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VALIDATION OF CANDIDATE GENES FOR SALT TOLERANCE IN RICE BY REAL-TIME PCR

A. R. F. Zahra[1], D. M. De Costa[1], W. A. J. M. De Costa[2]

[1]Department of Agricultural Biology, [2]Department of Crop Science, Faculty of Agriculture, University of Peradeniya

Salinity tolerance is a key trait for increasing and stabilizing rice production in salinity prone rice lands worldwide. Identification of the genes responsible for salinity tolerance and quantifying their expression would be useful for developing salt tolerant rice varieties. A previous study conducted by us identified several genes which are responsible for salt tolerance during Phase I (osmotic stress) and Phase II (ionic stress) of salt stress development in the known salt tolerant Sri Lankan rice variety At354.

Real-time PCR (qPCR) is a rapid and quantitative method which can be used to quantify the expression levels of candidate genes of salt tolerance. These validated candidate genes for salt tolerance could be identified as genes, which have the potential to be used in the development of salinity tolerant plants through molecular breeding. In the present study, three candidate genes *OsPHI-30* (homology to a putatively expressed salt-tolerant gene of rice), *OsPHI-147* (homology to putative Serine Decarboxylase) and *OsPHI-194* (homology to AP domain DRE binding factor) were quantified and validated by qPCR for their salinity tolerance during Phase I of salt stress development.

qPCR was carried out with a MiniOpticon qPCR Detection System (BIORAD Laboratories Inc.) with cDNA of salt-treated and non-salt treated (control) plants obtained from Phase I targeting the candidate genes for salt tolerance, harboured in the cDNA clones (i.e. *OsPHI-30, OsPHI—147* and *OsPHI-194*) and for the reference gene (α-tubulin) using SYBR green dye. The relative quantification values for each target gene were calculated by the standard curve method.

Relative fold expression levels of all three genes tested showed increases in salt-stressed At354 relative to the unstressed control. *OsPHI-147 (*Putative Serine Decarboxylase) showed the highest increase in the gene expression level over the control. These findings of increased expression levels confirmed the up-regulation of the selected candidate genes under salt-stress in the known salt-tolerant Sri Lankan rice variety At354.

Therefore, quantitative expression analysis of the cDNA clones *OsPHI-30, OsPHI-147* and *OsPHI-194* during Phase I of salt stress development by qPCR validated the contribution of these genes to tolerate osmotic stress during Phase I. Furthermore, our results revealed that these validated candidate genes for salt tolerance could be identified as genes, which have the potential to be used in over-expression studies to develop salinity tolerant plants through molecular breeding.

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