 mM or 428 mM.

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Tolerance of unadapted cells was determined by evaluating the effects of NaCl on fresh and dry weight accumulation. Cells were inoculated at a fresh weight density of 0.02 g ml^{-1} into 25 ml of medium contained in 125 ml Erlenmeyer flasks (Binzel *et al.* 1985).

Cells were harvested on Whatman No. 4 filter paper in a Büchner funnel under aspiration. The fresh weight was determined immediately and then cells were placed in an oven at 80°C for at least 24 h before dry weight was measured. Cell volume was estimated by subtracting the dry weight from the fresh weight and then dividing this value by the total number of cells in the sample (Bressan *et al.* 1982; Iraki *et al.* 1989a). Cell number was determined in a hemacytometer after aggregates were separated by treatment with 15% chromic acid solution at 65°C for 30 min. Cells were counted until the error was less than 5%.

Cell viability determinations. The dose dependent effects of NaCl on viability of *Nicotiana tabacum* L. var Wisconsin 38 (Binzel *et al.* 1985) and *A. nummularia* L. were determined by inoculating early stationary growth cells into fresh media (0.02 g fresh weight ml^{-1}) containing varying concentrations of salt. Viability was determined 48 hrs after inoculation by neutral red staining (LaRosa *et al.* 1987). Cells were judged to be viable if the vacuoles were stained, plastids were intact, and cyclosis was occurring.

Water relations determinations. Cell osmotic potentials were determined by plasmometry using NaCl solutions as standards (Binzel *et al.* 1985; Bressan *et al.* 1982). Water potentials (ψ) of the culture media were determined with a Precision Systems Inc. (Springfield, MA) automatic osmometer by the freezing point depression method. Calibration was accomplished using NaCl solutions. The ψ_p was calculated as the difference between cell ψ and osmotic potential (ψ_π) assuming that the cell ψ was in equilibrium with that of the medium (Binzel *et al.* 1985).

Results

Tolerance of A. nummularia cells to NaCl

Unadapted *A. nummularia* L. cells exhibited a dose-dependent response to NaCl with fresh weight gain inhibited to a greater degree than dry weight gain (Fig. 1). A similar response was observed with the glycophyte, tobacco (Binzel *et al.* 1985). *A. nummularia* L. does not appear to be substantially more tolerant to NaCl than tobacco based on cell growth comparisons (Binzel *et al.* 1985).

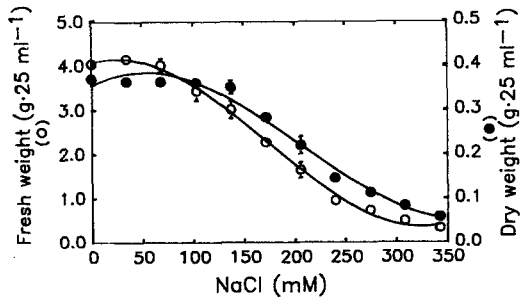

Figure 1. - Effect of NaCl on the maximum fresh (○) and dry (●) weight accumulation of unadapted *A. nummularia* cells after 10 days of growth. Each point represents the mean ± SD of three replicate samples from two different experiments.

Despite the fact that growth of *A. nummularia* L. cells was inhibited at moderate levels of NaCl (Fig. 1), the viability of the cells after 48 h was very high at these salt levels compared to the glycophyte tobacco (Fig. 2).

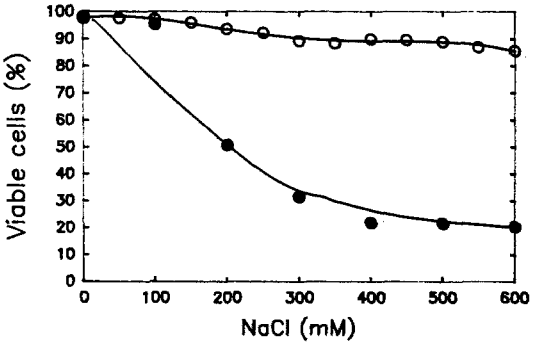

Figure 2. - Effect of NaCl on the cell viability of unadapted cells of *Atriplex nummularia* L. (○) and *Nicotiana tabacum* var. Wisconsin 38 (Binzel *et al.*, 1985) (●) after 48 h.

Cells adapted to NaCl for several generations grew much more compared to unadapted cells when inoculated into medium with a high level of NaCl (Fig. 3). Upon initial exposure to NaCl, the viability of unadapted cells did not decrease substantially (Fig. 2), however, after 4 days viability in medium with 342 and 428 mM NaCl decreased to a low of about 70 and 40%, respectively (data not shown). NaCl adapted cells did not exhibit any substantial decrease in cell viability when re-inoculated into medium containing the salt concentration to which they were adapted. These results indicate that *Atriplex* cells adapted to 342 and 428 mM NaCl exhibited increased salt tolerance relative to unadapted cells.

