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BARD

FINAL REPORT

PROJECT NO. US—535—82

Salinity Stress and Carbon Metabolism of Plants Differing in Stress Resistance

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1987

Standard BARD Cover Page for Scientific Reports

Date 9-30-87

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BARD Project No. US-535-82

Title of Research Project:

Salinity stress and carbon metabolism of plants differing in stress resistance.

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Name and Address of Affiliated Institutions:

Principal Institution: Purdue University

Cooperating Institution(s): Hebrew University

Project's starting date: June 1, 1983

Type of Report: 1st Annual _____ 2nd Annual _____ Final X

Signatures:

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Purdue Research Foundation

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ABSTRACT

We have developed a cell culture system to investigate the influence of adaptation to salinity (0.46 M NaCl; -25 bar) and drought (30% polyethylene glycol; -28 bar) on carbon partitioning and cell wall organization and chemical structure, and we developed a root tip culture system to investigate alteration in patterns of differentiation of plant tissues exposed to salinity. Our objectives were to use these systems to study physical and biochemical bases of why cells do not expand under osmotic stress.

With the cell culture system, we found that carbon utilization is predominantly through UDP-sugar pyrophosphorylase, and from measurements of a uracil nucleotide ratio, an index could be established to reflect the drain on available adenylate phosphoanhydride bond energy from formation of UDP-sugar required from polymer synthesis reactions. We found in parallel projects, marked changes in the chemical structure of the cell wall. Our studies support our hypotheses that extensibility of the cell wall is reduced specifically through inhibition of the synthesis and activity of endo-glucosidases responsible for cleavage of hemicellulosic xyloglucans, whereas inhibition of cellulose synthesis and polymerization of cell wall structural proteins is responsible for a decrease in absolute tensile strength of the cell wall.

With the root tip culture system, we found that, like in adapted cells in culture, salinity induces marked inhibition of cell expansion. Further, we found that meristematic activity in the pericycle associated with secondary root formation is enhanced by salinity. Differentiation of water and nutrient conducting tissues is also hastened, and from histochemical determination, lignification and suberization is induced earlier in cells exposed to salinity.

Together, our results suggest that exposure to salinity causes marked changes in the metabolism and in the chemical organization of the cell wall, and that control of discrete polymer assembly and turnover processes are directly involved with adaptation to osmotic stress.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

Understanding the biochemical, physiological, and genetic factors involved in adaptability of crop plants to saline soils has obvious practical value. As a result of selection, cultivated species are more sensitive to saline environments than ancestral plants. Some approaches toward improvement of crop plants have been to use traditional plant breeding to combine genomic characters for saline adaptability with those of productive cultivars, to select for stable, inheritable saline tolerance in productive cultivars through tissue culture techniques, and to understand the physiological and biochemical processes of tolerance so that genetic factors can be identified and isolated for eventual transfer via recombinant DNA technology.

We found early on that upon adaptation to water stress, tomato cells in culture partitioned reduced carbon into osmotic pools at the expense of the synthesis of the cell wall. Our research was directed toward elucidating biochemical markers for saline adaptation potential and the basis for control of this change in partitioning. Our specific objectives were four-fold:

1. To examine the fundamental organic solute and cell wall composition of growing radicles of cultivated and wild mesophytic species and halophytic species. Because development of a root culture system required for these studies, the group at Hebrew University concentrated on establishing the conditions for continuous culture of excised roots of *Pisum sativum*. At Purdue University, tobacco cell cultures adapted to saline stress of 2.5% NaCl (-25 bar) or water stress of 30% polyethylene glycol (-28 bar) were developed so that comparison of these very different kinds of osmotic stress could be made and plants could be regenerated to determine if the adaptive characters were stable upon tissue development.

2. To trace the path of carbon from [U-¹⁴C]-glucose into osmotic and cell wall pools and to determine the relative partitioning and the nature of solutes and wall polymers synthesized.

3. To examine the parameter of relative partitioning of [U-¹⁴C]-glucose by radicle cells as a function of salinity of the exogenous medium. This parameter was to yield a quantitative tool to assess the adaptability of the cells to saline stress. We would correlate changes in carbon

partitioning with morphological changes in the radicle tissue, and then compare root tissue of native and cultivated genotypes that differed in salinity tolerance.

