International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(6) June -2016

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THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 6, pp. 12148-12152, June, 2016

International Journal of Recent Scientific Research

Research Article

PLANT VACUOLAR PROTON PYROPHOSPHATASES (VPPases): STRUCTURE, FUNCTION AND MODE OF ACTION

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ARTICLE INFO

Article History:

Received 05th March, 2016
Received in revised form 21st April, 2016
Accepted 06th May, 2016
Published online 28th June, 2016

Key Words:

Vacuolar proton pyrophosphatases, motifs, structure, transmembranes, proton pump

ABSTRACT

Vacuolar proton-translocating inorganic pyrophosphatases (VPPases) are proton transporters activating vacuolar secondary transport systems by establishing proton gradient across the endomembrane. V-PPase, a simple proton pump with 13-16 transmembrane helices compactly folded in a rosette manner in two concentric walls. VPPases have three highly conserved motifs CS1, CS2 and CS3 which regulates the translocation of H^+ ions from cytosol to vacuolar lumen. The pumping of H^+ into vacuole builds electrochemical gradient which changes its pH and energizes various antiporters resulting in influx of Na^+, K^+, NO_3^-, Cl- from cytosol to vacuole and reduces the toxicity in cytosol. This review presents an overview on 3-D structure, motifs, function and working model of VPPases under salt stress.

INTRODUCTION

High salinity, drought and extreme temperatures adversely affect plant growth and development due to decreased photosynthetic activity, disturbed ion homeostasis, osmotic stress, and nutrient scarcity (Munns and Tester, 2008, Ashraf *et al*, 2008). Soil salinity is increasing every year because of poor water management, high evaporation, industrial discharge, agricultural run-off and previous exposure to sea water (Tuteja, 2007; Chang *et al*, 2012). Two important ways to reduce Na^+ damage is either by excluding Na^+ from cytoplasm to the outside or pumping Na^+ into vacuoles using Na^+/H^+-antiporters. Thereby the toxic level of Na+ is reduced in the cytoplasm (Li *et al*, 2010, Wei *et al*, 2011). The compartmentalization of Na^+ into vacuoles is an efficient strategy mediated by a vacuolar Na^+/H^+-antiporter. It increases vacuolar osmotic potential that favors uptake of water by the cells and better water retention in tissues under high soil salinity and also keeps Na^+ away from the sites of metabolism (He *et al*, 2005). The vacuolar Na^+/H^+-antiporter is driven by the electrochemical gradient of protons across the tonoplast generated by vacuolar H^+ pumps, H^+-ATPase (V-ATPase) and H^+-pyrophosphatase (VPPase) (Apse *et al*, 1999; Sze *et al*, 1999). V-ATPase and VPPase coexist on the plant vacuolar membrane and use ATP and inorganic pyrophosphate (PPi) respectively as energy sources for H^+ translocation. Generally, the proton pumps generate proton motive force (PMF) in the vacuole to decrease the pH to <3-4 (~30 mV membrane potential). To maintain the membrane potential and flux balance of ions in the vacuole, protons are pumped by proton pumps to the cytoplasm in exchange with ions like Na^+, K^+, Ca^{2+}, Cl^- and NO_3^- (Martinoia *et al*, 2007).

VPPase is an unique electrogenic proton pump, heat stable found in plants, algae, photosynthetic bacteria, protozoa and archaebacteria (Maeshima, 2000). V-PPase is a hydrophobic single-subunit protein of 80 kDa and utilizes a simple substrate pyrophosphate (PPi), which provides a high-energy phosphoanhydride bond for hydrolysis and translocation of protons (Maeshima, 2000; Gaxiola *et al*, 2007). PPi is synthesized in metabolic reactions such as DNA, RNA, sucrose and cellulose synthesis, or in conversion of pyruvate to phosphoenolpyruvate (PEP) by pyruvate phosphate dikinase. It energizes the transport of solutes such as betaine, polyols and sugars, amino acids (proline), across the vacuole membrane and accumulates in the cell by which plants protect themselves from damages under cold and osmotic stresses (Marty, 1999; Ratajczak, 2000; Chen and Murata, 2002).

