rank departments. There is continual pressure to fill gaps and improve academic rating schemes. Despite, or even because of controversy, the US News and World Report, and its counterparts, carry great weight and drive changes to meet their standards. The modest proposal above could recognize departments and schools that have succeeded in lifting the 'triple burden'.

Our analysis confirms the findings of various authors on women in science in different countries, that there is gender-related discrimination and stress in the practice of science. It is apparent that the stress is a product of similar reasons: a lack of universalism in science, undue burden of domestic responsibilities and lack of informal networking. Among these, while domestic burden is a product of gender stereotyping, the other two arise from the social organization of science. However, social norms related to gender infiltrate the organizations where science is practised, and the three are therefore interrelated. The common triple burden proves that the culture of science and the universal gender role schism is responsible for a low position of women in science across continents.

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Salt stress effects on the accumulation of vacuolar H^+-ATPase subunit c transcripts in wild rice, *Porteresia coarctata* (Roxb.) Tateoka

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The ion pump, vacuolar H^+-ATPase (V-ATPase) plays a major role in the maintenance of cellular pH, and influences the transport of cations into the vacuoles of plant cells. A cDNA clone (*PcVHA-c1*) encoding the c subunit of V-ATPase was isolated from the salt-tolerant wild rice, *Porteresia coarctata*. The DNA sequence of *PcVHA-c1* showed significant homology with V-ATPase subunit c of rice. The deduced amino acid sequence of *PcVHA-c1* and other reported c subunits were compared, and sequence relationships have been drawn to know their genetic relatedness. Southern analysis suggested the presence of multiple coding regions for subunit c in *P. coarctata*. Northern and Western analyses of salt-treated *P. coarctata* plants revealed that subunit c of V-ATPase is upregulated by NaCl treatment at both transcriptional and translational level.

Keywords: *Porteresia coarctata*, salt tolerance, subunit c, V-ATPase, wild rice.

TONOPLAST of the plant cell separates the vacuole from the cytoplasm. Specific proteins present in the tonoplast drive the transport of ions and other metabolites across it[1]. The selective transport properties of the tonoplast are central

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to the multifarious functions of the vacuole, and serve to retain toxic materials within the vacuolar sap while exchanging metabolically useful compounds with the cytosol. Therefore, in the past few years, there has been increasing emphasis on understanding the nature of transporters in the vacuolar membranes[2,3]. The primary active transport process in the tonoplast is the accumulation of H^+ by the action of two H^+ translocating enzymes[4], i.e. H^+-ATPase and H^+-PPase. The vacuolar H^+-ATPase (V-ATPase) is a universal component, and it is involved in acidification of intracellular compartments of eukaryotic cells[1,5]. The V-ATPase pumps protons from the cytoplasm to the lumen of the vacuole against the electrochemical gradient using the energy released by ATP hydrolysis, and regulates[1] the cytoplasmic pH.

V-ATPase is a multi-subunit complex composed of two functional domains, the peripheral V_1 cytoplasmic domain and the membrane integral V_0 domain[6]. The molecular mass of the V_1 domain is about 500 kDa, and it is responsible for ATP hydrolysis. It consists of at least eight different subunits of molecular weight 13–70 kDa (subunits A–H). The V_0 domain is a 250 kDa complex consisting of at least five different subunits of molecular weight 17–100 kDa (subunits a–d), and is involved in proton translocation[7]. The major components of V-ATPase, subunits A, B and c are found in all V-ATPases studied so far and thus seem to be essential subunits. The V_0 domain has been shown to contain the binding site for bafilomycin[8]. The 16 kDa polypeptide subunit c is particularly interesting as it is a major component of the membrane V_0 domain, and it plays a major role in forming the proton conductance pathway[8,9]. The 16 kDa polypeptide is highly hydrophobic, initially cloned from bovine adrenal cells[10] and has been widely studied in yeast[11] and also in higher plant systems[12].

The ability of plants to adapt and grow in a changing environment suggests that V-ATPase is subject to regulation by hormones and environmental factors[2], and some evidence is available for the enhancement of V-ATPase activity in response to stress due to chilling and salinity, where V-ATPase itself undergoes changes in its fine molecular structure[13]. The present investigation was therefore undertaken to understand the regulatory nature of the V-ATPase subunit c gene isolated from a salt-tolerant mangrove associated species *Porteresia coarctata*, long considered as a wild relative of cultivated rice. This communication deals with the structural and functional aspects of the V-ATPase subunit c from *P. coarctata* and its regulation under salinity stress.

P. coarctata plants growing in high saline conditions in the mangrove forests in the estuarine region of Pichavaram, Tamil Nadu, India were collected and grown in pots with clay soil in greenhouse under natural light condition. Young tillers produced in two months time were used for experimental studies.