4. To examine the relative partitioning of carbon from [U-¹⁴C]-glucose after exposure of radicles to prolonged saline stress. After culture of seedlings in media of non-lethal stress, the partitioning of carbon would be examined as a function of salinity to determine adaptability of the cells to changing external water status. The native and cultivated genotypes again would be examined so that partitioning could be compared as a function of external salinity and relative stress resistance.

DETAILED REPORT

Objectives 1 and 2. *Fundamental organic solute and wall composition of cells adapted to saline and water stress and the path of carbon from [U-¹⁴C]-glucose into osmotic and cell wall pools.*

Cells of tobacco (*Nicotiana tabacum* L., cv. W38) in liquid culture provided excellent models to study how adaptation to osmotic stress changes both osmotic solutes and cell wall structure in independent cells. These cells have been in continuous culture for over a decade, and sublines of these cells have been adapted to grow in at least 2.5% NaCl (-25 bar) or 30% polyethylene glycol 8000 (PEG; -28 bar). Description of how these cultures were developed has been published elsewhere (1,3,5). In addition to studies that were supported by this project, several on-going collaborative projects also involved studies with these culture systems. These studies include: 1) analysis of solutes contributing to osmotic adjustment, with emphasis on nitrogen metabolism, 2) cytological localization of sodium, potassium, and chloride ions as osmotic constituents of cytoplasm and vacuole, 3) the role of abscisic acid in adaptation to saline and water stress, 4) clonal variation in stress resistance in cells and plants regenerated from stress-adapted cells, and 5) the role of a 26 kD protein, called "osmotin" induced upon adaptation to stress (for review, see 2, 6-12).

We found that cells adapted to 2.5% NaCl or 30% PEG accumulated dry weight at about the same rate as unadapted cells, but much of the sugar was accumulated as osmoticum at the expense of the synthesis of cell wall material (see Handa, *et al.*, 1983). We examined controls of this partitioning. Because the nucleotide sugar, UDP-glucose, is the central donor of carbon from external or vacuolar stores to structural polymers, we examined the pathway of sucrose provided in the medium into UDP-glucose. There are two major routes in plants. One is catalyzed by sucrose synthase, where sucrose + UDP → UDP-glucose + fructose. If invertase is present, cleavage of sucrose to glucose and fructose would necessitate hexokinase and phosphoglucomutase to form glucose-1-phosphate, and UDP-glucose would be made in a reaction catalyzed by UDP-glucose pyrophosphorylase, where glucose-1-phosphate + UTP → UDP-glucose + PPi. It was not apparent which of these pathways were used by cell cultures grown on sucrose for many years, although growth on equivalent amounts of glucose was about equal to that of sucrose. We found to our surprise that most of the cell cultures completely hydrolyzed the sucrose within hours or days of subculture, well before the onset of logarithmic growth, and in its place appeared almost stoichiometric amounts of glucose and fructose. Thus, the major source of carbon for growing cells is hexose rather than sucrose, regardless of how the medium is prepared. In direct labelling studies, both glucose-1-phosphate and UDP-glucose quickly incorporated ^{14}C from glucose, while sucrose accumulated radioactivity at much slower rates.

In further studies, cell cultures of dicots (*Nicotiana tabacum*, *Daucus carota*, and *Petunia hybrid*) demonstrated a preference for glucose. Carrot cells in culture absorbed virtually all of the glucose before appreciable amounts of fructose were assimilated. Cells of graminaceous monocots (*Zea mays* and *Panicum miliaceum*) had particularly high levels of invertase, activities reaching greater than 10 times that of the dicot cultures. These monocots demonstrated a preference for fructose rather than glucose. Why these cells demonstrate this preference was not readily apparent, nor was its adaptive significance. (For more extensive reports of these findings, see Kanabus, *et al.*, 1986a).

