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Characterization of a Rice Gene Showing Organ-Specific Expression in Response to Salt Stress and Drought

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Protein changes induced by salinity stress were investigated in the roots of the salt-sensitive rice cultivar Taichung native 1. We found eight proteins to be induced and obtained partial sequences of one with a molecular mass of 15 kilodaltons and an isoelectric point of 5.5. Using an oligonucleotide probe based on this information, a cDNA clone, *salT*, was selected and found to contain an open reading frame coding for a protein of 145 amino acid residues. *salT* mRNA accumulates very rapidly in sheaths and roots from mature plants and seedlings upon treatment with Murashige and Skoog salts (1%), air drying, abscisic acid (20 μM), polyethylene glycol (5%), sodium chloride (1%), and potassium chloride (1%). Generally, no induction was seen in the leaf lamina even when the stress should affect all parts of the plant uniformly. The organ-specific response of *salT* is correlatable with the pattern of Na^+ accumulation during salt stress.

INTRODUCTION

To accommodate the growing demand for rice, more and more poorly irrigated land is being brought into cultivation. Inadequate drainage of the fields results in the progressive accumulation of salts in the soil, with the consequent decline in crop yield. Plant geneticists have been able to compensate partially for this loss by breeding for increased salt tolerance in commercial varieties of rice, but the mechanism by which tolerance is conferred remains unclear. If it were possible to identify the primary effects of salt on plant growth and the biochemical adaptation subsequently invoked, then it might be possible to use recombinant DNA technology to alter specific physiological processes in rice to increase its tolerance for salts.

Numerous metabolic changes have been noted to occur in different salt-sensitive plants subjected to ionic stress. Within 15 min of exposure to saline conditions, an Na^+/H^+ exchange process is activated so that K^+ can be pumped across the cell membrane (Watad et al., 1986) and Na^+ can be pumped into tonoplasts (Binzel et al., 1988; Garbarino and DuPont, 1989). Over the course of several days, the ratio of glycolipids to phospholipids can increase (Hirayama and Mihara, 1987), permitting more solutes to enter the cell and thus facilitate water entry, while the overall concentration of osmoprotectants including sugars, proline, and organic acids rises (Binzel et al., 1987; LaRosa et al., 1987). Abscisic acid also increases in osmotically stressed plants (Steward and Voetberg, 1985) and can induce, when applied exogenously, biochemical changes similar to those produced by salt (LaRosa et al., 1987). Such experiments have led to the conclusion that this hormone may normally be used to control part of the adaptation process.

The physiological changes of the adaptation process are accompanied by increases and decreases in a relatively small set of cellular proteins (Singh et al., 1985; Hurkman, Tanaka, and DuPont, 1988), some of which have been purified and characterized. The most extensively studied protein of this class has been referred to as osmotin (Singh et al., 1987a, 1987b). The biological activities of this 26-kD protein are not known, but its sequence resembles a maize α-amylase/trypsin inhibitor. Osmotin accumulates in vacuoles of salt-stressed plants (Singh et al., 1987a), although this increase may take more than 1 week to be detected by protein gel blotting (King, Hussey, and Turner, 1986). Osmotin has been found in several unrelated dicotyledonous plants but not in barley (King et al., 1986; Hurkman and Tanaka, 1987). However, two very different, osmotically regulated, cytoplasmic proteins have been identified in graminae. One, whose gene has been cloned from maize (Gómez et al., 1988), is likely to be an RNA-binding protein (Mortenson and Dreyfuss, 1989). The other gene, termed *rab21*, has been obtained from rice (Mundy and Chua, 1988) and shown to encode a glycine- and threonine-rich protein homologous to the cotton protein Lea-D11 (Dure et al., 1989).

Strikingly, none of the newly synthesized proteins appears to be an integral membrane component as expected if it was part of the ion pumps (Hurkman et al., 1988), nor

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is new protein synthesis necessary for increased Na^+ fluxes (Garbarino and DuPont, 1989). Thus, although it is presumed that these new proteins are essential for adaptation to high salt, the processes in which they might participate have not been assigned.

If an adaptive process is to be effective, it must be invoked before significant damage is done to the stressed tissues. To understand better the molecular events occurring during the early period, we have isolated one of the small set of proteins induced within 3 days of a salt treatment. Using the amino acid sequence obtained from one of these proteins, we have isolated a corresponding cDNA clone. With this clone as a probe, we show that the gene is rapidly induced by both salt and related osmotic stresses that mimic drought and that this induction is seen most clearly in the sheath tissues of rice plants, even when the stress is applied specifically to the roots. Moreover, it is generally not induced in the leaf laminae. This type of specificity demonstrates that there is not a uniform response to osmotic stress within the plant and may indicate differences in the types of adaptations used by different cells.