Genomic DNA was isolated from leaves of *P. coarctata* as described previously[14] and digested with different restriction enzymes according to the manufacturer's instructions. The DNA run on a 0.8% agarose gel was transferred to nylon membrane (Hybond N^+, Amersham Biosciences) by capillary transfer method[15]. Membranes were incubated in pre-hybridization solution (6X SSC, 5X Denhardt's reagent, 0.5% SDS, 50% formamide and 100 µg/ml denatured Salmon sperm DNA) at 42°C. After 3 h, the pre-hybridization solution was replaced with hybridization solution (6X SSC, 0.5% SDS, 50% formamide and 100 µg/ml denatured salmon sperm DNA). Hybridization was carried out overnight at 42°C with gentle rotation. The membrane was sequentially washed in 2X SSC, 0.1% SDS and 1X SSC, 0.1% SDS at room temperature for 15 min each, followed by a high stringency wash (0.1X SSC, 0.1% SDS) at 55°C for 30 min, and exposed to X-ray film with intensifying screens at –70°C. Southern analysis was performed using the full-length *PcVHA-c1* probe.

Total RNA from leaf and root tissues of *P. coarctata* was isolated following the protocol as previously described[16]. Total RNA (20 µg) was separated in a 1.4% formaldehyde agarose gel and transferred to nylon membrane (Hybond N^+, Amersham) by capillary transfer method[15]. Pre-hybridization was carried out for 1 h at 42°C in 50% formamide, 6X SSC (1X is 0.15 M NaCl, 0.015 M sodium citrate), 25 mM sodium phosphate buffer (pH 6.5), 10X Denhardt's solution (1X Denhardt's solution is 0.02% Ficoll, 0.02% PVP, 0.02% BSA) and 250 µg/ml denatured salmon sperm DNA. Hybridization was carried out overnight at 42°C in 50% formamide, 6X SSC, 25 mM sodium phosphate buffer (pH 6.5) and 250 µg/ml denatured salmon sperm DNA. The membranes were washed in medium stringency buffer (0.5X SSC, 0.1% SDS) at 55°C for 15 min, and exposed to X-ray film with intensifying screens at –70°C. Northern analysis was initially carried out with the full-length *PcVHA-c1* probe. The leaf RNA blot was then stripped and re-probed with *PcVHA-c1* UTR-specific fragment. The primers used for PCR amplification of the UTR region of V-ATPase are: V-ATPase forward- 5′-TTG CAG TAC CAA TCC GCA GTT-3′; V-ATPase reverse-5′-GGA GCA GCA AAT CCA GCC TA-3′. The program used for amplification of the UTR region using these primers included an initial denaturation step of 94°C for 1 min; 30 cycles of 94°C for 30 s, 66°C for 45 s and 72°C for 30 s; and a final extension of 72°C for 5 min. The amplified product was gel-eluted and labelled by random labelling method (Amersham Biosciences). The membranes were washed in high stringency buffer (0.1X SSC, 0.1% SDS) at 65°C for 15 min, and exposed to X-ray film with intensifying screens at –70°C. Relative transcript amount in Northern experiments was measured based on pixel intensity using a Imagemaster software (Amersham Biosciences).

Total RNA was isolated from the leaf tissue of *P. coarctata* plants treated with 0.5 M NaCl for 48 h and mRNA was purified by oligo-dT cellulose column chromatography. Double-stranded cDNA was synthesized from purified mRNA using Superscript II reverse transcriptase (Gibco-BRL cDNA synthesis kit) and ligated to pSPORT1 plasmid vector in the *Not*I and *Sal*I restriction sites. The ligation product was then transformed into *Escherichia coli* DH5α and maintained as plasmid DNA lots.

A homologous probe for V-ATPase subunit c gene from *P. coarctata* was obtained by PCR amplification using degenerate primers. The forward primer designed in the 5′ coding region (5′-GGAGCTGCTTATGGWACWGC-3′) and the reverse primer in the 3′ coding region (5′-AAGATGAGRATSAGGATCATGCC-3′) corresponded to conserved sequence domains after ClustalW alignment of V-ATPase subunit c sequences reported from other species. The double-stranded cDNA synthesized from *P. coarctata* was used as template. Thirty cycles of PCR were performed at 94°, 55° and 72°C for 1 min each. A specific amplification product of 0.35 kb fragment was gel-purified and used as a probe to screen *P. coarctata* cDNA library. A putative V-ATPase subunit c clone was obtained using screening methodology following the radioactive method of screening[15].

PcVHA-c1 was subcloned as a *Hin*dIII-*Not*I fragment in pSPORT2 vector (CNH7), and as a *Sal*I–*Hin*dIII fragment in pSPORT1 vector (VT3). The nucleotide sequence was obtained for both strands of the subclones by the Big-Dye terminator detection method using M13 forward and reverse primers on an ABI377 DNA sequencer (Applied Biosystems). The full nucleotide sequence of *PcVHA-c1* was aligned from the sequences of the subclones. BLAST algorithm was used for sequence analysis[17]. The protein sequence of *PcVHA-c1* was deduced using SeqAid II version 2.01(+) program. The amino acid sequence of *PcVHA-c1* and that of different subunit c protein sequences from GenBank were aligned using the multiple alignment program of CLUSTALW[18]. A cladogram was obtained by the tree-drawing program of DNASIS program.

