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Characterization and Determination of Ethylene Responsive Sensor Genes in Khao Dok Mali 105 (KDML105) Rice During Rice Tungro Bacilliform Virus (Chainat isolate) (RTBVCN) Infection

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ABSTRACT

Ethylene receptor family including ethylene responsive sensor 1 (*Os-ERS1*) and ethylene responsive sensor2 (*Os-ERS2*) were isolated and characterized. Sequence analysis showed that both sequences of *Os-ERS1* and *Os-ERS2* genes comparing with japonica variety had 99.4 and 98.2 percent identity, respectively. Southern analysis indicated that only one copy of both *Os-ERS1* and *Os-ERS2* was found in KDML105 rice genome which located on chromosome 3 and 5, respectively. In order to understand signaling system of ethylene in rice plant during Rice Tungro Bacilliform Virus Chainat isolate (RTBVCN) infection, RTBVCN infectious clone was *Agrobacterium* infiltrated into the meristem of two week old rice plant. Northern analysis was performed to determine the expression of three ethylene receptors (*Os-ERS1*, *Os-ERS2* and *Os-ETR1*) and four ethylene responsive factors (*Os-ERFG1*, *Os-ERFG2*, *Os-ERFG3* and *Os-ERFG4*). The result revealed that the expression of *Os-ERS1* and *Os-ETR2* are elevated by RTBVCN inoculation, whereas *Os-ERS2* is constitutively expressed. In transcriptional regulation step, the expression level of *Os-ERFG1* is strongly induced during RTBVCN infection. *Os-ERFG1* has been showed to play a major role in plant-pathogen interaction resulting in plant resistance. The result suggests the possible association of ethylene signaling in response to RTBVCN infection.

Key words: rice; ethylene; ethylene receptor; ethylene signal transduction, plant-pathogen interaction

INTRODUCTION

Although plants are exploited as sources of food and shelter by a wide range of pathogens including bacteria, fungi and virus. They have sophisticate responses to deal with these pressures by activating a battery of defense responses through signaling network. During infection, many of signal molecules such as jasmonic acid and ethylene are elevated and transferred through the complex of signal transduction pathways (Jane, 2001). Ethylene is well studied and a simple gaseous plant hormone that regulates many diverse plant processes, ranging from seen germination to organ senescence. Furthermore, it plays an important role in plant response to pathogen (Bleecker and Kende, 2000). Many studies have been proposed that the ethylene pathway primarily regulates resistance to pathogens, as mutants defective in ethylene biosynthesis or signaling

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show increased susceptibility to pathogens such as *Xanthomonas campestris*, *Pseudomonas syringae* and *Fusarium oxysporum* (Steven *et al*., 1998)

Ethylene is well studied and a simple gaseous plant hormone that regulates many diverse plant processes, ranging from seed germination to organ senescence. Furthermore, it plays important role in plant response to pathogen (Bleecker and Kende, 2000). Biosynthetic pathway of ethylene has been well characterized. Over the developmental control of ethylene biosynthesis, there are regulation of its perception and subsequent signal transduction pathway.

Therefore, ethylene has been a target for studying resistance mechanisms in the last decades. Mutants with either production or perception of ethylene increase susceptibility to several pathogens and pests (Corne *et al*., 2001). Furthermore, the application of exogenous ethylene was found to induce resistance or susceptibility depending on the plant pathogen interaction studies. Ellis and Turner (2001) reported that an *Arabidopsis* mutant with constitutive expression of defense related genes shown enhanted resistance to powdery mildew disease. This mutant had constitutively activated ethylene signaling pathway. For instance, several ethylene insensitive mutants of *Arabidopsis* have been reported to exhibit enhanced disease susceptibility to *Botrytis cinerea* (Thomma *et al*., 1999). This indicated that both ethylene perception and signaling are play significant roles in plant resistance.

Rice is the most important cereal crop of Thailand. It is not only a main staple food but also a major export crop. Khao Dok Mali 105 (KDML 105) is a premium rice variety famous for its fragrance and cooking quality. This variety is particularly susceptible to several diseases including ragged stunt, blast, and tungro. Among them, tungro disease causes severe reduction in rice yield. This disease is caused by a complex of two viruses, Rice Tungro Bacilliform Virus (RTBV) and Rice Tungro Spherical Virus (RTSV). Naturally, tungro disease is transmitted in a semi-persistant manner by leafhopper. RTBV transmission is dependent on the presence of RTSV. The leafhoppers have to feed on RTSV infected plants in order to transmit RTBV (Etienne *et al*., 2000). Conventional breeding of rice for resistance to tungro disease has proved only partially successful because it was not possible to distinguish between leafhopper and virus resistance. Furthermore, the resistance proved to be unstable in many lines. In most of these cases, the resistance was found to be against the insect vector. Agroinoculation provides a means of infecting plant with a virus genome without the use of insect vector (Sta *et al*., 1999).

In this study, agroinoculation technique was used to introduce infectious clone of RTBVCN into rice plants without insect vector and the molecular interaction by which plant response to the virus can be studied directly through ethylene signal transduction. To further study that how rice plant perceive and regulate the signal into defense response, ethylene perception genes (Ethylene Responsive Sensor 1, *Os-ERS1* and Ethylene Responsive Sensor 2, *Os-ERS2*) from Khao Dok Mali 105 (KDML105) rice were isolated and characterized. In order to detect the response of KDML105 rice to tungro disease. The expression of ethylene receptor genes of KDML105 rice before and during counteract with Rice Tungro Bacilliform Virus Chainat isolate (RTBVCN) were determined.