Because of the requirement of ATP and UTP as energy transducing compounds for synthesis

of UDP-glucose, we also examined the 'energy charge' of the adenylates, and comparable nucleotide ratios for the uracil compounds, in cells of *Daucus carota*. We found that a modified energy charge (calculated with the omission of AMP) decreased steadily from about 0.9 to 0.8 during the course of the culture cycle. The analogous uracil nucleotide ratio was considerably lower (0.85) during early culture, decreased to about 0.7 for the entire logarithmic phase, and returned to initial values as cells entered stationary phase. We suspected that the drain of uracil from UTP to UDP-glucose was the principal reason for this behavior, and the results indicated that energy coupling between ATP and UTP was permitted to fluctuate. The uracil nucleotide ratio may provide a useful index to assess the coupling between the energy available in phosphoanhydride bond in adenine nucleotides and the demand for sugar for polysaccharide synthesis through uridine diphosphate-sugar pools. (For a full report of these studies see Kanabus, *et al.*, 1986b)

Adaptation to osmotic stress resulted in several alterations in the organization and chemical structure of the cell wall. First, our earlier work (Handa *et al.*, 1983) demonstrated that adapted cells "overadjusted" osmotically and developed turgor pressures several-fold higher than typically found in unadapted cells. Despite the fact that these cells were "over-pressurized", cell expansion was severely inhibited in adapted cells. Cells adapted to either NaCl or PEG had cell volumes that were only 1/5 to 1/8 that of the unadapted cells (2). These observations indicated that either a change in the growth yield threshold or "extensibility" resulted in a stiffening of the wall matrix, and this stiffening occurred even though the amount of carbon expended by the cell for cell wall was reduced substantially in favor of accumulation of sugar as osmoticum. A nitrogen gas decompression technique was devised to determine the tensile strength of walls of single cells empirically (4). We found that the breaking pressures of plant cells in liquid culture were actually several fold higher than normal turgor pressures. The breaking pressures of adapted cells were actually lower than those of unadapted cells. These data have far reaching implications for appreciation of growth physics. Wall expansion must not be viewed as a critical turgor pressure required to pry the cellulose microfibrils apart mechanically, but rather a discrete biochemical process essentially independent of wall tensile strength. We sought to determine the nature of the

decrease in extensibility and the decrease in wall tensile strength.

Synthesis of the cell wall in either NaCl- or PEG-adapted tobacco cells was reduced to about 50% of that of unadapted cells. Cellulose synthesis was inhibited substantially in adapted cells. There were marked differences in the organization and chemical composition of the pectins. The proportions of total pectin in walls of unadapted and adapted cells were about the same, but a substantial amount of uronic acid-rich material from walls of cells adapted to either NaCl or polyethylene glycol was more easily extracted with cold sodium ethylenediamine tetraacetic acid solutions. Pectins extracted from walls of adapted cells by either cold sodium ethylenediamine tetraacetic acid or hot ammonium oxalate were particularly enriched in rhamnose. Thus, adaptation to stress resulted in proliferation of a loosely-bound shell of polygalacturonic acid and rhamnogalacturonan. The rhamnosyl units were more highly substituted with polymers containing arabinose and galactose, but these side groups were of greatly reduced molecular size. It was unlikely that the pectins, themselves, participated directly in the stiffening that prevents normal cell expansion, but subtle changes in gel properties of this matrix could influence the movement or association of wall enzymes, such as polysaccharide hydrolases, to or with their specific substrate. (For a full report of these studies, see Iraki, *et al.*, 1987a)

Hemicelluloses such as xyloglucan in dicots are considered that principal load bearing bonds that cement cellulose microfibrils and hydrolysis of these polysaccharides are implicated in wall expansion (13). For cells in liquid culture, the products of these hydrolyses may appear in the spent medium, and we examined in detail these extracellular polymers released by these cells. The chemical composition of extracellular polymers released by adapted cells of tobacco was compared to that released by unadapted cells. Unadapted cells released uronic acid-rich material of high molecular weight, arabinogalactan-proteins, low molecular weight fragments of hemicellulosic polysaccharides, and a small amount of protein. Cells adapted to grow in medium containing NaCl released arabinogalactan and large amounts of protein, but not the uronic acid-rich material, and cells adapted to grow in polyethylene glycol released only small amounts of an arabinogalactan of much smaller molecular size and some protein. Secretion of all material was nearly blocked by

polyethylene glycol, but when cells were transferred to a medium containing iso-osmolar mannitol, they again released extracellular polymers at rates similar to those of unadapted cells. Like cells adapted to NaCl, however, these cells released arabinogalactan and large amounts of protein, but only small amounts of the uronic acid-rich material. Furthermore, accumulation of low molecular weight polysaccharide fragments was significantly reduced in both NaCl- and polyethylene glycol-adapted cells, and specifically, the material comprised lower proportions of xyloglucan fragments. Our results indicated that adaptation to simulated saline or water stress resulted in inhibition of release of uronic acid-rich material into the culture medium, and inhibition of cell expansion was correlated with inhibition of hydrolysis of hemicellulosic xyloglucan. (For a full report of these studies, see Iraki, *et al.*, 1987b)