Microsomal fractions were isolated from leaf tissue of control and 0.5 M NaCl-treated *P. coarctata* plants following the reported method[19]. *P. coarctata* plants grown in greenhouse were kept initially in nutrient solution for a week and then replaced with fresh nutrient solution (control) or nutrient solution supplemented with 0.5 M NaCl (treated) for 48 h and leaves harvested from controls and treated plants for the isolation of microsomal fractions. About 200 g fresh control and treated leaves were homogenized at 4°C with a commercial warring blender in 400 ml homogenization buffer (50 mM Hepes-bis-Tris propane, pH 7.4; 250 mM sorbitol; 0.5% BSA and 0.1 mM phenylmethylsulfonyl fluoride). The homogenate was filtered through four layers of cheesecloth and centrifuged at 14,000 *g* for 20 min to remove cell debris and mitochondria. The supernatant was then centrifuged at 80,000 *g* for 45 min to pellet microsomal membranes. The pellet was layered onto a discontinuous sucrose gradient (16, 22, 36 and 45%) and centrifuged at 90,000 *g* for 5 h. The tonoplast membrane layer between 16 and 22% was collected and diluted in homogenization buffer and precipitated with 20% TCA, washed with cold acetone and finally resuspended in 25 mM Hepes-bis-Tris propane (pH 7.4), 250 mM sorbitol and 0.1 mM phenylmethylsulfonyl fluoride. Protein concentration was determined using the manufacturer's instructions (Bangalore Genei, India).

The open reading frame of *PcVHA-c1* was cloned into pET 28a(+) expression vector in the *Sal*I and *Not*I restriction site (pETSc 9). The recombinant clone pETSc 9 was transformed into *E. coli* BL 21 (DE3), and induced with 1 mM IPTG. The over-expressed protein was purified through nickel affinity binding column[20]. The purified protein (250 µg) was injected into rabbit as a first sensitization dose. After 20 days, 125 µg protein was injected as challenge dose. Three challenge doses were given at 20 days intervals. Antiserum was collected 20 days after the third challenge dose, and used to detect *P. coarctata* subunit c. Monoclonal antibodies for subunits A and B were obtained from Heven Sze, University of Maryland, USA. Western analysis and immunostaining were carried out following methods described earlier[19].

In this study, we have constructed a cDNA plasmid library from *P. coarctata* and maintained in *E. coli* DH5α. The cDNA library consisted of 1×10^5 clones represented in 50 lots of 2000 colonies each. An analysis performed with randomly selected colonies from the cDNA library showed that the size distribution of cDNA inserts in the library ranged from 0.5 to 2.5 Kb. The range and size-distribution of clones was a good indicator that the *P. coarctata* cDNA library could be a good source for isolation of genes differing in size and function.

A full-length cDNA clone designated *PcVHA-c1* (*Portersia coarctata* Vacuolar H^+-ATPase c-subunit gene number 1), coding for the V-ATPase subunit c was obtained from *P. coarctata* cDNA library. The insert size of *PcVHA-c1* was 876 bp. DNA sequencing of the clone revealed a translation initiation codon, ATG at 72–74 bp, and a stop codon, TAA at 567–569 bp. The open reading frame (ORF) was flanked by a 71 bp 5′-untranslated region (UTR), and 307 bp 3′-UTR. A polyadenylation signal, AATAAA was found between 794 and 799 bp. A poly $(A)^+$ tail of 44 bp length was detected at the 3′-end. The longest ORF of *PcVHA-c1* translated a protein (designated PVC1 based on the corresponding cDNA *PcVHA-c1*) of 165 amino acids with a calculated molecular mass of 16.5 kDa (Figure 1). The deduced protein sequence showed significant homology with V-ATPase subunit c of *Oryza sativa* (GenBank accession no. U27098) and *Avena sativa* (GenBank accession no. M73232). The amino acid sequence was 99 and 98% identical with *O. sativa* and *A. sativa* respectively (Figures 2 and 3). However, the nucleotide sequences of the coding region showed 92% homology with *O. sativa*, and 88%

