EXPRESSION DETERMINATION OF ETHYLENE SINGNAL TRANSDUCTION GENES IN KHAW DOK MALI 105 (KDML105) RICE DURING RICE TUNGRO BACILLIFORM VIRUS (RTBV) INFECTION

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A Thesis Submitted in Partial Fulfillment of The Requirements for the Degree of Master of Science (Agricultural Biotechnology) Graduated School, Kasetsart University 2005 Yada Mukjang 2005: Expression Determination of Ethylene Signal Transduction Genes in Khaw Dok Mali 105 (KDML105) Rice During Rice Tungro Bacilliform Virus (RTBV) Infection. Master of Science (Agricultural Biotechnology), Major field Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Mrs. Parichart Burns, PhD. 61 pages ISBN***

Ethylene responsive sensor 1 (Os-ERS1) and ethylene responsive sensor 2 (Os-ERS2) were isolated from Khaw Dok Mali 105 (KDML105) rice. The Os-ERS1 and Os-ERS2 cDNA sequences were 2,072 and 2,950 nucleotides in length which encoded 636 and 635 amino acids, respectively. Southern analysis indicated that only one copy of both Os-ERS1 and Os-ERS2 was found on chromosome 3 and 5, respectively. Analysis of amino acid sequence revealed that Os-ERS1 and Os-ERS2 putative proteins show the highest level of 98.6% and 99.4% identity comparing Oryza sativa japonica variety from Genbank database. Three domains including transmembrane, GAF and histidine kinase domain were found in both Os-ERS1 and Os-ERS2 putative proteins. In order to understand the signaling system of ethylene during Rice Tungro Bacilliform Virus (RTBV) infection, we monitored the dynamics of four ethylene receptors and four ethylene responsive factor genes using Northern analysis technique. Results indicated that transcription levels of five out of eight genes (Os-ERS2, Os-ETR1, Os-ETR2, Os-ERFG1 and Os-ERFG2) were detected at low level or none at all before inoculation. After RTBV inoculation, the level of expression of those genes was elevated with significant difference in their patterns. Os-ERS1, Os-ETR2, Os-ERFG3 and Os-ERFG4 shown to be rhythmic pattern that highest level at 2-4 hr after RTBV infection. Interestingly, four isoforms of Os-ERFG1 gene is presented while the other ethylene responsive factor genes are shown only single isoform. Effect of Agroinoculation technique was observed at low level in all of those genes excepted on Os-ERFG2 which shown to up-regulate by Agrobacterium inoculation. The result suggesting the possible association of ethylene signaling with RTBV infection.

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Student' signature

Thesis Advisor's signature

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LIST OF ABBREVIATIONS (Cont'd)

SSC	=	Standard saline citrate
TAE	=	Tris acetate EDTA
TCS	=	Two-component regulatory system
UV	=	Ultraviolet
Vol	=	Volume
X-gal	=	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside

LIST OF ABBREVIATIONS

ACC	=	1-Aminocyclopropane-1-carboxylic acid
bp	=	Base pair
CTR	=	Constitutive triple response
dH_2O	=	Distilled water
DEPC	=	Diethylpyrocarbonate
DIG-11-UTP	=	Digoxigenin-11-uridine-5'-triphosphate
DNA	=	Deoxyribonucleic acid
EDTA	=	Ethylenediaminetetraacetic acid
EIN	=	Ethylene insensitive
ETR	=	Ethylene triple response
ERS	=	Ethylene responsive sensor
ERF	=	Ethylene responsive factor
g	=	Gram
h	-	Hour
IPTG	-	isopropyl-beta-D-thiogalactopyranoside
JA	=	Jasmonic acid
LB	=	Luria bertani broth
LBA	=	Luria bertani agar
mg	=	Milligram
ml	=	Millilitre
MAPK	=	Mitogen activated protein kinase
nt	=	Nucleotide (s)
OD	=	Optical density
PCR	=	Polymerase chain reaction
RNA	=	Ribonucleic acid
rpm	=	Revolutions per minute
RT	=	Room temperature
SA	=	Salicylic acid
SDS	=	Sodium dodecyl sulphate