Molecular Cloning and Characterization of a Vacuolar H^+-Pyrophosphatase Gene from the Xerophyte *Zygophyllum xanthoxylum*

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Molecular cloning and characterization of a vacuolar H^+-pyrophosphatase gene from the xerophyte *Zygophyllum xanthoxylum*

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Introduction Drought and salinity are the two severe abiotic stresses result in reduction of output of agriculture worldwide . Increased vacuolar Na^+ compartmentalization and vacuolar solute accumulation can confer plants salt-and drought-tolerance . Overexpression of vacuolar H^+-pyrophosphatase (VP) gene can potentiate the active transport of Na^+ into vacuole by establishing the H^+ electrochemical potential difference between the vacuole lumen and the cell cytosol (Gaxiola et al . , 2001) . A homologous gene (*ZxVP1-1*) of vacuolar H^+-pyrophosphatase was cloned and characterized from an endemic genera plant *Zygophyllum xanthoxylum* , which lives in the desert area of middle Asia with distinctive mechanism on drought resistance .

Materials and methods The core fragment of *ZxVP1-1*was cloned by RT-PCR . The full-length cDNA ends of *ZxVP1-1* were isolated by RACE system . Sequence analysis was run on the software DNAstar . Homology comparison was analyzed by using DNAMAN software .

Results The full-length cDNA of vacuolar H^+-pyrophosphatase gene from *Z . xanthoxylum* was obtained by assembling the core cDNA fragment , 5 -RACE fragment and 3 -RACE fragment . The cDNA consists of 2695 bp with a 5 -untranslated region of 206 bp , an open reading frame of 2262 bp that encodes a protein of 753 amino acids with a calculated molecular weight of 80 KDa , and a 3 -untranslated region of 224 bp containing a ploy (A) tail . It was named as *ZxVP1-1* and deposited in the GenBank database (accession no . EU103625) . The deduced amino acid sequence of ZxVP1-1 shares a high homology with those of higher plants , ranging from 85 .5% homology with that of *Vitis vinifera* VvVP to 78 .2% with that of *Triticum aestivum* TaVP , and contains the consensus PPi-binding domain ^{238}KAADVGADLVGKVE^{250} . Moreover , the other two core *ZxVP* cDNA fragment named *ZxVP1-2*(accession no . EU103626) and *ZxVP1-3*(accession no . EU103627) with the length of 898bp were obtained . Semi-quantitative RT-PCR analysis showed that the expression of *ZxVP1-1* in *Z . xanthoxylum* was induced and regulated by salt stress .

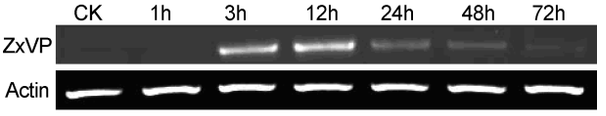

Figure 1 *Expression patterns of ZxVP1-1 under salt stress . Plants were irrigated with 150mM NaCl , and total RNA was isolated from the leaves of Z . xanthoxylum at six different time points (0 ,1 ,3 ,12 ,24 ,48 and 72 h) . 4 μg aliquot of total RNA was subjected to Semi-quantitative RT-PCR analysis .*

Conclusions The results indicated that the gene *ZxVP1-1* isolated from *Z . xanthoxylum* is a vacuolar H^+-pyrophosphatase . This gene may play important roles in the drought tolerance , and the mechanism is under further investigation .

Reference

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