RESULTS

Selection of Proteins Highly Expressed in Salt-Stressed Roots

Repeated cycles of flooding and evaporation leave behind a mixture of salts in the rice paddy soils, some of which can be more deleterious to plants than others (Ben-Hayyim, Kafkafi, and Ganmore-Neumann, 1987; Hassan and Wilkins, 1988). To mimic the complex nature of this stress in the laboratory, rice was grown hydroponically in half-strength Hoagland solution and challenged with the addition of 2.2 times the normal concentration of Murashige and Skoog salts (Murashige and Skoog, 1962) (1% MS salts). Plants stressed in this way begin wilting and browning within 3 days to 4 days.

Proteins were extracted from roots harvested after 4 days of treatment with 1% MS salts and separated by two-dimensional gel electrophoresis. After staining with Coomassie Blue dye, at least nine protein species were consistently more abundant in stressed tissues and three consistently less abundant, as shown in Figure 1. On the basis of its abundance and apparent separation from other protein species, the 15-kD, 5.5 isoelectric point (pI) protein was chosen for further investigation.

Two protein gels were prepared, each containing approximately 100 mg of total protein. After electrophoresis, these were electroblotted to either glass fiber filters coated with poly (4-vinyl-*N*-methylpyridiniumiodide) or Immobilon® membranes and stained with fluorescamine or Coomassie Blue dye, respectively, so that the 15-kD protein spots could be identified and isolated (Bauw et al., 1987). The protein blotted to the glass filters was used immediately for sequencing to identify the amino acid-terminal residues. These were found to be TLVKI. The protein transferred to Immobilon® was digested in situ with trypsin, and the peptides were eluted and separated by HPLC chromatography (Bauw et al., 1988). One peptide was selected for

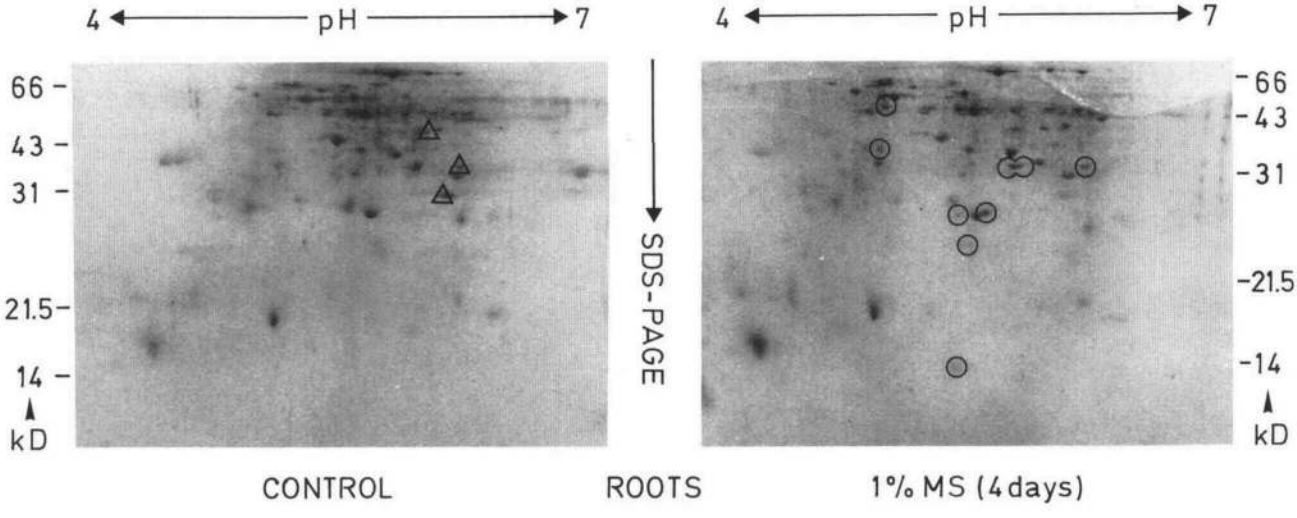

Figure 1. Comparison of Protein Patterns of Roots of Control and MS-Stressed Plants.

Analysis of protein patterns by two-dimensional gel electrophoresis (isoelectric focusing) of proteins isolated from roots of control plants (left) and roots of plants grown for 4 days in medium supplemented with 1% MS salts (right). Gels have been treated with Coomassie stain. Proteins whose abundance increased during stress are indicated with open circles (○); proteins whose abundance reduced during stress are indicated with open triangles (△). Numbers give the positions of the molecular mass protein markers.

sequencing and gave the sequence SGTLIDAIGIYVHP. The fact that the sequence terminated with proline rather than lysine or arginine, as tryptic peptides should, indicated that this might correspond to the carboxyl terminus of the protein.