MATERIALS AND METHODS

Plant material

Oryza sativa variety Khao Dok Mali 105 (KDML 105) was selected for this experiment. Rice was grown in netted house at Kasetsart University, Kamphaeng Sean Campus, Nakhon Pathom province.

Isolation of homologue ethylene receptor genes, *Os-ERS1* and *Os-ERS2*

Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed by preparing a mixture containing 5 μg of total RNA, 1 μl of formamide, 30 pmole of Os-ERS1 and Os-ERS2 specific primer and 1 mM of each dNTPs. The mixture was heated at 65°C for 5 min and quenched on chilled (-80°C) absolute ethanol. The master mix containing 1X RT-buffer (25 mM Tris-HCl pH8.3, 5 mM $MgCl_2$, 50 mM KCl) 2 mM DTT, 25 U of RNase out (Invitrogen) and 10 U of SuperscriptIII (Invitrogen), was added to a total volume of 20 μl. The reaction was incubated at 42°C for 50 min, followed by heating at 70°C for 15 min to inactivate the reverse transcriptase and used as a template for PCR amplification.

In PCR reaction, primer pairs of ERS1-4 (sense: 5'-AAAACHACWCTTGTKGAG-3'), ERS1-9 (antisense: 5' CATTTCATGRTTCAKD AC 3'), ERS2-3 (sense: 5'CAGGTAGCGGTC GCATTGTC 3') and ERS2-10 (antisense: 5' CCAGCTCAAGACTCCCAT 3') were used to amplify. 5'-RACE and 3'-RACE strategies were employed to obtain the full-length cDNA sequence of ERS1 and ERS2. The sequence of gene specific primer was as follows: 5'ERS1 (sense: 5' TAAACATTGGGTATGGATG3'),5'ERS1 Nested (antisense: 5' AGCAAGTCAGGGATAA TATG 3'), ERS2-3F1-B (sense: 5' GGATGGAT CATGTGATTGC 3'), 5' ERS2R1-A (antisense: 5' TTTTGCTCCATTAGTAG 3'), 3'ERS1-F1 (sense: 5' CGAGCAATCGTTTCTTT 3'), and 3' ERS2-F2-A (antisense: 5' AGTGGCAGTGGA TTG 3') Nucleotide sequences of Os-ERS1 and Os-ERS2 were cloned into pDrive vector (QIAGEN) and determined using DNA sequencer (Amplied Biosystem). The sequences were analyzed using BLAST program (NCBI) and ClustalW (EBI). The phylogenetic tree was constructed using the MegAlign program.

***Agrobacterium* inoculation**

An infectious clone of RTBVCN (Chainat isolate) was introduced into rice plants by agroinoculation technique. Ten microliter of *Agrobacterium* clone of RTBV suspension were plated on 2X-YT media containing 50 μg/ml of kanamycin and 50 μg/ml of rifampicin as a selective agent and incubated at 28°C for 48 hr. The *Agrobacterium* cells were scraped from the media surface and resuspended in deionized water. Ten microliters of *Agrobacterium* suspension (10^{10} C.F.U/ml) was injected at the base of a stem of a two-week old rice plant using one milliliter epidemic syringe.

Detection of viral DNA in rice plants

The presence of RTBVCN (Chainat) genome in inoculated rice was determined by PCR analysis. Small pieces of infected leaves were ground in liquid nitrogen to fine powder. Twenty microliter of 2 M NaOH was added. One microliter of upper phase was transferred into 500 μl of 1 M Tris-HCl, pH9.0 and used as template for PCR mixing containing 1X PCR buffer, 10 mM each of dNTPs, 2.5 U of *Taq* DNA polymerase, and RTBVCN specific primer. PCR was performed under to the following condition: 94°C for 4 min and 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1.30 min.

RNA gel blot analysis

Total RNA was extracted from rice leaves at various times after RTBVCN infection using seed extraction method as described by Naito *et al*. (1994). The concentration and purity of total RNA was determined using spectrophotometer (Ultraspec 500/1100). For Northern blot analysis 25 μg of total RNA was used. The RNA was separated on 1% agarose/formaldehyde gel and transferred to nylon membrane positively charge (Roche). The membrane was hybridized with PCR-DIG labeling probe.