Release of protein into the medium was significant for perhaps another reason. Amino acid composition of the total protein indicated that substantially amounts of hydroxyproline were present. Analysis of the cell wall indicated that the hydroxyproline content had decreased to about 10% that of unadapted cells. These preliminary results obtained near the conclusion of this project indicated that the structural proteins, "extensin" and proteins associated with extensin, failed to polymerize under osmotic stress and, instead, escaped into the medium. The adapted cells may have inadvertently provided unique models to study the soluble precursors to these structural proteins.

Objectives 3 and 4. *Morphological changes during development of pea roots in liquid culture under saline stress, and carbon partitioning in these root tissues.*

Growth and differentiation of higher plants under conditions of severe osmotic stress is modified in at least two levels of complexity. For example, cells exposed to saline stress must adjust osmotically, expending energy to either exclude the salt and accumulate innocuous cellular osmotica, or partition the salt in the vacuolar compartment. Cells must then obtain sufficient additional carbon to divide and grow, building structural and functional cell walls compatible with the altered metabolism required for survival. Somatic cells in liquid culture are advantageous for

examining the basic biochemistry and physiology associated with these alterations. At the second level, the multicellular organism must undergo a severely altered morphogenesis, proceeding beyond mere osmotic adjustment, to produce a plant capable of survival under continued saline stress. To establish a framework to study biochemistry and physiology of adaptation to stress at the organismal level, roots of pea seedlings were excised and grown in liquid shake culture. Considerable effort was devoted to maintain the root tips in continuous culture so that the influence of prolonged exposure to saline could be assessed. With this system, we found that salinity induced several anatomical and morphological differences. Roots became constricted just above the apcx, but the region above this constriction thickened, and the root tip curved about 90°. Because cell expansion was inhibited substantially, cell differentiation began much closer to the tip. Despite this inhibition of expansion, mitotic activity of the pericycle was increased markedly. The secondary meristems developed, but initiation of root elongation was inhibited. From

histochemical staining, increased lignification and suberization also was evident at the constriction zone. (For a more complete description of this study, see Solomon, *et al.*, 1987a).

Because of the unique anatomical and morphological alterations that accompanied differentiation, a basic metabolic study was undertaken to examine both salt accumulation and carbon metabolism. While growth in saline medium resulted in increased sodium uptake in all tissues, potassium was preferentially taken up by root apical cells. In differentiating cells, brief exposure to salinity induced increased amounts of free sugar, cellulose, uronic acid, and protein per cell at the expense of respiratory carbon. These results contrasted those obtained for cells of tobacco in culture, and perhaps reflected the advance of differentiation resulting from the inhibition of cell expansion in the apical region. (For a more complete description of these results, see Solomon, *et al.*, 1987b).

During the course of studies of the anatomical and ultrastructural changes induced by salinity, we found that mitochondrial damage was apparent in meristematic cells, and these cells showed advanced vacuolization despite failure to expand. Interestingly, amyloplasts of cells of the

root cap were tightly clumped around the cell nuclei. A relationship between this phenomenon and the alteration in geotropic response was considered. (For a more complete description of this study, see Hodson and Mayer, 1987.)

The biochemical events surrounding the apparent advance in lignification and suberization was investigated toward the end of the project. Peroxidases, which are considered responsible for formation of diphenyl cross-links and generation of phenoxy free radicals necessary for synthesis of these substances, were studied in the roots exposed to saline medium. Our preliminary results showed that salinity induced an increase in the pelletable (cell wall) fraction of peroxidases. Because enhancement of the activity of these peroxidases could contribute to the inhibition of cell expansion, we are now concentrating on purification and characterization of the activities of the many cell wall peroxidases as a fundamental step toward understanding control of their expression.