Isolation of a cDNA for the Salt-Induced Protein

An oligonucleotide probe was prepared based upon the sequence of the putative COOH terminus and used to isolate a clone from a cDNA library prepared from roots of salt-treated plants. Upon sequencing, the clone (designated *sal*T) was found to contain a reading frame beginning with methionine, followed by the amino-terminal residues determined by protein sequencing, and ending with the expected carboxyl terminus, as illustrated in Figure 2. This reading frame was followed by 244 bases and ended with 14 adenosine residues. No readily discernable polyadenylation addition sequence could be found within the preceding 60 bases.

As shown in Figure 3, the cDNA clone was hybridized to rice nuclear DNA digested with EcoRI or HindIII. Both digests show between 3 and 5 hybridizing fragments,

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    CAAGAAAATTTAAGCGACGACGAAGAGT ATG ACG CTG GTG AAG ATT GGC CTG TGG

GGT GGA AAT GGA GGG TCA GCT CAG GAC ATC AGT GTG CCA CCC AAG AAG CTT

CTA GGC GTG ACA ATC TAC AGC TCA GAT GCA ATC AGA TCC ATT GCC TTC AAC

TAT ATC GGT GTG GAT GGA CAG GAA TAT GCC ATT GGT CCA TGG GGT GGG GGC

GAA GGC ACC TCT ACA GAG ATT AAA TTG GGC TCC TCT GAG CAT ATT AAG GAG

ATT TCT GGA ACC CAT GGC CCA GTC TAT GAT CTG GCT GAC ATT GTC ACC TAT

CTT AAG ATC GTG ACA AGT GCT AAT AAT ACA TAC GAG GCT GGA GTC CCA AAT

GGA AAG GAA TTC AGC ATT CCA CTG CAA GAC TCT GGC CAT GTC GTT GGA TTC

TTT GGA AGG TCT GGA ACG CTT ATC GAC GCA ATT GGC ATC TAC GTC CAC CCT
                           ATI GA(C/T) GCI ATI GGI ATI TA(C/T) GTI CA(C/T) CC

TGA TTCCCAGTGGTCAAAGAATTACTACCTACTACCATATCTACGAAATAATGTGCCATGGTGTTG

TTGTACTTCATGCAATCCCCCCATTGTCTGTGTACGTGTTGTACCGGTCCGTAGTACAATAAAGTTG

GTGATGTATGTTCCCCAGTTGACTCTTTAATATTTACTACAACAAGTTGGGGTCATTTGTACATGTT

CAGCACCTGGTCCGCTCACTTTATGTGTTTCTCATATTGGTATCACTAAAAAAAAAAAAAA
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Figure 2. Nucleotide Sequence of *sal*T.

Sequence as determined from the isolated cDNA insert. The putative reading frame is shown separated into nucleotide triplets ending with the TGA stop codon. Also indicated is the redundant synthetic oligonucleotide used to isolate the *sal*T clone. Identical bases between the cDNA sequence and the oligonucleotide are indicated by a dot.

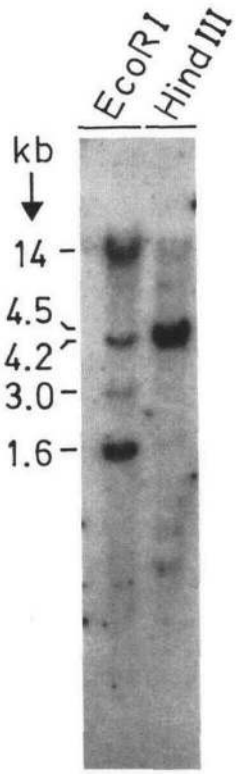

Figure 3. Rice Genomic DNA Digests Hybridized with *sal*T.

O. sativa genomic DNA was digested with EcoRI and HindIII and hybridized with the entire cDNA insert of *sal*T.

indicating either a small, multigene family or the presence of several introns disrupting the genomic sequences. If there are multiple copies of the gene, one must assume either that they are highly conserved or that only one is expressed in the root since no heterogeneity was detected in the sequenced peptides.

The protein sequence did not resemble any sequence present in either the Protein Identification Resource Sequence Data Bank or the University of Geneva Protein Sequence Data Bank. Like several other osmotically regulated proteins (Gómez et al., 1988; Mundy and Chua, 1988), it has glycine-rich repeats, as shown in Figure 4, but consisting of a motif not found elsewhere. When the initial methionine is cleaved off as the peptide sequence indicated, then the translation product would lack sulfur-containing amino acids, and have a molecular mass of 15.1 kD with a pI of 5.0, in agreement with the electrophoretic properties of the original rice protein. This agreement indicates that the protein is not extensively modified, although there is a potential N-glycosylation site (NNT) immediately following a β-sheet at amino acid position 102. The absence of apparent targeting signals indicates that transport to organelles or to the cell wall is unlikely.