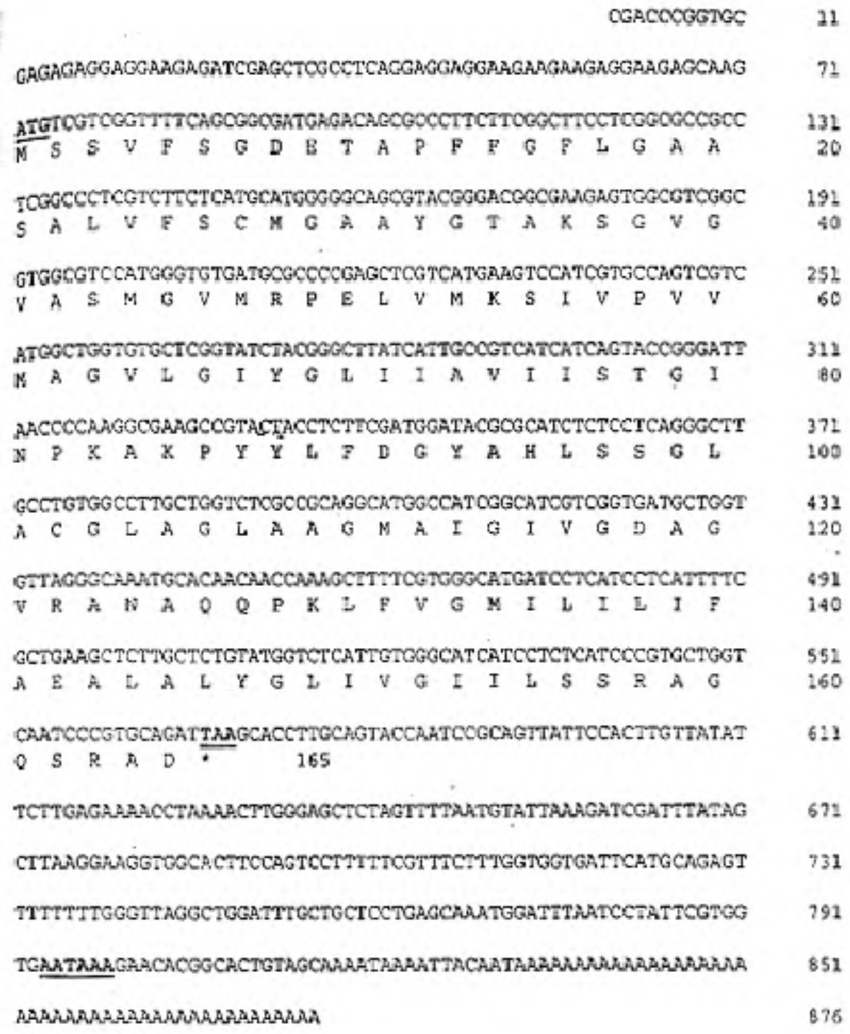

Figure 1. Nucleotide sequence and deduced amino acid sequence of *PcVHA-c1* cDNA. The deduced amino acid sequence is shown in single letter code below the first nucleotide of each codon. The putative translation start site ATG is indicated in bold and underlined. The TAA termination codon is indicated by an asterix and underlined. The polyadenylation signal AATAAA is also underlined.

homology with *A. sativa*. The nucleotide sequence of *PcVHA-c1* was submitted to GenBank under the accession number AF286464.

Southern analysis showed the presence of more than one hybridizing fragment with all the restriction enzymes, even after stringent hybridization washes using full-length *PcVHA-c1* probe (Figure 4). The fragments ranged in size from 0.63 to 24 Kb. It produced seven fragments with *Eco*RI, *Sac*I; six with *Dra*I, *Hin*dIII; five with *Pvu*II, *Bgl*II. These results indicated the possibility that a multigene family encodes the subunit c V-ATPase in *P. coarctata*.

Transcript level for V-ATPase subunit c in leaves and roots of *P. coarctata* was determined by Northern blot analysis. *PcVHA-c1* probe used for preliminary Northern analysis correspond to the entire coding region of subunit c, and therefore, the resulting transcript signal reflected the sum of all isoforms of V-ATPase subunit c. Northern analysis on RNA isolated from leaves indicated that there is no significant increase in the subunit c transcript level until 5 h after salt treatment. However, increase in the transcript level was two-fold after 24 h and three-fold after 48 h. The subunit c transcript level declined after 10 h of salt withdrawal (Figure 5). In roots, the transcript level of V-ATPase subunit c increased two-fold after 5 h, indicating the response in roots was immediate and more pronounced than in leaf tissues. After salt withdrawal, the transcript levels started decreasing. The level of transcript declined to 2.5-fold after 10 h of withdrawal from salt stress (Figure 5). Furthermore, we wanted to analyse the expression with *PcVHA-c1* 3′-UTR-specific primers as probe, and we observed a similar trend. Again, we observed that the transcript levels did not increase until 5 h of salt stress leaf in tissues (Figure 5). Our results with leaf RNA blot confirmed that the upregulation of subunit c transcripts observed upon NaCl stress in *P. coarctata* leaf and root tissues was specific for *PcVHA-c1* and also salt-specific.

