Vacuolar pyrophosphatases confer anoxia tolerance in rice by pumping protons across the tonoplast

This dissertation is submitted for the degree of Doctor of Philosophy in the Macquarie University by

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March 2009

Contents

Abstract

Vacuolar pyrophosphatase (V-PPase) is induced by a number of abiotic stresses and is thus thought to play a role in plant adaptation. This thesis reports on the regulation of six V-PPase genes in rice (*Oryza sativa* L.) coleoptiles under anoxia, using cultivars with varying flood tolerance at the germination stage to test this hypothesis. Quantitative PCR time courses showed that one vacuolar pyrophosphatase (*OVP3*) was consistently induced by anoxia, particularly in the flood-tolerant cultivar Amaroo where it rose 20-fold in 2 h. Regulation of *OVP3* expression under anoxia was investigated by analysis of putative *OVP* promoters. The putative *OVP3* promoter contained more previously identified anoxia-inducible motifs than the promoters of the other five *OVP* genes. GUS activity in transgenic rice plants transformed with the *OVP3* promoter region linked to the GUS reporter gene was induced by anoxia but not by salt or cold: GUS staining was visible mainly in the stele of seminal roots. Transgenic *Arabidopsis* plants overexpressing *AVP1* (an *Arabidopsis* vacuolar pyrophosphatase) showed increased anoxia resistance as measured by survival and growth rate during the recovery period in air after anoxic treatment. Transgenic rice plants overexpressing *OVP3* had higher anoxia tolerance which was supported by reduced solute leakage, more polarised membrane potentials and higher cytosolic pH under anoxia, compared to wild type roots. Membrane potentials of knockouts of the *OVP* gene family were depolarised under anoxia compared to wild type plants. I conclude that *OVP3* is a key gene determining anoxia tolerance in rice seedlings via its effect on membrane properties. The importance of *OVP3* is significant as a single gene determinant of anoxia tolerance.

Acknowledgments

This research project would not have been possible without the support of many people. First I would like to express my gratitude to my supervisor, Assoc. Prof. Brian Atwell who was abundantly helpful and offered invaluable assistance, support and guidance. My deepest gratitude is also due to my associate supervisor Dr. Rachel A. Burton. Without her knowledge and assistance this study would not have been successful.

My special thanks also go to Dr. Qisen Zhang for his advice and crucial contribution to this research.

I would also like to thank all members in Geoff Fincher's laboratory. I am much indebted to Neil J. Shirley for his valuable technical support on quantitative PCR. The discussions and cooperations with all of my colleagues have contributed substantially to this work. This research was made possible through the generosity of the Australian Centre for Plant Functional Genomics, School of Agriculture, Food and Wine, University of Adelaide, Australia.

I am very grateful to Mark Tester and his group for rice transformation. Many thanks go in particular to Olivier Cotsaftis for expert assistance with rice transformation and Gehan Safwat El-Hussieny for kindly providing *Arabidopsis* transgenic plants.

I have also been benefited by outstanding works on membrane potential measurements under the help of Stephen Tyerman with his particular skill in handling precisely delicate equipments and plasma membrane isolation under the help of Wendy Sullivan.

Statement of Candidate

I certify that the work in this thesis entitled "**Vacuolar pyrophosphatases confer anoxia tolerance in rice by pumping proton across the tonoplast"** has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

Qinxiang Liu

5 January 2009

One part of this study has been accepted for publication in *Plant Molecular Biology* in 2009. This paper is titled "*Expression of vacuolar pyrophosphatase (OVP3) is under control of an anoxia-inducible promoter in rice*" and co-athored by Qisen Zhang, Neil Shirley, Rachel Burton and Brian Atwell.

List of Abbreviation

V-PPase	Vacuolar pyrophosphatase
PDC	Pyruvate decarboxylase
ADH	Alcohol dehydrogenase
LDH	Lactate dehydrogenase
OVP3	*Oryza sativa* vacuolar pyrophosphatase 3
AVP1	*Arabidopsis thaliana* vacuolar pyrophosphatase 1
ANP	Anaerobic proteins
PPDK	Pyruvate - phosphate dikinase
ATP	Adenosine 5'-triphosphate
AMP	Adenosine monophosphate
UTP	Uridine 5'-triphosphate
UDP	Uridine diphosphate
DCCD	*N*,*N*'-dicyclohexylcarbodiimide
CAM	Crassulacean acid metabolism
ARE	Anaerobic response element
GapC4	Glyceraldehye-3-phosphate dehydrogenase 4 gene
BLAST	Basic Local Alignment Search Tool
CDD	Conserved domain database
HMM	Hidden markov model
NCBI	National center for biotechnology information
MEME	Mulptile EM for motif elicitation
TFBSs	Transcription factor-binding sites