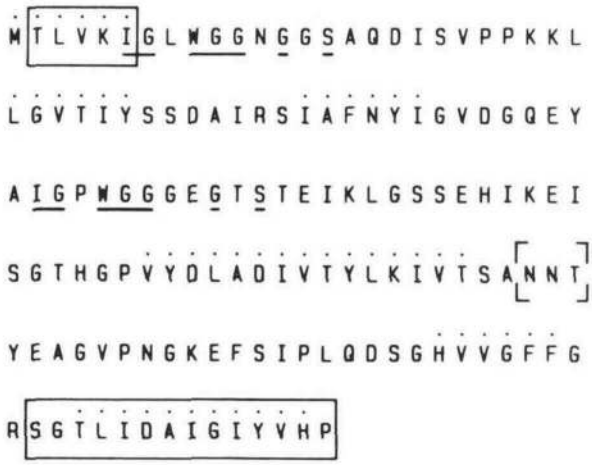

Figure 4. Deduced Amino Acid Sequence of the *sal*T Protein.

The amino acid sequence obtained from cDNA produces a 15.1-kD protein with a pI of 5.0. The boxed residues shown correspond to those determined by NH_2-terminal and tryptic peptide sequencing. Dots above amino acids indicate residues with a theoretical ability to form β-sheet structures. The open box represents a putative N-glycosylation site. An interspersed internal repeat found in the sequence is underlined.

*sal*T Messenger Levels Are Increased Rapidly by Excess Salts

The *sal*T cDNA hybridized to an RNA species of approximately 800 nucleotides present in small amounts in roots and sheaths of hydroponically grown rice (see Figure 5A). After 3 days of treatment with 1% MS salts, there was a slight increase in expression in leaf laminae and a considerable increase in the amount of RNA in roots and sheaths. This demonstrates that salt stress did not produce either a uniform or a continuously graded systemic induction of *sal*T messenger. On the contrary, the greatest difference between induced and uninduced tissues was seen in the sheath that was not in direct contact with the salt solution.

Genes may be induced during stress either as part of a primary response to minimize damage or because damage has been done resulting in secondary effects (for example, generalized protein denaturation or inappropriate gene expression resulting from inactivated regulatory molecules). The more rapidly a gene is induced, the less likely it is to be because of secondary effects. For this reason, the speed of induction of *sal*T mRNA was measured after plants were placed in 1% MS salts. As seen in Figure 5B, near-maximal expression is reached in the sheath between 2 hr and 6 hr after the stress is applied. Maximum root expression is only seen after 1 day (data not shown). Analysis of protein gels showed that the *sal*T peptide was easily detected with Coomassie Blue dye 3 days to 4 days after salt treatments; however, we do not know whether the rate of protein accumulation differs in different organs.

Based on this experiment, it would seem that the *sal*T protein may be produced as part of the primary response to salt stress and not symptomatic of prolonged damage. Further support for this interpretation came from experiments with the regulatory molecule abscisic acid (ABA), which has been found to induce a variety of osmotically regulated genes (Singh et al., 1985; Gómez et al., 1988; Mundy and Chua, 1988) and to accelerate osmotic adjustment (LaRosa et al., 1987). If *sal*T were induced solely by the damage resulting from osmotic shock, then it should not respond to ABA treatments. Instead, it was seen that 20 μM ABA was as effective at inducing *sal*T in each part of the plant (see Figure 6) as the more obviously deleterious treatment with salt shown in Figure 5A. Even 0.4 μM ABA, which is not inhibitory to rice callus growth (Torrizo and Zapata, 1986), stimulated *sal*T RNA accumulation in the roots after 3 days. However, unlike the response to stress, 0.4 μM ABA induced a gradient of expression, greatest in roots where the hormone was applied and least in the lamina. This could correspond to the internal ABA gradient generated by applying the hormone externally or to the sensitivity of the ABA receptors throughout the plant.

The mixture of ions present in MS salts and in saline soils forces the plant to compensate both for new osmotic conditions and for the specific inhibitory effect that some inorganic ions can have when present in excess. For this reason, we tested whether rice plants responded similarly to high concentrations of potassium and sodium. The former is one of the major cations of MS medium, whereas the latter is virtually absent. Eight-day-old seedlings were given a 7-day treatment with 0.3% and 1% of either NaCl or KCl. Before isolation of the RNA for RNA gel blot

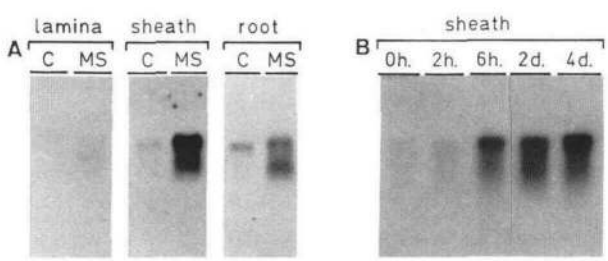

Figure 5. RNA Gel Blot Analysis of Expression of *sal*T under MS Stress.