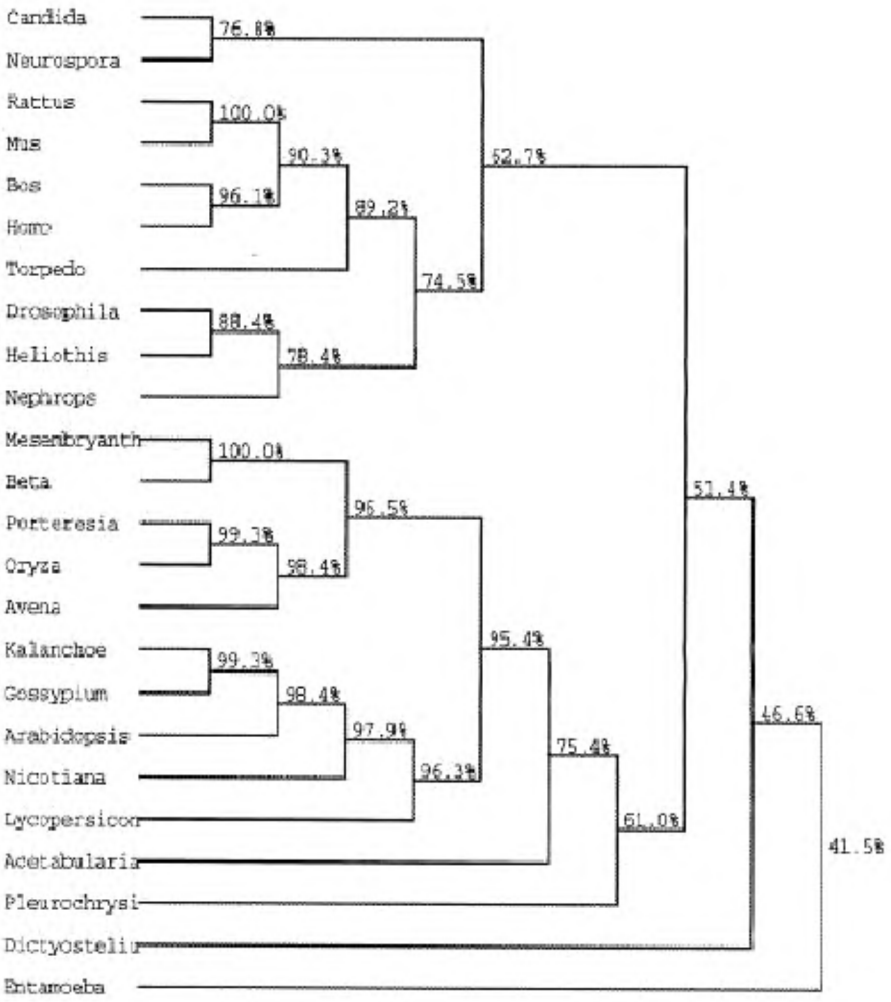

Figure 2. Phylogenetic relationships deduced from the protein sequences of V-ATPase subunit c from *Porteresia coarctata* (present study) and other species. The protein sequences were aligned using CLUSTALW program and the output from this program was used to generate a cladogram using the tree-drawing program of DNASIS. *Entamaoeba histolytica* (GenBank accession no. U01057), *Dictyostelium discoideum* (X90516), *Pleurochrysis carterae* (U81519), *Acetabularia acetabulum* (AB003938), *Lycopersicon esculentum* (AF010228), *Nicotiana tabacum* (X95751), *Arabidopsis thaliana* (L44585), *Gossypium hirsutum* (U13669), *Kalanchoe daigremontiana* (U16244), *Avena sativa* (M73232), *Oryza sativa* (U27098), *P. coarctata* (present study), *Beta vulgaris* (X98851), *Mesembryanthemum crystallinum* (X94999), *Nephrops norvegicus* (S40059), *Heliothis virescens* (L16884), *Drosophila melanogaster* (X77936), *Torpedo marmorata* (X52002), *Homo sapiens* (M62762), *Bos taurus* (P23956), *Mus musculus* (M64298), *Rattus norvegicus* (D10874), *Neurospora crassa* (P31413), *Candida tropicalis* (U02877).

Equal quantity of protein (10 µg) from microsomal fractions of control and NaCl-treated *P. coarctata* was transferred to PVDF membrane. The immunostaining experiment showed that the monoclonal antibodies for subunits A and B strongly hybridized with a 70 and a 60 kDa in protein *P. coarctata*. Increase in the intensity of the 70 and 60 kDa proteins was observed in salt-treated plants. Western blot and immunostaining experiments using both polyclonal and monoclonal antibodies of V-ATPase with *P. coarctata* indicated increase of translational product of 70 kDa (subunit A), 60 kDa (subunit B) and 16.5 kDa (subunit c) of V-ATPase in 0.5 M NaCl-treated *P. coarctata*. Polyclonal antibodies raised against PVC1 protein showed cross-reaction with a 16.5 kDa protein, and significant increase in its expression was observed in salt-treated plants (Figure 6).

The multimeric enzyme V-ATPase plays a major role in the maintenance of cellular pH, and it influences the transport of cations into the vacuoles of plant cells. The proteolipid domain of vacuolar H^+-ATPase plays a major role in H^+-transport in microvesicles and other acidic organelles. A full-length cDNA clone (*PcVHA-c1*) corresponding to V-ATPase subunit c was isolated from the *P. coarctata* cDNA library. Deduced amino acid sequence revealed that *PcVHA-c1* cDNA clone encoded 165 amino acids and the calculated molecular mass was 16.5 kDa. These results are in correspondence with that of V-ATPase subunit c isolated from other species such as *O. sativa*[21] and *A. sativa*[12].