RESULTS AND DISCUSSIONS

In this study, *Os-ERS1* and *Os-ERS2* cDNA were cloned and sequenced from Khao Dok Mali 105 (KDML105) rice using RT-PCR technique. 5′ RACE and 3′ RACE strategies were employed to obtain the full-length cDNA sequences. The *Os-ERS1* cDNA sequence was 2,072 nucleotides in length which encoded 363 amino acids (Figure 1). The cDNA sequence of *Os-ERS2* was 2,950 nucleotides in length and the open reading flame (ORF) encoded 635 amino acids (Figure 2). Amino acid comparison between two varieties rice, KDML105 and japonica variety, was done using ClustalW program. The deduced amino acid sequences of both *Os-ERS1* and *Os-ERS2* of KDML105 displayed high homology to japonica varieties (accession number AF013979 and AF460181) (Figure 3 and 4). When compare Os-ERS1 and Os-ERS2, the percentage of homology is at 58.3 and 68.9 at nucleotide and amino acid, respectively. The C-terminus showed lowest similarity (Figure 5 and 6). When their nucleotide and amino acid sequences were compared with other plant ERSs, the phylogenic tree indicated that monocot *Os-ERS1* and *Os-ERS2* were in separate sub-clusters to dicot plants and *At-ERS2* was in separate cluster to other plant ERSs (Figure 7 and 8). Moreover, *Os-ERS1* and *Os-ERS2* were in separate sub-cluster. Although there is limited number of ethylene receptor genes isolated from monocotyledonous species so far, the results illustrated that there is a presence of a sub-cluster containing ethylene receptor members from monocot species.

Amino acid sequences of *Os-ERS1* and *Os-ERS2* were analyzed for their functional domains. Three domains including transmembrane (ethylene binding), histidine kinase and GAF domain were present in both *Os-ERS1* and *Os-ERS2* (Figure 9). The transmembrane and histidine kinase domains are involved in affinity to ethylene binding and efficiency to transmit the signal to downstream components, while the function of the GAF region are not known (Chen *et al*., 2005). However, it must play some role in signal transduction as it joins the ligand binding portion or the receptor to the proposed signal transmission domain and is one of the most highly conserved domains within the ethylene receptor family. In *Arabidopsis*, hydrophobic segment IV of transmembrane domain was found on *At-ERS2*, whereas it is not presented on *Os-ERS1* and *Os-ERS2* of both KDML105 and japonica variety (Figure 9). This extra domain might affect ethylene affinity. Moreover, amino acid residue on H and F motif of histidine kinase region of KDML105 rice were differenced from japonica variety (Figure 11). Therefore, the conserved leucine (L) residue on H motif (consensus sequence, —HE—PL) of both *Os-ERS1* and *Os-ERS2* of KDML105 were substituted from leusine (L) to be methionine (M). Consensus proline (P) amino acid sequence on F motif (consensus sequence, -F-PF-) of both KDML105 and japonica variety were substituted to be lysine (K) and arginine (R), respectively. The implication is probably in conformational of both *Os-ERS1* and *Os-ERS2* which might be affecting on their affinity and efficiency to receive and transduce the signal into the cell. As reported in Qu and Schaller (2004) reported that, site directed mutation eliminated histidine kinase activity had a modest effect upon the ability of the receptor to repress ethylene responses.

The copy number of *Os-ERS1* and *Os-ERS2* in rice genome was determined by Southern analysis. Using Os-ERS1 probe, a single band of approximately at 3 kb and 9 kb was observed in *Bam*HI and *Hind*III lanes, respectively. When the Southern blot was hybridized with Os-ERS2 specific probe, the *Msp*I lane showed a single band of approximately 3 kb (Figure 10). When cDNA of Os-ERS1 and Os-ERS2 was analyzed using blastn (NCBI), the data indicated that Os-ERS1 and Os-ERS2 are located on chromosome number 3 and 5, respectively.

In order to monitor the dynamic of ethylene signaling pathway in response to Rice Tungro Bacilliform Virus (RTBV) infection, Northern analysis was performed. In this study, the expression of three putative receptor genes and four groups of ethylene responsive factors (ERFs) were investigated. The three ethylene receptors including Os-ERS1, Os-ERS2 and os-ETR2 were