(A) Analysis of total RNA from different organs of rice grown in control medium (C) and grown for 3 days in medium supplemented with 1% MS salts (MS). Each lane contained 10 μg of RNA and was hybridized simultaneously to *sal*T cDNA insert.

(B) Analysis of total RNA from sheaths of rice plants treated for various times with 1% MS. Stress was applied for 0 hr, 2 hr, 6 hr, 2 days, and 4 days. Each lane contained 10 μg of RNA.

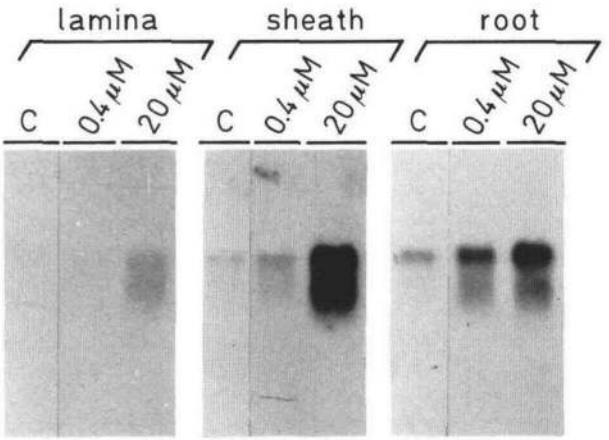

Figure 6. RNA Gel Blot Analysis of Expression of *salT* in Response to ABA.

Total RNA from different organs was analyzed from control plants (C) and plants treated for 3 days with 0.4 μM and 20 μM ABA in the hydroponic medium. Each lane contained 10 μg of RNA prepared and hybridized simultaneously with treatments shown in Figure 5A.

analysis, the average length of the sheaths of 15 seedlings was measured to determine growth inhibition. As seen in Figure 7, 0.3% NaCl has nearly no effect on the growth rate of the seedlings, whereas 0.3% KCl has a slight stimulatory effect. At this concentration, the seedlings are exposed to at least as much K^+ and Cl^- and several orders of magnitude more Na^+ as is found in 1% MS salts. Despite this, the levels of *salT* mRNA did not change (Figure 7). This shows that the gene is not induced to eliminate any of these three ions selectively. On the other hand, when 1% NaCl or KCl was used, seedling growth was drastically reduced and *salT* messenger accumulated in the sheath. High levels of NaCl also induced the expression of the gene in leaf lamina. These results might indicate that the final salt concentration (regardless of whether it is MS, NaCl, or KCl) is at least as important at determining the level of *salT* expression as the particular composition of the salt.

Osmotic Stress Alone Can Enhance *salT* Gene Expression

The net influx of ions into salt-treated plants reduces the concentration of free water within the cell. To an extent, therefore, salt concentrations induce the same stresses as drought. To determine whether *salT* was induced during water deprivation as well as during salt stress, plants were gently dried and allowed to stand at room temperature without water for 3.5 hr and then assayed. As seen in Figure 8A, uniform drying did not induce *salT* uniformly: little gene expression was detected in the leaf blades, in comparison with the response in roots and sheaths. In addition, messenger levels in the sheaths and roots remained high even after plants were returned to the hydroponic medium for 3 days. This may indicate that the physiological consequences of dehydration are not easily reversed and, consequently, plants need more than 3 days to recover.

The response to dehydration appears to be even more rapid than the response to salinity. It was seen (Figure 8B) that the level of *salT* RNA begins to increase in the root after 30 min and reaches a maximum within 1 hr. Maximal *salT* expression in the sheath was also seen after 1 hr but there was no induction in laminae (data not shown).

Drought can be mimicked by treatments with polyethylene glycol (PEG). PEG is not readily taken up by cells; thus, it reduces the external free water concentration without altering the ionic composition of the cell. The treatment, however, is localized to the roots, so the effects should be more severe there. Yet, after 3 days, there was increased gene expression in both sheath and roots (Figure 8A), although the response was not as great as that

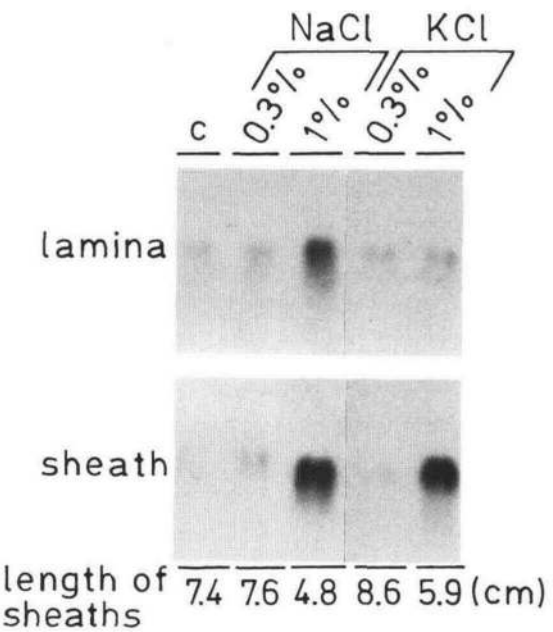

Figure 7. Correlation between Growth and Expression of *salT* in Rice Seedlings in Response to Various NaCl and KCl Concentrations.