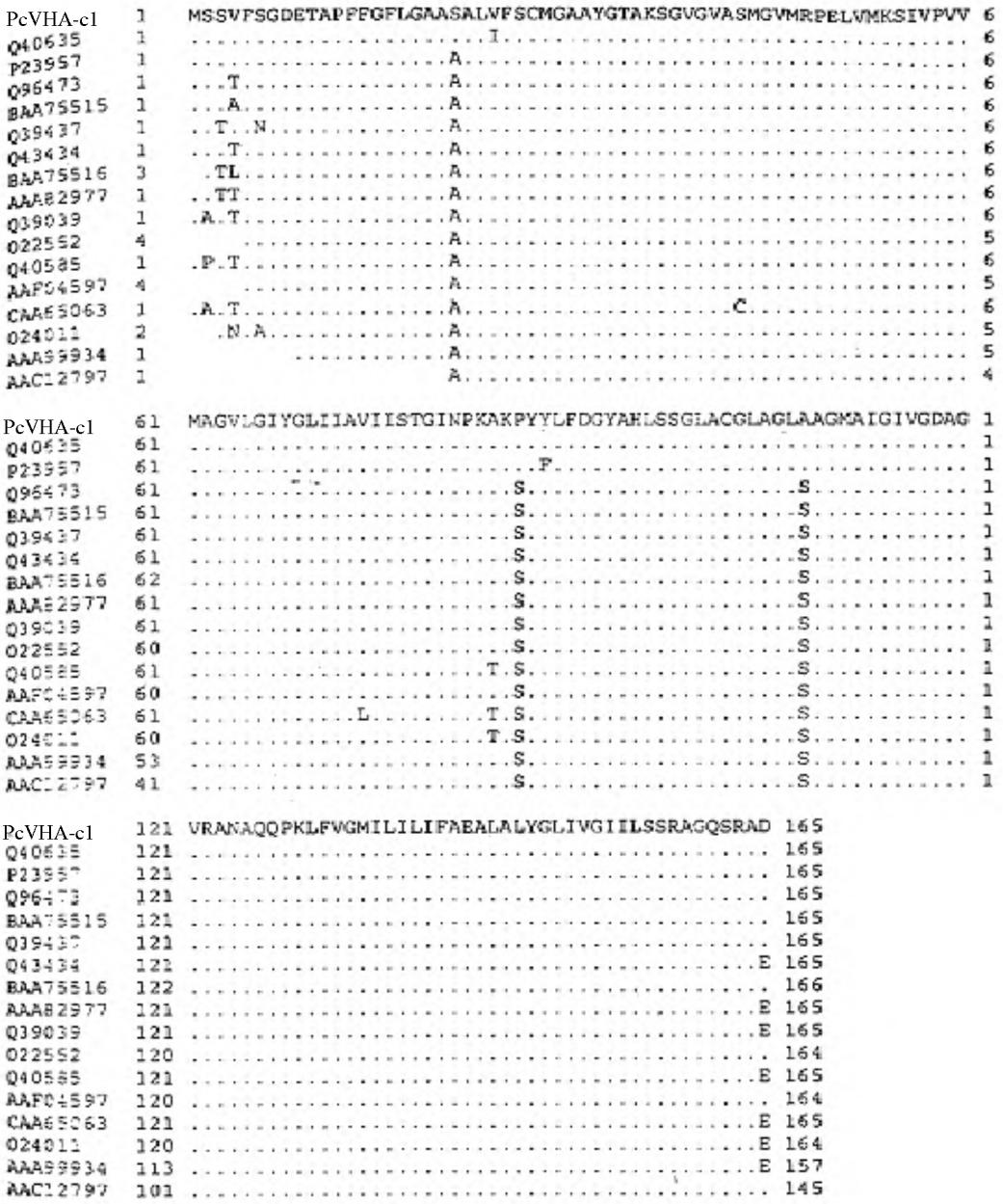

Figure 3. Comparison of amino acid sequence of plant V-ATPase subunit c aligned using BLAST multiple alignment program (NCBI). Dots indicate amino acid residues that are identical with the deduced amino acid sequence of *PcVHA-c1*. The deduced amino acids of *PcVHA-c1* show similarities with the following plant species. GenBank accession numbers are given in brackets. *O. sativa* (Q40635); *A. sativa* (P23957); *K. daigremontiana* (Q96473); *Citrus unshiu* (BAA75515, BAA75516); *M. crystallinum* (Q39437); *G. hirsutum* (Q43434, AAA82977), *A. thaliana* (Q39039, AAA99934); *N. tabacum* (Q40585, CAA65063); *Dendrobium crumenatum* (AAF04597), *L. esculentum* (O24011); *Vigna radiata* (AAC12797, O22552).

The CLUSTALW algorithm showed significant identities among V-ATPase subunit c sequences from different plant species; fungal species, insects, animals, yeast and amoebae. The difference between the proteolipid from plants, fungi and animals resides within the carboxyl-terminus domain. The proteolipid carboxyl terminus in animal species is characterized by a short stretch of three to four hydrophilic amino acids. However, this hydrophilic region is extended by an additional seven amino acids in plants[22]. This heptapeptide terminates with an Asp residue (D) in moderately salt-tolerant plant species such as *O. sativa* and *A. sativa*; and salt-tolerant species *Kalanchoe daigremontiana*, *Mesembryanthemum crystallinum* and *P. coarctata*. It terminates with a glutamate residue (E) in salt-sensitive species such as *Arabidopsis thaliana*, *Gossypium hirsutum*, *Lycopersicon esculentum* and *Nicotiana tabacum* (Figure 3). How much of this difference in amino acid residues contributes to functional differences in V-ATPase