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TTAACATTGG  GTATGGATGG  ATGTGACTGC  ATCGAGCCAC  TATGGCCAAC  TGATGAGCTC  CTCATCAAGT   70
              M  D  G     C  D  C     I  E  P     L  W  P  T     D  E  L     L  I  K
ATCAGTACAT  CTCGGACTTC  TTCATAGCCC  TCGCATATTT  CTCGATCCCG  TTGGAACTAA  TTTATTTCGT  140
Y  Q  Y  I     S  D  F     F  I  A     L  A  Y  F     S  I  P     L  E  L     I  Y  F  V
GAAGAAGTCA  TCCTTCTTCC  CATACAGATG  GGTTTTGATC  CAGTTTGGTG  CATTTATAGT  CCTATGTGGA  210
  K  K  S     S  F  F     P  Y  R  W     V  L  I     Q  F  G     A  F  I  V     L  C  G
GCGACTCATC  TCATAAGCCT  GTGGACTTTC  ACCACACACA  CAAAGACTGT  TGCTATGGTC  ATGACAGTCG  280
A  T  H     L  I  S  L     W  T  F     T  T  H     T  K  T  V     A  M  V     M  T  V
CAAAGGTTTC  AACGGCTGTT  GTGTCCTGTG  CGACAGCTTT  GATGCTTGTA  CATATTATCC  CTGACTTGCT  350
A  K  V  S     T  A  V     V  S  C     A  T  A  L     M  L  V     H  I  I     P  D  L  L
GAGCGTGAAA  ACAAGGGAAT  TGTTTCTGAA  GAATAAAGCT  GAACAGCTTG  ATAGGGAGAT  GGGCTTGATT  420
  S  V  K     T  R  E     L  F  L  K     N  K  A     E  Q  L     D  R  E  M     G  L  I
AGGACACAGG  AAGAGACTGG  GAGGCATGTT  AGGATGCTTA  CCCATGAAAT  CAGAAGCACT  CTTGATAGAC  490
R  T  Q     E  E  T  G     R  H  V     R  M  L     T  H  E  I     R  S  T     L  D  R
ATACGATTTT  GAAGACTACA  CTTGTTGAGC  TAGGAGGGAC  CTTGGGCCTG  GAAGAATGTG  CCCTATGGAT  560
H  T  I  L     K  T  T     L  V  E     L  G  G  T     L  G  L     E  E  C     A  L  W  M
GCCATCAAGA  AGCGGTTCAA  GTCTTCAGCT  TTCTCATACT  CTCCGCCACC  AGATTACTGT  TGGGTCAACT  630
  P  S  R     S  G  S     S  L  Q  L     S  H  T     L  R  H     Q  I  T  V     G  S  T
GTATCAATTA  ATCTTCCTGT  TGTCAATCAA  GTGTTCAGTA  GCAACAGAGC  AATTATAATA  CCCCACACAT  700
V  S  I     N  L  P  V     V  N  Q     V  F  S     S  N  R  A     I  I  I     P  H  T
CTCCTTTGGC  ACGGATCCGA  CCTCTTGCAG  GGAGATATGT  TCCACCAGAA  GTGGCTGCAG  TGCGAGTTCC  770
S  P  L  A     R  I  R     P  L  A     G  R  Y  V     P  P  E     V  A  A     V  R  V  P
TCTTCTACAT  CTTTCAAACT  TTCAAATAAA  TGATTGGCCA  GAGCTTTCAG  CAAAGAGCTA  TGCAATCATG  840
  L  L  H     L  S  N     F  Q  I  N     D  W  P     E  L  S     A  K  S  Y     A  I  M
GTTCTGATGC  TTCCATCTGA  TAGCGCAAGA  AAATGGCATG  TGCATGAGTT  GGAGCTTGTT  GAGGTCGTGG  910
V  L  M     L  P  S  D     S  A  R     K  W  H     V  H  E  L     E  L  V     E  V  V
CTGATCAGGT  TGCAGTTGCA  CTTTCTCATG  CAGCTATTCT  TGAAGAGTCC  ATGCGTGCGC  GTGATCTGCT  980
A  D  Q  V     A  V  A     L  S  H     A  A  I  L     E  E  S     M  R  A     R  D  L  L
AATGGAGCAA  AATGTTGCTC  TGGATTTAGC  TCGACGTGAG  GCTGAAATGG  CTATCCGTGC  TCGTAATGAT 1050
  M  E  Q     N  V  A     L  D  L  A     R  R  E     A  E  M     A  I  R  A     R  N  D
TTCCTGGCTG  TTATGAATCA  TGAAATGAGA  ACACCAATGA  ATGCAATAAT  AGCCCTTTCG  TCCTTACTTT 1120
F  L  A     V  M  N  H     E  M  R     T  P  M     N  A  I  I     A  L  S     S  L  L
TGGAAACCGA  ACTTACTCCT  GAGCAGCGCT  TGATGGTGGA  AACAGTCTTG  AAAAGCAGCA  ATCTTTTAGC 1190
L  E  T  E     L  T  P     E  Q  R     L  M  V  E     T  V  L     K  S  S     N  L  L  A
AACACTCATC  AATGATGTGT  TAGATCTTTC  CAAACTTGAG  GATGGTAGCC  TTGAATTGGA  GATTAAAGCA 1260
  T  L  I     N  D  V     L  D  L  S     K  L  E     D  G  S     L  E  L  E     I  K  A
TTCAATCTTC  ATGCTGTTTT  CAAGGAGGTA  ATGAGTTTCA  TCAAGCCAAT  CGCAGCCATC  AAGAGACTGT 1330
F  N  L     H  A  V  F     K  E  V     M  S  F     I  K  P  I     A  A  I     K  R  L
CTGTGTCAGT  TATGTTGGCA  CCAGACTTGC  CATTATGTGC  CATCGGTGAT  GAAAAGAGGC  TAATGCAAAC 1400
S  V  S  V     M  L  A     P  D  L     P  L  C  A     I  G  D     E  K  R     L  M  Q  T
TATTTTGAAT  ATCTCTGGCA  ATGCTGTTAA  GTTTACAAAG  GAGGGCCACA  TCACACTTGT  AGCTTCTGTT 1470
  I  L  N     I  S  G     N  A  V  K     F  T  K     E  G  H     I  T  L  V     A  S  V
GTGAAGGCTG  ACTCTTTAAG  AGAATTCAGA  ACCCCAGATT  TTCACCCAAC  TGCAAGCGAT  GACAATTTTT 1540
V  K  A     D  S  L  R     E  F  R     T  P  D     F  H  P  T     A  S  D     D  N  F
ATTTGAAAGT  TCAGATTAAA  GATACGGGCT  GTGGCATTAG  CCCTCAGGAT  CTACCTCAGG  TATTTACAAA 1610
Y  L  K  V     Q  I  K     D  T  G     C  G  I  S     P  Q  D     L  P  Q     V  F  T  K
GTTTCCTCAA  TCTCAGCCTG  GAGGAAACAG  GGGATACAGT  GGTAGTGGTC  TTGGCCTTGC  CATTTGCAAG 1680
  F  P  Q     S  Q  P     G  G  N  R     G  Y  S     G  S  G     L  G  L  A     I  C  K
AGGTTTGTTA  CTTTGATGGG  AGGGCACATC  TGGTTGGATA  GCGAAGGAAC  AGGAAGAGGC  TGCACAGTAA 1750
R  F  V     T  L  M  G     G  H  I     W  L  D     S  E  G  T     G  R  G     C  T  V
CGTTTGTCAT  CCAGCTTGGC  ATATGCGATA  ATACGAATGC  ATATCAGCAG  AAGCTGATTC  CTCTAGTCTG 1820
T  F  V  I     Q  L  G     I  C  D     N  T  N  A     Y  Q  Q     K  L  I     P  L  V  W
GCCGAGCAGT  GGGGATGCTG  ATTTTGTTGG  TCCGGTGCCA  AATGCTCCTA  ATGAAGAGAA  AGGTCAGGCT 1890
  P  S  S     G  D  A     D  F  V  G     P  V  P     N  A  P     N  E  E  K     G  Q  A
TCTCTGAATC  TCGGTATCAG  AGAAGTATAT  TGAGCTACTG  TAAATTGATC  GACGGCATTG  TATCAAGTAG 1960
S  L  N     L  G  I  R     E  V  Y     *
GGCCTGGTTA  GTTTGCCTCT  AATTTTGTGC  CTAGCCTTGT  GATAGCTGGA  ATATATTTGT  ACAAATAATG