RNA gel blot analysis of *salT* messenger in seedlings grown for 7 days in control medium (C) and in control medium supplemented with 0.3% and 1% NaCl or KCl. Each lane contains 10 μg of RNA. Below each lane is the average length (n = 15) of sheaths of control plants and of plants grown in control medium supplemented with 0.3% and 1% NaCl or KCl.

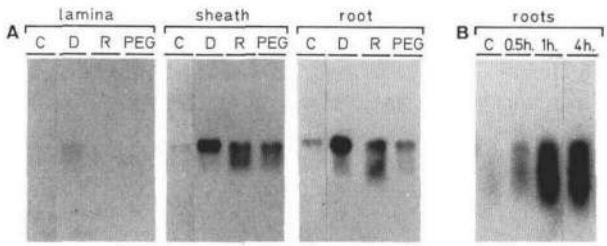

Figure 8. RNA Gel Blot Analysis of the Expression of *sal*T in Response to Osmotic Stress.

(A) Total RNA (10 μg) was analyzed from different organs of rice plants grown in control medium (C), air-dried for 3.5 hr (D), air-dried for 3.5 hr and then rewatered by transferring back to the control medium (R), and plants grown for 3 days in medium supplemented with 5% PEG (PEG). RNA was prepared and analyzed simultaneously with experiments shown in Figure 5A.
(B) Analysis of total RNA from roots of control (C) plants and of plants air-dried for 0.5 hr, 1 hr, and 4 hr.

seen when plants were actually deprived of water; this might depend upon the concentration of PEG used.

These experiments indicated that *sal*T mRNA was produced in large amounts only in certain regions of the plant. One additional stress was found to elevate messenger levels more evenly, extending the zone of high expression from the sheath into the leaf blade. This stress consisted of a temperature shift from 27°C to 37°C. After 4 hr, *sal*T mRNA increased as with other stresses in both root and sheath. However, after 3 days, strong expression could be seen in the upper leaf, as illustrated in Figure 9. Because heat stress is inadvertently accompanied by increased water loss, it is not clear whether high temperature or rapid water loss plays the greater role in inducing *sal*T throughout the plant; however, *sal*T was not induced in suspension culture cells shifted from 28°C to 42°C for 3 hr (data not shown).

DISCUSSION

Physiological studies have demonstrated that a complex array of processes is employed by plants to adapt to saline environments. The first defense is presumably the selective exclusion of excess ions by the roots. When this fails, the affected cells attempt to sequester excess ions in tonoplasts to minimize damage to sensitive proteins (Binzel et al., 1988). This defense is augmented by increases in the concentrations of various osmoprotectants including proline, sugars, and organic acids (Handa et al., 1983; Steward and Voetberg, 1985; Binzel et al., 1987). Several studies, however, have indicated that proline levels do not necessarily correspond to the tolerance of plants for salt and may be induced only after damage has been sustained (Bhaskaran, Smith, and Newton, 1985; Chandler and Thorpe, 1987; Moftah and Michel, 1987). It may be that some of the heat shock proteins produced by salt stress (Heikkila et al., 1984; Harrington and Alm, 1988) similarly are needed to cope with damaged cellular components.

These physiological changes can occur in any cell type exposed to adverse conditions. There is additional evidence, however, for a coordinated response between the cells of different portions of the plant. Maize, for example, accumulates 7 times to 10 times more Na^+ in the mesocotyl than in the shoot, and 2 times to 3 times more in the mesocotyl than in the root (Drew and Läuchli, 1987). Rice, although morphologically distinct, concentrates salt in an equivalent gradient. Yeo and Flowers (1982) showed that the rice sheath harbored 4 times more Na^+ than the leaf laminae.