Comparison of growth characteristics of unadapted and NaCl adapted Atriplex cells

NaCl adaptation resulted in a reduction in the rate and maximum fresh weight gain of *A. nummularia* L. cells (Fig. 3A). A similar, albeit somewhat less substantial, decrease in dry weight accumulation also occurred (Fig. 3B). Glycophyte cells exhibited an analogous decrease in fresh weight accumulation as a result of NaCl adaptation, however, dry weight gain was relatively unaffected (Binzel *et al.* 1985).

The decreased rates of fresh and dry weight gain that occurred as a result of NaCl adaptation of *A. nummularia* L. cells were attributable to a reduction in the rate (from 2.2 to 4.0 days doubling time) and total number (3.6 to 3.2 total cell doublings) of cell divisions (Fig. 4A). Reduction in fresh weight gain that occurred coincident with salt adaptation of *A. nummularia* L. cells was not the result of reduced rate, (1.04×10^{-8} and 1.03×10^{-8} ml day^{-1} for unadapted cells and cells adapted to 342 mM NaCl, respectively) or maximal extent of cell expansion (Fig. 4B) as it was for tobacco (Binzel *et al.* 1985; 1989; Bressan *et al.* 1990; Iraki *et al.* 1989a; Singh *et al.* 1989). The rate of cell expansion and maximum cell size of unadapted *A. nummularia* L. cells were similar to NaCl adapted

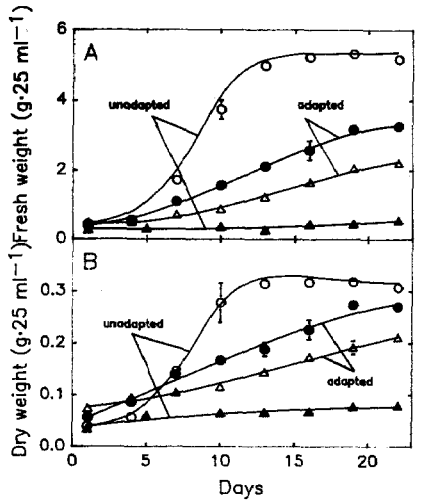

Figure 3. - Fresh (A) and dry (B) weight accumulation of unadapted *A. nummularia* cells in medium without (○) or containing 428 mM NaCl (▲) and *A. nummularia* cells adapted to 342 (●) or 428 mM (△) NaCl in medium with the respective level of salt. Each point represents the mean ± sd of two replicate samples from two different flasks.

tobacco cells and were considerably less than for unadapted cells of tobacco (Bressan *et al.* 1990; Iraki *et al.* 1989a; Singh *et al.* 1989). However, these results must be interpreted with the assumption that the culture media or conditions do not limit the full expansion potential of the *A. nummularia* L. cells.

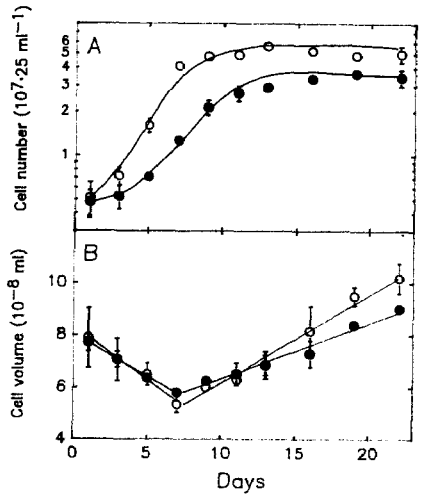

Figure 4. - Cell number (A) and volume (B) of unadapted (○) and 342 mM NaCl adapted cells (●) during a culture growth cycle. Cell volume was calculated as (fresh weight − dry weight/cell number).

Water relations characteristics of the cell lines

During growth the cells exhibited a cyclic period of osmotic adjustment that resulted in a turgor increase that was dissipated as the cells underwent expansion (compare Fig. 4B and Fig. 5C). The most negative ψ_πs coincided approximately with the time when the cell volume was minimal, i.e. period of most active cell division. NaCl adapted *A. nummularia* L. cells