TACAGCAGAT  GGGACTGCAT  AATGCAGCTA  TGCACCTGGG  AT
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Figure 1 Nucleotide sequence and the deduced amino acid sequence of putative ethylene responsive sensor 1 (*Os-ERS1*) from KDML105 variety rice. Numbers on the right refer to nucleotide residues. The translation start site is underlined. The asterisk denotes the stop codon.

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ATGGATGGAT  CATGTGATTG  CATTGAGCCG  CTCTGGCAGG  CGGGTGATCT  CCTTGTAAAG  TATCAGTACA     1
 M  D  G    S  C  D  C    I  E  P    L  W  Q    A  G  D  L    L  V  K    Y  Q  Y
TATCTGACTT  CTTTATTGCG  CTCGCTTACT  TCTCGATCCC  TTTGGAGCTC  ATATATTTCG  TTAAGAAGTC    70
I  S  D  F    F  I  A    L  A  Y    F  S  I  P    L  E  L    I  Y  F    V  K  K  S
AGCATTCTTC  CCTTACCGAT  GGGTGCTTAT  ACAATTCGGC  GCATTCATTG  TTCTTTGTGG  GGCAACCCAC   140
   A  F  F    P  Y  R    W  V  L  I    Q  F  G    A  F  I    V  L  C  G    A  T  H
CTGATAAATT  TGTGGACTTT  TGCCATATAT  ACCAAGAGTA  TAGCTGTGGT  ACTGACAGTG  GCGAAAGCAG   280
 L  I  N    L  W  T  F    A  I  Y    T  K  S    I  A  V  V    L  T  V    A  K  A
CGACAGCGGT  TGTTTCGTGC  ATCACAGCTT  TGATGCTTGT  GCATATAATT  CCTGATTTGT  TGAATGTGAA   350
A  T  A  V    V  S  C    I  T  A    L  M  L  V    H  I  I    P  D  L    L  N  V  K
GTTGAGAGAG  AGATTTCTGA  AGGATAAGGC  TGATGAGCTT  GATAGAGAGA  TGGGGATTAT  AAGAACACAA   420
   L  R  E    R  F  L    K  D  K  A    D  E  L    D  R  E    M  G  I  I    R  T  Q
GAGGAGACAG  GAAGACATGT  CCACATGCTG  ACCCATGAGA  TAAGAAGCAC  ACTTGACAGG  CACACCATTC   490
 E  E  T    G  R  H  V    H  M  L    T  H  E    I  R  S  T    L  D  R    H  T  I
TGCGAACTAC  GCTCGTCGAG  CTGGGAAGGA  CTCTTGCTTT  AGCGGAGTGT  GCCCTGTGGA  TGCCAACACG   560
L  R  T  T    L  V  E    L  G  R    T  L  A  L    A  E  C    A  L  W    M  P  T  R
CTCTGGATCG  GCCCTTCAGC  TCTCTCATAC  GATATATAAC  AGCGCAGCAA  TTGGATCAGT  TGTTCCTATC   630
   S  G  S    A  L  Q    L  S  H  T    I  Y  N    S  A  A    I  G  S  V    V  P  I
AACCTTCCCA  TTGTCAGTAA  GGTTTTTAAT  AGTAACCGTG  TAGTAAAAAT  TCCGCATACC  TCCCCGTTAG   700
 N  L  P    I  V  S  K    V  F  N    S  N  R    V  V  K  I    P  H  T    S  P  L
CTTCGATAAC  GGCTGACAAA  AGCAGGTATG  TGCCACCAGA  GGTTGTGGCT  ATCCGTGTTC  CACTGCTGCA   770
A  S  I  T    A  D  K    S  R  Y    V  P  P  E    V  V  A    I  R  V    P  L  L  H
CCTTACGAAT  TTCCAAATAA  ATGATTGGCC  TGAGCTATCT  GCAAAAAGCT  TTGCAGTAAT  GGTTTTGATG   840
   L  T  N    F  Q  I    N  D  W  P    E  L  S    A  K  S    F  A  V  M    V  L  M
CTCCCTCCAG  ACAGTGCAAG  AGAATGGCGG  CCGCATGAAC  GGGAGCTTGT  TGAAGTCGTC  GCTGATCAGG   910
 L  P  P    D  S  A  R    E  W  R    P  H  E    R  E  L  V    E  V  V    A  D  Q
TAGCGGTCGC  ATTGTCTCAT  GCTGCCATTT  TGGAAGAGTC  CATGCGGGCC  CGTGATCTAC  TAATGGAGCA   980
V  A  V  A    L  S  H    A  A  I    L  E  E  S    M  R  A    R  D  L    L  M  E  Q
AAACATTGCT  CTTGATGCAG  CACGGCGGGA  GGCAGAAATG  GCTATTTGTG  CCTGTAATGA  TTTTCTTGCT  1050
   N  I  A    L  D  A    A  R  R  E    A  E  M    A  I  C    A  C  N  D    F  L  A
GTAATGAACC  ATGAAATGCG  GACTCCTATG  CGAGCAATCG  TTTCTTTGTC  CTCTCTCCTT  TTAGAAACAA  1120
 V  M  N    H  E  M  R    T  P  M    R  A  I    V  S  L  S    S  L  L    L  E  T
ATCTTAGTGC  TGAACAACGC  CTAATGGTTG  AGACTATACT  AAAGAGTAGC  GATCTTCTGG  CAACTCTTAC  1190
N  L  S  A    E  Q  R    L  M  V    E  T  I  L    K  S  S    D  L  L    A  T  L  T
GAATGATGTT  TTGGACGTTT  CAAAGCTTGA  GAATGGGAGT  CTTGAGCTGG  AAATTGCACC  TTTTAATTTG  1260
   N  D  V    L  D  V    S  K  L  E    N  G  S    L  E  L    E  I  A  P    F  N  L