The pattern of Na^+ accumulation matches the pattern of *sal*T expression. After salt treatments, the messenger levels from this gene were highest in the sheath and lowest in the leaf laminae. Additionally, the response time and rate of accumulation of the RNA were comparable with the rate of accumulation of Na^+ in maize mesocotyls (Drew and Läuchli, 1987) and within the estimated rate of Na^+ accumulation for rice sheaths (Yeo and Flowers, 1982). This rapid rate of induction might indicate that the gene is not responding to damage that has occurred, but rather is produced in response to changing salt concentrations to prevent damage and, as such, help the cells to become

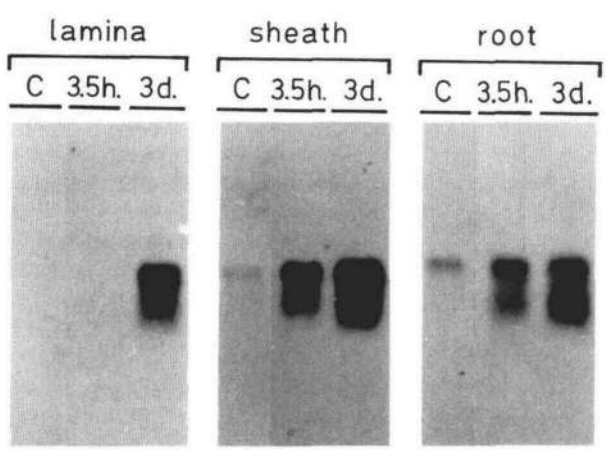

Figure 9. RNA Gel Blot Analysis of the Expression of *sal*T after Shifting Plants from 24°C to 37°C.

Total RNA (10 μg) was analyzed from different organs of control plants (C) and from plants shifted to 37°C for 3.5 hr or 3 days. RNA was prepared and analyzed simultaneously with samples shown in Figure 5A.

more tolerant. The fact that ABA induces the gene without inducing osmotic damage and without growth impediment (Torrizo and Zapata, 1986) is also consistent with this interpretation.

Both water deprivation and treatment with PEG induced *saI*T messenger accumulation without necessarily adding to ion pools throughout the plant. Both conditions produced the same RNA distribution as that induced by different salts. This may mean that water is lost more readily from the sheath than elsewhere, although the roots would seem to be the more vulnerable portion of the plant. The converse might, therefore, be more likely. That is, that water is rapidly drawn from the sheath to replace that lost from leaves or roots, or that salts are transferred from leaves and roots into the sheath to maintain a critical osmotic balance in the two more important tissues. As the availability of water declines, *saI*T could be induced for some form of osmoprotection. However, we have no direct evidence that rice can adapt successfully to salt stress during the time that *saI*T is produced.

The only condition that induced significant *saI*T expression within 3 days in the laminae was prolonged heat treatment. This does not necessarily imply that the gene is a member of the heat shock regulon. Most heat shock genes are induced rapidly, within 1 hr of treatment (Kanabus, Pikaard, and Cherry, 1984), yet *saI*T was not induced in the upper leaf areas even after 3.5 hr. Because the magnitude of the response to heat in the roots and sheath is comparable with that due to water deprivation alone (Figures 8A and 8B), it is quite possible that induction is not due to heat per se, but reflects the progressively deteriorating ionic balance as more and more water is lost through transpiration.

It is not possible to assign a particular function to this protein, nor even, at this time, to be certain that it is directly involved in osmotic adjustment. It appears to lack transit sequences for export into organelles or to the cell surfaces, and, thus, is probably not an internal component of a vesicular pump or external component of the cell wall. It is also evident that many different salts and ionic conditions increase *saI*T gene expression (Figures 5 and 7; and B. Claes, A. Caplan, and R. Dekeyser, unpublished results) so that the protein is not likely to be involved in excretion or detoxification of a specific ion.

The restricted distribution of Na^+ protects the most photosynthetically active parts of the leaf from salt-induced damage and has been shown to provide a fairly reliable physiological indicator for salt tolerance in rice (Yeo and Flowers, 1983). Being able to monitor changes in gene expression in the sheath tissue may help identify limiting steps in this aspect of the adaptive process. Such limitations may go undetected if one looks solely at the physiology of undifferentiated callus (Bhaskaran et al., 1985). Productive changes can be genetically engineered into rice only after the limiting steps in the adaptive processes occurring at both the cellular and organismal levels have been identified.

METHODS

Plant Material and Experimental Conditions

Rice seeds (*Oryza sativa*, var. Indica, cv Taichung native 1) were obtained from the International Rice Research Institute (Manila, Philippines). Seeds were sown on vermiculite impregnated with half-strength Hoagland solution (Hoagland and Arnon, 1938) and grown at 27°C for 14 days. Plants were then transferred and cultured hydroponically for 6 more weeks in half-strength Hoagland solution. The induction studies were carried out by adding the appropriate concentrations of the indicated products to a half-strength Hoagland solution. Plants were then maintained in these hydroponic cultures for various times. Drying was performed for the indicated periods by putting the plants on paper towels at room temperature. Rewatering of plants occurred by putting the plants back in the half-strength Hoagland solution.

If seedlings were used, they were germinated and grown in sterile jars on a medium containing 0.8% agar (Difco), 1% sucrose, and 0.5% MS salts (Murashige and Skoog, 1962). Induction experiments were carried out by transferring seedlings to the same medium containing the appropriate concentration of salts.