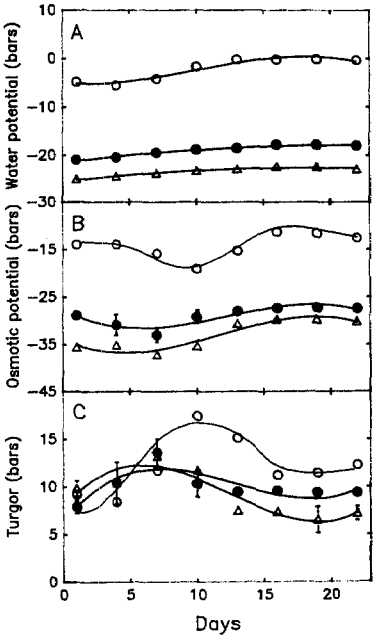

Figure 5. - Water potential (A), osmotic potential (B) and turgor pressure (C) of unadapted (○) and NaCl adapted cells (●, 342 or △, 428 mM) during a growth cycle.

adjusted osmotically to the extent that ψ_p was reestablished; 12.1, 10.0 and 9.5 bars averaged through the growth cycle for unadapted cells and cells adapted to 342 and 428 mM, respectively. Turgor "over adjustment" did not occur in a manner analogous to salt adapted glycophyte cells (Binzel *et al.* 1985), for which ψ_p increased substantially with the level of adaptation. In fact with *A. nummularia* L. cells, at the end of the growth cycle, ψ_p values decreased slightly as a function of the level of NaCl adaptation.

Discussion

Cells of *A. nummularia* L. appear to have a lower degree of tolerance to NaCl *in vitro* than cells of the halophytic grasses *Distichlis spicata* and *Spartina pectinata* (Warren and Gould 1982; Warren *et al.* 1985) when tolerance is measured as the ability to grow after initial exposure to NaCl. Within the growth conditions in this study, the responses of *A. nummularia* L. cells to NaCl were similar to the glycophyte tobacco (Binzel *et al.* 1985). However, *A. nummularia* L. cells are more capable of surviving high concentrations of NaCl than tobacco cells.

A primary response of *A. nummularia* cells to NaCl involves a reduction or cessation of growth. During this period, however, the cells must initiate the adaptive mechanisms that facilitate survival and growth in the saline environment. Consequently, determinations of tolerance, based on growth over a particular finite period after initial exposure to NaCl, may not reflect the degree of salt tolerance of *A. nummularia* L.

Reduced ability to gain fresh weight as a result of salt adaptation of *A. nummularia* L. cells is due to a decreased cell division rate. Growth inhibition of leaf cells elicited by water deficit has been attributed to reduced rates of cell division (Van Volkenburgh 1987), however, decreased cell division may be mediated by a reduction in the rate of cell expansion that occurs between the mitotic events (Bressan *et al.* 1990). Although it remains unclear what factor is limiting cell division of *A. nummularia* L. cells after salt adaptation, it could be that carbon utilization has been altered to adversely affect cell division. Tobacco cells adapted to NaCl exhibited a reduced number of cell divisions that could be enhanced by an increase in the supply of reduced carbon (Schnapp *et al.* 1990).

Salt adaptation of *A. nummularia* L. does not result in reduced rate of cell expansion or reduced maximal cell size as it does in the glycophyte, tobacco (Binzel *et al.* 1985; 1989). These results indicate that changes in the wall extensibility properties that accompany salt adaptation of glycophyte cells that may limit cell expansion (Iraki *et al.* 1989a) do not occur as a result of adaptation of the halophyte, *A. nummularia* L. It would be of interest to determine if the substantial changes in wall composition and turnover of wall constituents that occur during salt adaptation of glycophytes (Iraki *et al.* 1989b,c) occur in halophytes.

It is still uncertain if halophytes in general continue cell expansion at unreduced rates after adjusting to NaCl. However, the normal expansion rate of halophyte cells appears to be inherently slow relative to that of cells of the glycophyte, tobacco (Bressan *et al.* 1990; Iraki *et al.* 1989a; Fig. 3). This slow rate of cell expansion could be a fundamental salt tolerance mechanism contributing to the maintenance of water status and the regulation of ion pools. As discussed previously, the requirement to maintain low concentrations of ions in the cytoplasm and a limited capacity to transport ions from the cytoplasm to the vacuole, may necessitate greatly reduced cell expansion rates in the presence of high external levels of NaCl (Bressan *et al.* 1990; Hasegawa *et al.* 1990).

We do not know yet if *A. nummularia* L. and other halophytes will eventually respond to higher levels of NaCl by reducing cell expansion rates. In addition, the effects of desiccation, rather than salinity, on cell expansion in halophytes have not been determined. We have suggested in the past that cell expansion of halophytes may be affected differently by desiccation and salinity stress (Singh *et al.* 1989; Binzel *et al.* 1989).

Since glycophytes have evolved in environments lacking salinity, it is probable that rapid cell expansion rates were, from an evolutionary perspective, more advantageous in nonstress environments. Also, it is clear that selection for increased productivity in managed agriculture clearly favors those genotypes whose cells have greater expansion rates.

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