DESCRIPTION AND RESULTS OF COLLABORATION

For projects described in this report, Drs. Carpita and Bressan headed research involving use of the tobacco cell suspension cultures, and Drs. Poljakoff-Mayber and Mayer conducted work establishing the root tip culture and metabolism studies associated with this system. Dr. Carpita and a post-doctoral associate, Dr. Jan Kanabus, visited Hebrew University in August, 1987. During that time, we discussed at length the similarities and differences in the biochemical and physiological responses of cells and tissues to saline environments. We also conducted work together to establish basic carbohydrate analyses and to demonstrate some special considerations for use of radiolabelled sugars for assessing the partitioning of carbon into osmotic and cell wall pools.

As a result of this study, each laboratory initiated comprehensive studies of how saline environments induced specific alterations in cell wall metabolism. Both groups realized the importance of cell wall metabolism and its involvement in the self-imposed failure to expand under conditions of osmotic stress, and this fostered close communication. We anticipate that even closer

collaboration in the future as we intend to provide extended stays in each laboratory by post-doctorals and other professionals to share techniques in carbohydrate chemistry, enzyme purification, and use of molecular cloning techniques in study many of these problems in plant cell development.

EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH REGARD TO THE ORIGINAL RESEARCH PROPOSAL

As a direct result of this work, laboratories at Purdue University and Hebrew University established comprehensive programs to study how osmotic stress specifically alters cell wall metabolism. To our pleasure, several interesting and exciting results were obtained which not only advanced our understanding of how osmotic stress inhibits plant growth, but also deepened our appreciation for the kinds of polymers that impart specific physical and mechanical wall properties. We found that stress does not merely inhibit cell expansion osmotically. The cells accumulated massive amounts of organic solutes quickly and even overcompensated for the osmotic stress. Thus, the cells physically are fully capable of expansion, but do not as a result of a self-imposed restriction in wall extensibility. We have identified at least one group of polysaccharides, the xyloglucans, that participate directly in control of extensibility. Interestingly, this control of cell wall "stiffening" is not related to the actual tensile strength of the wall. The decrease in extensibility occurred despite almost a 50% reduction of carbon expended for wall synthesis, and measurement of tensile strength of the wall empirically confirmed that the walls of adapted cells were actually weaker than those of unadapted cells. We found also that, unlike unadapted cells, adapted cells released large amounts of protein enriched in hydroxyproline into the medium, and gel electrophoresis demonstrated that several specific polypeptides were especially enriched in adapted cells. Concomitantly, the amounts of hydroxyproline in structural protein in the cells decreased markedly upon adaptation to stress. These data indicate that adapted cells released precursors to extensin, the structural proteins of the wall, and that failure to polymerize these precursors resulted in decrease in the tensile strength of the wall. These results obtained in the last year of our project have permitted us to narrow our focus of study from knowing only that physical properties of the cell wall were modified upon adaptation to stress to where we have identified specific changes in carbohydrate and protein components, and we expect to continue studies of enzymic turnover of

polysaccharides in adapted cells, including repression of specific xyloglucan endo-glycosidases, and the primary structure of novel wall proteins identified in the adapted cells and peroxidases involved in polymerization of precursor polypeptides and aromatic compounds during differentiation.