Protein Analysis

The protein extraction procedure was done as described by Hurkman and Tanaka (1986) and slightly modified according to Bauw et al. (1987).

Two-dimensional gel electrophoresis was carried out for the first dimension under isoelectric focusing conditions according to O'Farrell (1975) and O'Farrell, Goodman, and O'Farrell (1977) with minor modifications according to Bravo (1984). In the second dimension we used the discontinuous SDS-PAGE gel system (Laemmli, 1970).

For protein sequence analysis, the separated proteins were electroblotted onto poly(4-vinyl-*N*-methylpyridiniumiodide) (P4VMP)-coated glass fiber and stained with fluorescamine (1 mg/L) or to Immobilon® membranes and stained with Coomassie Blue dye (Bauw et al., 1987). NH_2-terminal amino acid sequencing was performed as described by Bauw et al. (1987) on protein bound to the P4VMP-coated glass fiber.

Sequencing of internal peptides was carried out after trypsin digestion of the protein bound to the Immobilon® membranes (Bauw et al., 1988). The amino acid sequence analysis was performed using a 470A gas-phase sequenator equipped with a 120A on-line phenylthiohydantoin amino acid analyzer (Applied Biosystems Inc., Foster City, CA).

With the open reading frame from the *saI*T cDNA clone we screened the Protein Identification Resource Sequence Data Bank (release No. 18, September 1988) and the University of Geneva Protein Sequence Data Bank (Swissprot, release No. 9, December 1988) using the FASTDB program (release No. 5.3) supplied by Intelligenetics Inc. (Mountain View, CA).

cDNA Cloning and Isolation of *saI*T cDNA

A cDNA library was prepared using kits obtained from Pharmacia LKB Biotechnology Inc. (Uppsala, Sweden) and Promega Co. (Madison, WI) from poly(A)$^+$ RNA isolated from rice roots stressed with 1% Murashige and Skoog salts for 3 days. After second-

strand synthesis and methylation, EcoRI linkers were added. The cDNA was purified from excess linkers according to Pharmacia. The cDNA was cloned into the EcoRI site of pSP65 (Melton et al., 1984) and transformed into SCS-1 bacteria. Oligonucleotides were synthesized by the phosphoramidite method with a 380A Applied Biosystems DNA synthesizer. The oligonucleotide utilized was a 29-mer with three mixed C/T bases and inosine (Ohtsuka et al., 1985) at the other six positions of codon degeneracy (Figure 2). Oligonucleotides were labeled at the 5′ end with γ-^{32}P-ATP and T4 polynucleotide kinase (Bethesda Research Laboratories) (Maniatis, Fritsch, and Sambrook, 1982) and used to screen the cDNA library. The cDNA library was divided into 24 pools of bacteria from which miniprep DNA was prepared (Birnboim and Doly, 1979) and digested with various enzymes. These digests were transferred onto nylon filters (Amersham) and hybridized against the oligonucleotide at 40°C in 15% formamide, 5 × SSC, 5 × Denhardt's, 1% SDS, and 2 μg/mL heparin. Washing was done at 42°C in 5 × SSC, 0.5% SDS. In this way, 82,000 clones were screened. Colonies from hybridizing pools were screened against the oligonucleotide under the same conditions until a pure colony could be isolated.

DNA Sequencing Analysis

Double-stranded DNA nucleotide sequence was generated with the dideoxy chain termination method of Sanger, Nicklen, and Coulson (1977). The T7 sequencing kit from Pharmacia employing 7-deaza mixes was used. Labeling was done with α-^{35}S-dATP (10 mCi/mL). The products of the sequencing reactions were electrophoresed on 5% acrylamide, 50% urea, 1 × 27 mM Tris-HCl, pH 8.9, 9 mM $Na_2B_4O_7$, 0.05 mM EDTA field gradient gel system (0.25 mm to 0.8 mm). Gels were dried on plates as described by Garoff and Ansorge (1981). Autoradiograms were exposed for about 40 hr.

RNA Gel Blot Analysis

Total RNA was prepared by the method of Jones, Dunsmuir, and Bedbrook, (1985). RNAs were separated through formaldehyde gels and blotted onto nylon filters (Amersham) and hybridized to randomly primed probes from the cDNA insert using standard protocols (Maniatis et al., 1982).

DNA Hybridization

Genomic DNA from greenhouse-grown rice was prepared according to Timberlake (1978) and digested with EcoRI and HindIII according to the manufacturer's instructions (Bethesda Research Laboratories). DNA was separated on 0.8% agarose gel and transferred to nylon filters (Amersham). Hybridization to probes from the cDNA insert fragment was performed in 3 × SSC, 5 × Denhardt's, 0.5% SDS, and 100 μg/mL denatured salmon sperm DNA at 60°C. Washing conditions were 1 × SSC, 0.5% SDS at 60°C.

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