CATTCCACCT  TTACAGATGT  GGTTAATTTG  ATTAAGCCAG  TAGCAGCGTG  CAAGAGGCTC  TCGGTTATGG  1330
 H  S  T    F  T  D  V    V  N  L    I  K  P    V  A  A  C    K  R  L    S  V  M
TCACTTTGGC  ACCAGAGTTA  CCTCTACATG  CTATTGGTGA  TCAAAAGCGA  TTGATGCAAA  TAATCCTAAA  1400
V  T  L  A    P  E  L    P  L  H    A  I  G  D    Q  K  R    L  M  Q    I  I  L  N
TGTTGCCGGG  AACTCCATTA  AGTTCACAAA  GGAGGGTCAT  GTTTCGATTA  CAGCTTCTAT  GGCCAGACCA  1470
   V  A  G    N  S  I    K  F  T  K    E  G  H    V  S  I    T  A  S  M    A  R  P
GATGCTTTGA  GAGGTCCACA  TGAACCTGAC  TACCATCCAG  TTGTCTCTGA  TGGATTCTTT  TACCTGGCTG  1540
 D  A  L    R  G  P  H    E  P  D    Y  H  P    V  V  S  D    G  F  F    Y  L  A
TTCAGGTAAA  AGACACAGGC  TGCGGAATCA  GCCCTCAGGA  TATGCCCCAC  ACATTCAGAA  AGTTTGCACA  1610
V  Q  V  K    D  T  G    C  G  I    S  P  Q  D    M  P  H    T  F  R    K  F  A  H
CCCTGAAAAC  GCAGGCAAAT  GGAACAGTGG  CAGTGGATTG  GGGCTGGCCC  TTTCCAGAAG  ATTTGTCAGT  1680
   P  E  N    A  G  K    W  N  S  G    S  G  L    G  L  A    L  S  R  R    F  V  S
CTAATGGAAG  GTAACATCTG  GCTCGAGAGC  GAAGGCGTCG  GGAAGGGCTG  TACCGCGATG  TTCTTTGTGA  1750
 L  M  E    G  N  I  W    L  E  S    E  G  V    G  K  G  C    T  A  M    F  F  V
AACTTGGCAT  GCCTGAGAAA  CCAAATGCAA  ACCTCCGAAG  AATGGCACCG  CACCCTCTAC  AGCCAAACCA  1820
K  L  G  M    P  E  K    P  N  A    N  L  R  R    M  A  P    H  P  L    Q  P  N  Q
AGGAGCTGGA  GGCCCTGATG  CCCTCAGTAT  ATCCATAATG  GACAGCAACC  CGAGGGTTCC  TCGGGTTCGC  1890
   G  A  G    G  P  D    A  L  S  I    S  I  M    D  S  N    P  R  V  P    R  V  R
TACCAATCAA  GCGTATGAGA  AGCACCGATA  AACCATCGCC  ATGCCAAGGC  AGAGCATCCG  ACCCAGTTTT  1960
 Y  Q  S    S  V  *
GCCATCACAG  TTTACAGCTC  CATTTGCAGC  TCTCTTTTTG  ATGTATGGAT  GGTAGATGTT  GTTCTTGTCC  2030
ATCAGTGGAG  GTACCTTGGG  CATGCTGGGA  TAAGTTTCAT  TGGCATTCCT  GAATCTTGAT  TTCAGTTGTG  2100
GGGGTGATTG  GGGGGTCTTA  AGTGGTGACA  ATTGGGAAAG  CTGATGTAGT  ATGCTGTTGT  CTGCACGAGT  2170
CTGAAGTTAC  TGGGAAGTGC  CAATTTACTG  CAAGTGACAG  TGAACATGAA  GGTCTATGGC  CATCTTACAT  2240
CTCTGATACC  CTGAGCTATA  GAAGAGCTTG  GACGAACGTG  TTTATTTTTC  TTACTTTTTT  CCCTAGATTA  2310
GGGCTACAAC  CTGGAAGGGA  TCGATCTACT  AGCTGATTGC  AGTATCATTT  GTACTTTTTG  TTTTTTTTCT  2380
NAAATAAGAG  TATCAGTGGN  AANTGTAAGT  GTTACTGCAG  TGATAANAAG  AACAAAAAAA  TAAAACAAA   2449
```

Figure 2 Nucleotide sequence and the deduced amino acid sequence of putative ethylene responsive sensor 2 (*Os-ERS2*) from KDML105 variety rice. Numbers on the right refer to nucleotide residues. The translation start site is underlined. The asterisk denotes the stop codon.

determined at various times after RTBV inoculation. Comparison to non-infected rice, transcription of Os-ERS1 and Os-ETR2 were strongly enhanced by RTBV inoculation, whereas Os-ERS2 had been constitutively expressed. Moreover, effect of *Agrobacterium* inoculation and wounding (water injection) was observed at low level in Os-ERS1 while Os-ETR2 was activated in response to both *Agrobacterium* and water injection. Only Os-ERS2 was not responded and the expression pattern was different. These may reflect their functional difference.

In nuclear event, Northern analysis revealed that transcriptional levels of all four groups of ethylene responsive factors were elevated by RTBV inoculation. Interestingly, Os-ERFG1 and Os-ERFG4 were strongly induced after the infection of many pathogens. Many studied reported that ERFG1 is induced after the infection of many pathogens and constitutively expression of ERFG1 enhances the resistance of Arabidopsis plants to several fungi (Berrocal *et*