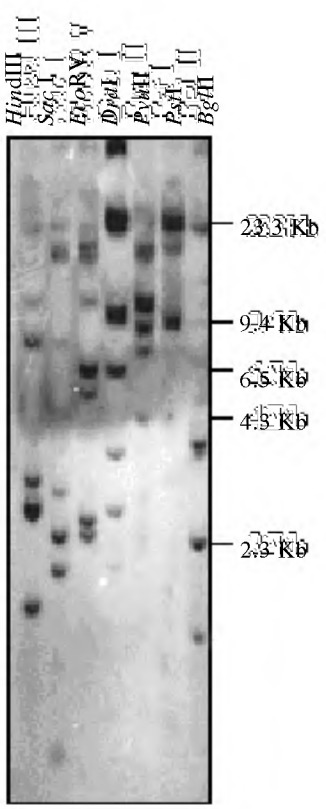

Figure 4. Genomic DNA gel blot analysis for *PcVHA-c1*. Genomic DNA of *P. coarctata* digested with different restriction enzymes and probed with *PcVHA-c1*.

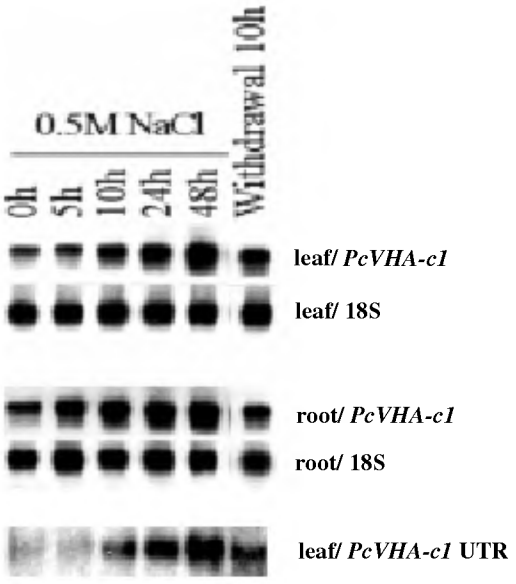

Figure 5. Effect of salt treatment (500 mM NaCl) on the expression of *PcVHA-c1* in leaves and roots of *P. coarctata*. Total RNA isolated from leaf and root tissue of *P. coarctata* plants treated with 0.5 M NaCl for different duration was probed with *PcVHA-c1* coding region. Total RNA from leaf and root tissue of *P. coarctata* plants treated with 0.5 M NaCl for 48 h and then transferred to normal growth medium (withdrawal of salt stress) for 10 h (shown in the figure). Blots were probed with 18S to determine uniformity in RNA loading. The leaf RNA blot was then stripped and reprobed with *PcVHA-c1* UTR region.

subunit c activity in plants remains to be elucidated; nevertheless, this was an interesting observation from our study. Apart from the amino acid differences at the N- and C-terminal ends of the coding regions, the plant and fungal sequences were consistently longer at the C-terminus compared to those reported from animals (figure not shown). The position of the membrane-spanning α-helices predicted by TMAP analysis[23] on deduced amino acid sequences from *PcVHA-c1* agreed with detailed structural models[24] of V-ATPase subunit c. Glu-142 in *P. coarctata* proteolipid IV hydrophobic domain, like *O. sativa*[21], oat[12], *K. daigremontiana*[25], *M. crystallinum*[26] or Glu-139 in bovine[10] or Glu-137 in *Saccharomyces cerevisiae*[11] is the site for DCCD inhibition. The sensitivity of the V-ATPase to DCCD has been documented. It has been reported that exposure to this inhibitor leads to covalent modification of the 16 kDa proteolipid[27,28].

Southern analysis of *P. coarctata* genomic DNA using *PcVHA-c1* as probe suggests that a multigene family may encode the V-ATPase subunit c of *P. coarctata*. The complex genomic organization of this gene could be due to existence of more than one copy of this gene or less likely due to the tetraploid nature of *P. coarctata* ($2n = 4x = 48$). Multigene family has been reported for the c subunit of the V-ATPase in *A. sativa*[12], *A. thaliana*[29], *Beta vulgaris*[30], *K. daigremontiana*[25], *M. crystallinum*[26], and from a polyploid species *G. hirsutum*[22]. In contrast, *S. cerevisiae*[11], *Drosophila melanogaster*[31], *Neurospora crassa*[32] all possess a single gene copy for the V-ATPase subunit c polypeptide. This suggests that there may be greater degree of specificity