Homologous Regions in Vppase

VPPases are highly conserved among land plants and less among archaeon, protozoan and bacteria. Liu *et al*, (2011) reported highest similarity of VPPase isolated from *Suaeda corniculata*. Similarity of VPPases was reported with *Kalidium foliatum* (96%), *Suaeda salsa* (94%), *Chenopodium rubrum* (89%), *Beta vulgaris* (89%), *Chenopodium glaucum* (88%) and

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Arabidopsis thaliana (87%). Dong *et al*, (2011) reported that MdVHP1 shared high similarity (87%) with VPPases of tobacco, grapevine and *Arabidopsis* and exhibited the highest identity withVPPase of peach (94%). Sequence alignment also showed that MdVHP1 and MdVHP2 shared 80% of sequence identity. Hu *et al*, (2012) observed that VPPase isolated from *Halostachys caspica* showed high sequence similarity with other VPPases of Chenopodiaceae, and shared 95% nucleotide sequence identity with that of *Kalidium foliatum*. Blast performed and phylogenetic tree construction using neighbor joining method using 28 species showed 86-91% identity among land plants and less homology to land precursor and bacteria (Fig 1).

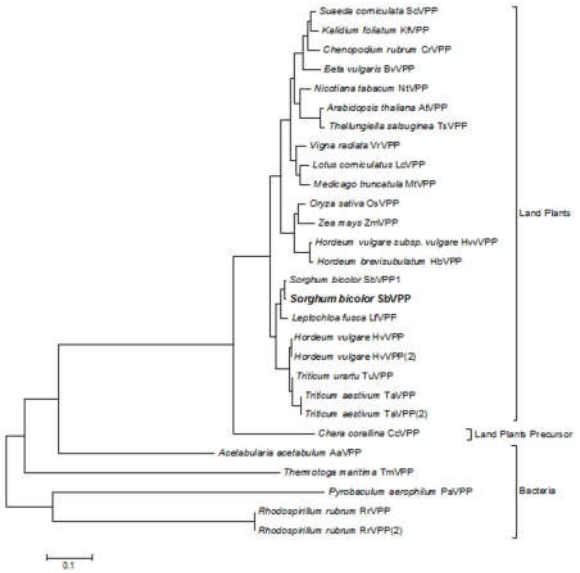

Figure 1 The phylogenetic tree of 28 VPPase proteins indicating the close relationship among the land plant V-PPases constructed by neighbor joining method.

Functional motifs in VPPases

VPPases have highly conserved motifs: CS1, CS2 and CS3 motifs (Rea and Poole, 1993; Baltscheffsky *et al*, 1999; Maeshima, 2000; Mimura *et al*, 2004; Suneetha, 2015). The CS1 motif has consensus sequence of DVGADLVGKVE and functions as catalytic site by hydrolyzing PPi (Rea and Poole, 1993; Takasu *et al*, 1997; Maeshima, 2001; Mimura *et al*, 2004; Liu *et al*, 2011). The CS1 region is exposed to the cytosolic side of the tonoplast (Tanaka *et al*, 1993). Suneetha (2015) reported that the CS2 motif has consensus sequence GSAALVSL and is located in a hydrophilic loop. CS2 motif is highly conserved and is similar to rhodopsin like G-protein coupled receptors (GPCRs) with calcium signaling signature property that senses the high cytosolic Ca^{2+} levels and transduces the extracellular signal to the site of action (Suneetha, 2015). CS3 is located in the carboxy-terminal region and has consensus sequence GDTIGD exposed to the cytosol and plays a critical role in catalytic function in combination with CS1 and CS2 (Liu *et al*, 2011; Rea *et al*, 1992). The position of these conserved regions change from one plant VPPase to others. For instance DDPR and VGDN are located at 271, 285 amino acids position in mung bean whereas the amino acids positions are 266, 280 in *S.corniculata* and 266, 281 in *S. color* (Fig. 2).

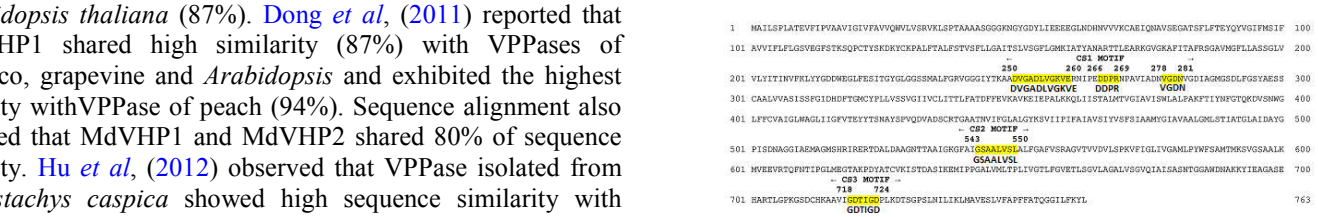

Figure 2 VPPase protein sequence showing CS1 (250-260), CS2 (543-550) and CS3 (718-724) motifs