LIST OF PUBLICATIONS RELATED TO THE RESEARCH PROJECT

- Handa, S., R. A. Bressan, A. K. Handa, N. C. Carpita, P. M. Hasegawa. 1983. Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. *Plant Physiology* **73**, 834-843.
- Kanabus, J., N. C. Carpita, and R. A. Bressan. 1985. Carbon utilization by carrot cells in culture. *Plant Physiology* **77**(S), 13.
- Iraki, N. M., N. Singh, and N. C. Carpita. 1985. Organization of pectins in walls of tobacco cells adapted to salt or drought stress. *Plant Physiology* **77**(S), 134.
- Solomon, M., E. Gedalovitch, A. M. Mayer, and A. Poljakoff-Mayber. 1986. Changes induced by salinity to the anatomy and morphology of excised pea roots in culture. *Annals of Botany* **57**, 811-818.
- Kanabus, J., R. A. Bressan, and N. C. Carpita. 1986a. Preference for glucose or fructose among monocot and dicot cell lines in liquid culture. *Plant Physiology* **80**(S), 38.
- Iraki, N. M. and N. C. Carpita. 1986. Extracellular polysaccharides of *Nicotiana tabacum* cell cultures in relation to adaptation to drought and saline stress. *Plant Physiology* **80**(S), 97.
- Kanabus, J., R. A. Bressan, and N. C. Carpita. 1986b. Carbon assimilation in carrot cells in liquid culture. *Plant Physiology* **82**, 363-368.
- Solomon, M., R. Ariel, M. J. Hodson, A. M. Mayer, and A. Poljakoff-Mayber. 1987. Ion absorption and allocation of carbon resources in excised pea roots grown in liquid medium in absence or presence of NaCl. *Annals of Botany* **59**, 387-398.
- Hodson, M. J. and A. M. Mayer. 1987. Salt-induced changes in the distribution of

- amyloplasts in the root cap of excised pea roots in culture. *Annals of Botany* **59**, 499-503.
- Iraki, N. M., N. Singh, R. A. Bressan, and N. C. Carpita. 1987a. Changes in composition of cell walls of tobacco cells upon adaptation to osmotic stress. *Plant Physiology*, in preparation.
- Iraki, N. M., R. A. Bressan, and N. C. Carpita. 1987b. Extracellular Polysaccharides and Proteins of Tobacco Cell Cultures in Relation to Adaptation to Water and Saline Stress, *Plant Physiology*, in preparation.

REFERENCES

1. Binzel, M. L., P. M. Hasegawa, A. K. Handa, R. A. Bressan. 1985. Adaptation of tobacco cells to NaCl. *Plant Physiol* **79**, 118-125.
2. Binzel, M., P. M. Hasegawa, D. Rhodes, S. Handa, R. A. Bressan. 1987. Solutes involved in the osmotic adjustment of tobacco cells adapted to NaCl. *Plant Physiol*, in press
3. Bressan, R. A., A. K. Handa, S. Handa, P. M. Hasegawa. 1982. Growth and water relations characteristics of cultured tomato cells during adjustment to low external water potentials. *Plant Physiol* **70**, 1303-1309.
4. Carpita, N. C. 1985. Tensile strength of cell walls of living cells. *Plant Physiol* **79**, 484-488.
5. Handa, A. K., S. Handa, R. A. Bressan, P. M. Hasegawa. 1982. Characteristics of cultured tomato cells after prolonged exposure to medium containing polyethylene glycol. *Plant Physiol* **69**, 514-521.
6. Handa, A. K., R. A. Bressan, S. Handa, P. M. Hasegawa. 1983. Clonal variation for tolerance to PEG-induced water stress in cultured tomato celss. *Plant Physiol* **72**, 645-653.
7. Handa, S., R. A. Bressan, P. M. Hasegawa, A. K. Handa. 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. *Plant Physiol* **80**, 938-945.

8. LaRosa, P. C., A. K. Handa, P. M. Hasegawa, R. A. Bressan. 1985. Absciscic acid accelerates adaptation to cultured tobacco cells to NaCl. *Plant Physiol* 79, 138-142.
9. LaRosa, P. C., P. M. Hasegawa, D. Rhodes, R. A. Bressan. 1987. Osmotic adjustment stimulated by abscisic acid and its involvement in adaptation to NaCl in cultured tobacco cells. *Plant Physiol*, *in press*.
10. Rhodes, D., S. Handa, R. A. Bressan. 1986. Metabolic changes associated with adaptation of plant cells to water stress. *Plant Physiol* 82, 890-903.
11. Singh, N. K., A. K. Handa, P. M. Hasegawa, R. A. Bressan. 1985. Proteins associated with adaptation of cultured tobacco cells to NaCl. *Plant Physiol* 79, 126-137.
12. Singh, N. K., P. C. LaRosa, A. K. Handa, P. M. Hasegawa, R. A. Bressan. 1987. Hormonal regulation of protein synthesis associated with salt tolerance in plant cells. *Proc Natl Acad Sci USA* 84, 739-743.
13. Taiz, L. 1984 Plant cell expansion: regulation of cell wall mechanical properties. *Ann Rev Plant Physiol* 35, 585-657.