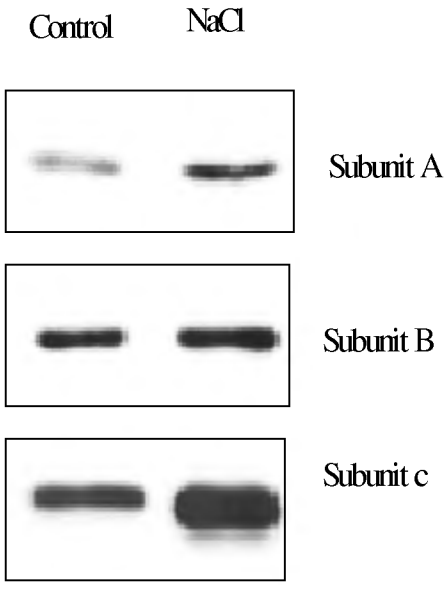

Figure 6. Western blot analysis of different V-ATPase subunits. Proteins prepared from microsomal fractions of leaf tissue from control and salt-treated (0.5 M NaCl for 48 h) *P. coarctata* plants were probed with monoclonal antibodies for subunits A and B, and polyclonal antibody for subunit c.

and/or regulation of expression of this polypeptide in plants compared to other organisms[25].

Changes in the transcript levels for the different subunits of V-ATPase have been reported when the plants are exposed to salinity stress[30,33]. In this study, increase in V-ATPase subunit c transcript levels after salt treatment was consistently observed in both leaves and roots of *P. coarctata* plants with full-length cDNA and later confirmed with gene-specific *PcVHA-c1* UTR probe. Transcripts for *PcVHA-c1* were found to be specific to NaCl treatment, as transcript levels declined after withdrawal from NaCl stress. Studies have been made to understand salt effects on V-ATPase transcript expression in plants. In *M. crystallinum*, transcript levels of V-ATPase subunits, particularly of subunit c, are markedly increased by salinity stress in mature leaves[33].

Earlier studies have shown the coordinate upregulation of V-ATPase subunits upon imposed salt stress. For example, studies have revealed a significant increase in levels of V-ATPase subunits A and B in salt-treated sugar beet plants using polyclonal antiserum directed against the V-ATPase holoenzyme of *K. daigremontiana*[30]. There are also reports which suggest that the increase in transcripts for V-ATPase subunit c in *Tortula ruralis* upon salt stress is not reflected in abundance in protein level using polyclonal antibodies[34]. We therefore wanted to study the effect of salt stress on the individual subunits, i.e. on PVC1 subunit protein using polyclonal antibody and on A and B subunits using monoclonal antibodies. We also observed an increase in the translational product of subunit A, subunit B and PVC1 subunit protein in leaves of *P. coarctata* plants treated with 0.5 M NaCl using Western blot and immunostaining experiments. However, only a modest increase in subunits A and B was observed in this study. On the contrary, induction of PVC1 was more pronounced upon salt stress. Though we did not rule out the possibility of an additive effect of the isoforms of subunit c in our Westerns, our study clearly shows that PVC1 protein accumulates with salt stress. This is corroborated by studies which show the intra membraneous particles build-up of subunit c in salt-stressed plants[35]. These studies support the idea that induction of V-ATPase is involved in osmotic adjustment in plant cells.

The present communication therefore suggests that there is a contributory role for the V-ATPase subunits in the salt stress response in *P. coarcata*. Furthermore, the present study and other studies[36] provide a basis for analysing the regulatory/functional role of transporters in a salt-tolerant species like *P. coarcata*.

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Differential expression of tomato ACC oxidase gene family in relation to fruit ripening

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The enzyme ACC oxidase (ACO) catalyses the terminal step in the ethylene biosynthesis pathway and is encoded by a small multigene family in tomato. We studied the expression of the ACO gene family during various stages of fruit ripening using the multiplex RT–PCR approach in three tomato cultivars differing in their rate of ripening. The *ACO1* gene expression, compared to other isoforms of the *ACO* gene family, showed a strong correlation with the rate of fruit ripening and ethylene evolution. The results revealed *LEACO1* as the predominant ripening-regulated isoform, and demonstrated the potential of the multiplex RT–PCR technique to analyse the rate of ripening in diverse tomato genotypes.

Keywords: ACC oxidase, fruit ripening, multiplex RT–PCR, tomato.

THE gaseous phytohormone ethylene orchestrates many aspects of plant growth and development, including fruit ripening, leaf and flower senescence and abscission, seed germination and plant responses to environmental stimuli[1,2]. Fruit ripening is a genetically programmed specialized phase of plant senescence and requires differential gene expression that is triggered, modulated and coordinated by ethylene. The terminal ethylene-forming enzyme (EFE), now confirmed as ACC oxidase, is encoded by at least four members of a multigene family in tomato[3] and appears to be primarily located in the cell wall of ripening fruits[4]. The enzyme apparently is a dioxygenase belonging to the superfamily of iron-ascorbate oxidases, expressed constitutively in most vegetative tissues[1] and found to be induced during fruit ripening[5]. In view of the mounting evidence for major changes in ACC oxidase transcript abundance in the ripening tomato fruit, the present study was undertaken to investigate the differential expression pattern of ACC oxidase gene family during fruit ripening in three selected Indian tomato cultivars, which differed in their rate of ripening.

The level of ACC oxidase gene expression during fruit ripening was monitored by multiplex RT–PCR[6] analysis in three commercially important Indian tomato cultivars, Pusa Ruby, Pusa Sheetal and Pusa Uphar, selected on the basis

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