D Structure of VPPase

VPPase gene encodes a polypeptide with 761 to 771 amino acids with molecular mass ranging from 80 to 81 kDa. VPPase from *H. capsica* encodes 764 amino acids, apple encodes 771, *S. corniculata* encodes 764 and *S. bicolor* encodes 763 amino acids. Suneetha (2015) predicted that *S. bicolor* VPPase has 16 transmembrane regions using TMred and TMHMM (Fig. 3, 4). The sequence has 16 inside to outside helices orientations and 16 outside to inside helices orientations of the transmembranes. All the 16 helices are having scores above 500 when the transmembrane helix is chosen with minimum of 14 to maximum of 23.

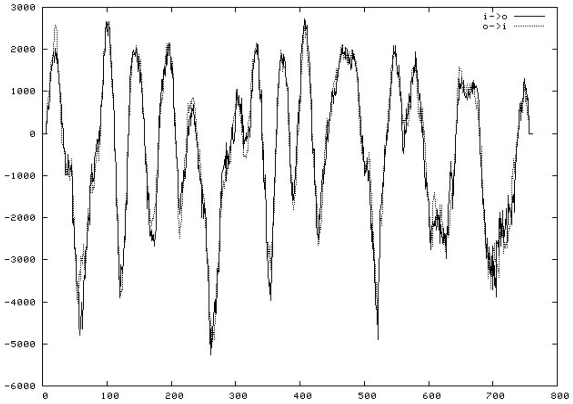

Figure 3 TMpred output representing transmembrane helices for VPPase sequence.

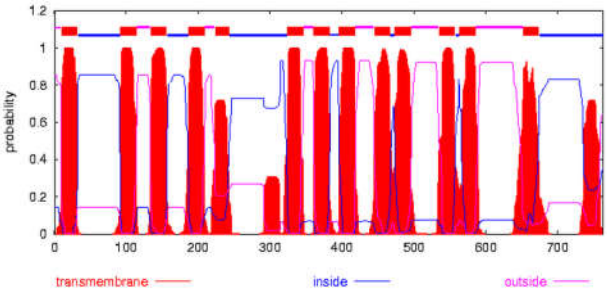

Figure 4 TMHMM posterior probabilities of VPPase sequence

Suneetha (2015) reported that the VPPase is vacuolar membrane bound protein compactly folded in rosette manner in two concentric walls (Fig. 5). The core has six transmembrane helices surrounded by ten transmembrane helices which form the inner and outer walls of the pump which is displayed in cylinders (Fig. 6a). Two short helices are present on the cytosolic side, two helices and two anti-parallel β strands are present on the luminal side of the protein (Fig. 6b). The core of the model has one IDP molecule surrounded by five Mg^{2+} ions

which are essential for the activity of V-PPases and one K^+ ion which acts as stimulator (Fig. 6c). The above elements are highly conserved among the V-PPases which forms a hydrophobic door to the hydrophilic surroundings of the vacuolar lumen. The hydrophobic gate prevents the reflux of H^+ ions and helps in maintaining the translocation of H^+ from cytosol to vacuolar lumen (Fig. 7).