```
ERS1-KDML105        MDGCDCIEPLWPTDELLIKYQYISDFFIALAYFSIPLELIYFVKKSSFFPYRWVLIQFGA 60
ERS1-Japonica       MDGCDCIEPLWPTDELLIKYQYISDFFIALAYFSIPLELIYFVKKSSFFPYRWVLIQFGA 60
                    ************************************************************

ERS1-KDML105        FIVLCGATHLISLWTFTTHTKTVAMVMTVAKVSTAVVSCATALMLVHIIPDLLSVKTREL 120
ERS1-Japonica       FIVLCGATHLINLWTFTTHTKTVAMVMTVAKVSTAVVSCATALMLVHIIPDLLSVKTREL 120
                    ***********.************************************************

ERS1-KDML105        FLKNKAEQLDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGGTLGLEECA 180
ERS1-Japonica       FLKNKAEQLDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKTTLVELGGTLGLEECA 180
                    ************************************************************

ERS1-KDML105        LWMPSRSGSSLQLSHTLRHQITVGSTVSINLPVVNQVFSSNRAIIIPHTSPLARIRPLAG 240
ERS1-Japonica       LWMPSRSGSSLQLSHTLRHQITVGSTVSINLPVVNQVFSSNRAIIIPHTSPLARIRPLAG 240
                    ************************************************************

ERS1-KDML105        RYVPPEVAAVRVPLLHLSNFQINDWPELSAKSYAIMVLMLPSDSARKWHVHELELVEVVA 300
ERS1-Japonica       RYVPPEVAAVRVPLLHLSNFQINDWPELSAKSYAIMVLMLPSDSARKWHVHELELVEVVA 300
                    ************************************************************

ERS1-KDML105        DQVAVALSHAAILEESMRARDLLMEQNVALDLARREAEMAIRARNDFLAVMNHEMRTPMN 360
ERS1-Japonica       DQVAVALSHAAILEESMRARDLLMEQNVALDLARREAEMAIRARNDFLAVMNHEMRTPMN 360
                    ************************************************************

ERS1-KDML105        AIIALSSLLLETELTPEQRLMVETVLKSSNLLATLINDVLDLSKLEDGSLELEIKAFNLH 420
ERS1-Japonica       AIIALSSLLLETELTPEQRLMVETVLKSSNLLATLINDVLDLSKLEDGSLELEIKAFNLH 420
                    ************************************************************

ERS1-KDML105        AVFKEVMSFIKPIAAIKRLSVSVMLAPDLPLCAIGDEKRLMQTILNISGNAVKFTKEGHI 480
ERS1-Japonica       AVFKEVMSFIKPIAAIKRLSVSVMLAPDLPLCAIGDEKRLMQTILNISGNAVKFTKEGHI 480
                    ************************************************************

ERS1-KDML105        TLVASVVKADSLREFRTPDFHPTASDDNFYLKVQIKDTGCGISPQDLPQVFTKFPQSQPG 540
ERS1-Japonica       TLVASVVKADSLREFRTPDFHPTASDDNFYLKVQIKDTGCGISPQDLPQVFTKFPQSQPG 540
                    ************************************************************