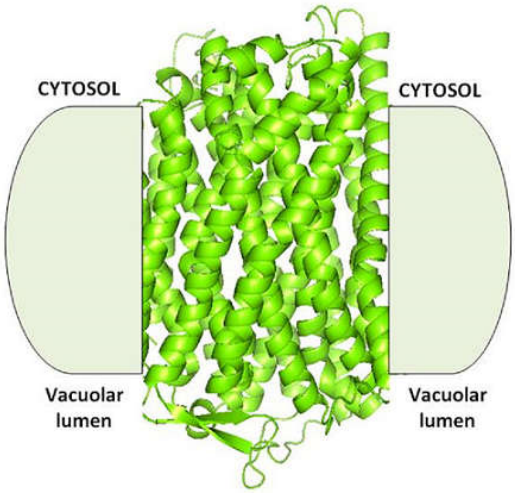

Figure 5 VPPase protein compactly folded as membrane bound protein

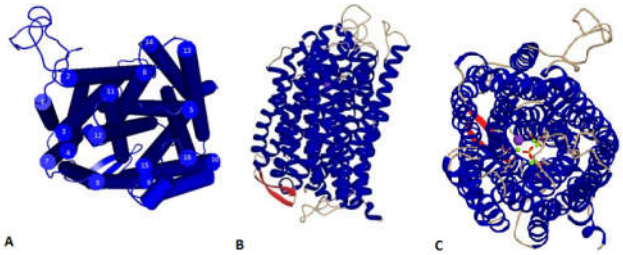

Figure 6 3-D structure of VPPase

a) Sixteen transmembrane helices are shown in blue cylinders with six helices in the core surrounded by 10 transmembrane helices to form inner and outer walls of the pump.
b) Ribbon structure of V-PPase containing 16 transmembranes colored in blue and two antiparallel β strands coloured in red
c) V-PPase model is rotated by 60 to visualize the core of the model showing Mg^{2+} and K^+.

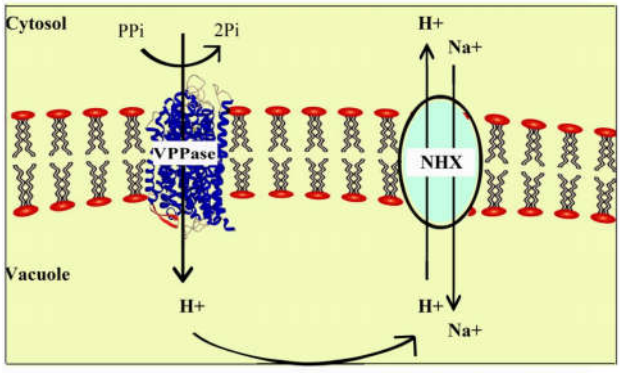

Figure 7 Working model of the VPPase showing the pumping of protons into vacuole to generate electrochemical gradient against which sodium is taken in under stress conditions

The space fill representation of VPPase model is considered to analyze electrostatic surface potential and is indicated by colors: red which represents negative, blue represents positive and white represents neutral potentials (Fig. 8a). The core of model which contains IDP binding site is represented within the circle the core of VPPase (Fig. 8b).

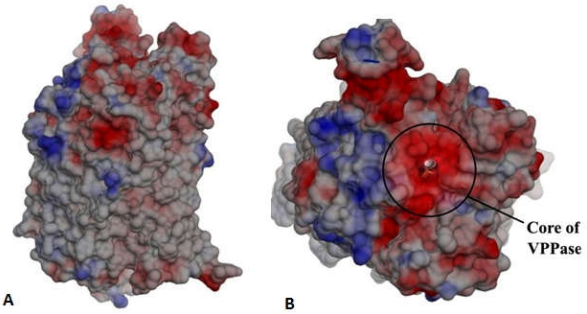

Figure 8 Spacefill representation of modeled VPPase

a) The model showsng electrostatic surface potential with negative potential represented in red color, positive in blue and neutral in white color
b) The core of model contains IDP binding site is represented within the circle the core of VPPase

Metal geometry in VPPases

Suneetha (2015) reported that five bivalent Mg^{2+} ions, one K^+ are present surrounding the IDP (imidodiphosphate) playing an essential role in activating V-PPases by transphosphorylation reaction involving ATP's. The IDP molecule is the regulatory factor in membranes of various tissues with possible biological significance in regulating V-PPases. Each Mg^{2+} ions interacts with surrounding amino acids like Aspartic acid (ASP), Asparagine (ASN), Glutamic acid (GLU) and 2PN (IDP) molecules. The K^+ ion acts as stimulator is surrounded by amino acids like Aspargine (ASN), Glycine eeGLY) and 2PN molecule. The metal geometry and the coordinating amino acids distance from the each Mg^{2+} metal ion and K^+ is as shown by Fig. 9

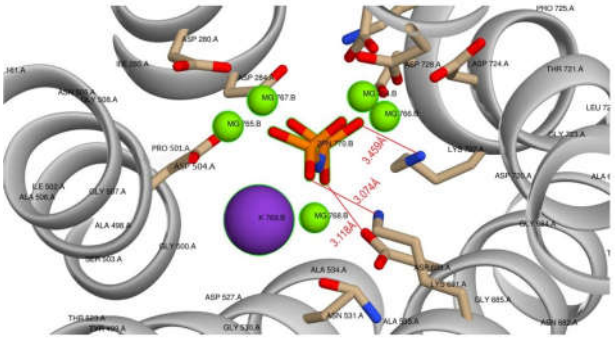

Figure 9 The metal geometry and coordinating amino acid from IDP-binding residue (red) to the five Magnesium ions (green)

Suneetha (2015) predicted phosphate binding sites using Phosfinder and reported eleven phosphate binding site. The location of phosphate binding sites are represented as yellow color balls with numbers and interacting residues with green color (Fig.10).

VPPase mode of action

VPPase contains three highly conserved motifs CS1 - DVGADLVGKVE (250-260), DDPR (266-269), VGDN (278-282); CS2 - SAALVSL (544-550) and CS3 - GDTIGD (719-724) [22]. CS1 motif segment (DDPR, VGDN) and CS3 motif form the core catalytic domain. CS1 motif segment (DVGADLVGKVE) is essential for hydrolyzing PPi and

transporting protons (Maeshima 2001). CS2 motif is highly conserved and similar to rhodopsin like G-protein coupled receptors (GPCRs) with calcium signaling signature property. CS2 senses the high cytosolic Ca^{2+} levels and transduces the extracellular signal to the site of action.

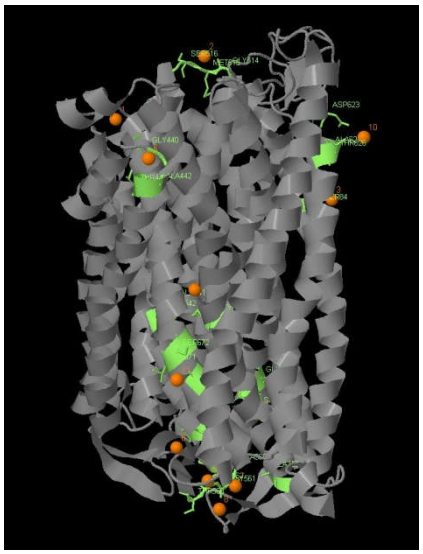

Figure 10 Eleven phosphate binding site are identified on the modeled VPPase using Phosfinder. The location of phosphate binding sites are represented yellow colored balls with numbers and interacting residues are in green color

The freely available cytosolic Ca^{2+} may be phosphorylated to Ca-PPi by Ca^{2+} dependent membrane bound protein kinase (Johannsen *et al*, 1996). The substrate PPi of Ca-PPi is exchanged with Mg^{2+} to form Mg-PPi at the core catalytic site (Fig. 11). The above elements are highly conserved among the VPPases that form hydrophobic door to the hydrophilic surroundings of vacuolar lumen. The hydrophobic gate prevents the reflux of H^+ ions and helps in maintaining the translocation of H^+ from cytosol to vacuolar lumen.

The core of VPPase has one IDP molecule surrounded by five Mg^{2+} ions which regulates the VPPase activity. The core also has one K^+ ion which acts as a stimulator in coordination with the surrounding amino acids. The pumping of H^+ into the vacuole builds electrochemical gradient (PMF) which changes its pH to 2-4 units (equivalent to -120 to -240 mV) (Stanislav *et al*, 2010). This PMF can energize various antiporters like Na^+ and K^+, H^+ exchanger, NO_3^- and Cl^-:H^+ exchanger resulting in the influx of Na^+, K^+, NO^{3-}, Cl^- from cytosol to vacuole.

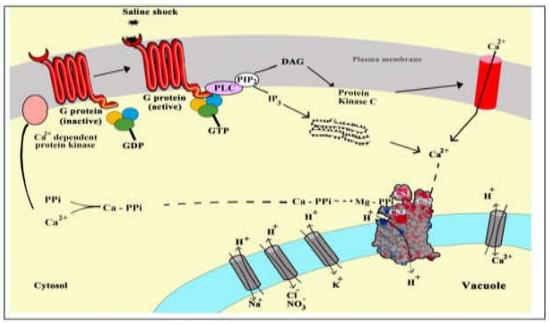

Fig. 11 Mode of action of VPPase in salt stress

This influx reduces the toxicity in cytosol and protects the cell against deleterious effects which are caused due to abiotic stress. Thus, this overall signalling web appears to play an important role in providing stress tolerance to plants.

Acknowledgements

GS, NNR and CS are grateful to GITAM University, Visakhapatnam, India for providing necessary facilities to carry out the research work and for extending constant support in the preparation of the review.

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How to cite this article:

Suneetha G., Nageswara Rao N., Reddy and Surekha Ch. 2016, Plant Vacuolar Proton Pyrophosphatases (Vppases): Structure, Function and Mode of Action. *Int J Recent Sci Res.* 7(6), pp. 12148-12152.

TSSN 0976-3031