ERS1-KDML105        GNRGYSGSGLGLAICKRFVTLMGGHIWLDSEGTGRGCTVTFVIQLGICDNTNAYQQKLIP 600
ERS1-Japonica       GNRGYSGSGLGLAICKRFVTLMGGHIWLDSEGTGRGCTVTFVIQLGICDNTNAYQQKLIP 600
                    ************************************************************

ERS1-KDML105        LVWPSSGDADFVGPVPNAPNEEKGQASLNLGIREVY 636
ERS1-Japonica       LVWPSSGDADFVGPVPNAPNEEKGQASLKSRYQRSI 636
                    **************************:   :.
```

Figure 3 Amino acid sequence alignment of *Os-ERS1* from KDML105 variety rice compared with *Oryza sativa* japonica variety (Accession number AF013979) using ClustalW program.

al., 2002: Chen *et al*., 2002). This might indicated that the transcription response is triggered by specific pathogen factors. It is clear from this study that ethylene receptors and responsive factors involving in rice responses to viral infection.

It is clear from this study that several ethylene receptors and responsive factors involving in rice responses to viral infection. Although it seems redundant and complicate, studies in plants and Drosophila indicated that overlapping sets of synergistic and antagonistic physiological responses are likely serve purposes of monitoring and integrating multitude of inputs to reach the action at cellular and whole-organism levels (Burack and Shaw, 2000; Lohrmann and Harter, 2002). A recent study demonstrated the ethylene and cytokinin signal integration in *Arabidopsis* through a response regulator, *Arabidopsis* response regulator 2 (ARR2) (Hass *et al*., 2004). Further studies into plant response to viral pathogen are urgently needed to understand for better manage and control the plant diseases.

```
Os-ERS2-KDML105      MDGSCDCIEPLWQAGDLLVKYQYISDFFIALAYFSIPLELIYFVKKSAFFPYRWVLIQFG 60
Os-ERS2-Japonica     MDGSCDCIEPLWQADDLLVKYQYISDFFIALAYFSIPLELIYFVKKSAFFPYRWVLIQFG 60
                     **************.*********************************************

Os-ERS2-KDML105      AFIVLCGATHLINLWTFAIYTKSIAVVLTVAKAATAVVSCITALMLVHIIPDLLNVKLRE 120
Os-ERS2-Japonica     AFIVLCGATHLINLWTFAIYTKTIAVVLTVAKAATAVVSCITALMLVHIIPDLLNVKLRE 120
                     **********************:************************************

Os-ERS2-KDML105      RFLKDKADELDREMGIIRTQEETGRHVHMLTHEIRSTLDRHTILRTTLVELGRTLALAEC 180
Os-ERS2-Japonica     RFLKDKADELDREMGIIRTQEETGRHVHMLTHEIRSTLDRHTILRTTLVELGRTLALAEC 180
                     ************************************************************

Os-ERS2-KDML105      ALWMPTRSGSALQLSHTIYNSAAIGSVVPINLPIVSKVFNSNRVVKIPHTSPLASITADK 240
Os-ERS2-Japonica     ALWMPTRSGSALQLSHTIYNSAAIGSVVPINLPIVSKVFNSNRVVKIPHTSPLASITADK 240
                     ************************************************************

Os-ERS2-KDML105      SRYVPPEVVAIRVPLLHLTNFQINDWPELSAKSFAVMVLMLPPDSAREWRPHERELVEVV 300
Os-ERS2-Japonica     SRYVPPEVVAIRVPLLHLTNFQINDWPELSAKSFAVMVLMLPPDSAREWRPHERELVEVV 300
                     ************************************************************

Os-ERS2-KDML105      ADQVAVALSHAAILEESMRARDLLMEQNIALDAARREAEMAICACNDFLAVMNHEMRTPM 360
Os-ERS2-Japonica     ADQVAVALSHAAILEESMRARDLLMEQNIALDAARREAEMAICACNDFLAVMNHEMRTPM 360
                     ************************************************************

Os-ERS2-KDML105      RAIVSLSSLLLETNLSAEQRLMVETILKSSDLLATLTNDVLDVSKLENGSLELEIAPFNL 420
Os-ERS2-Japonica     RAIVSLSSLLLETNLSAEQRLMVETILKSSDLLATLTNDVLDVSKLENGSLELEIAPFNL 420
                     ************************************************************

Os-ERS2-KDML105      HSTFTDVVNLIKPVAACKRLSVMVTLAPELPLHAIGDQKRLMQIILNVAGNSIKFTKEGH 480
Os-ERS2-Japonica     HSTFTDVVNLIKPVAACKRLSVMVTLAPELPLHAIGDQKRLMQIILNVAGNSIKFTKEGH 480
                     ************************************************************

Os-ERS2-KDML105      VSITASMARPDALRGPHEPDYHPVVSDGFFYLAVQVKDTGCGISPQDMPHTFRKFAHPEN 540
Os-ERS2-Japonica     VSITASMARPHALRGPHEPDYHPVVSDGFLYLAVQVKDTGCGISPQDMPHTFRKFAHPEN 540
                     **********.******************:******************************

Os-ERS2-KDML105      AGKWNSGSGLGLALSRRFVSLMEGNIWLESEGVGKGCTAMFFVKLGMPEKPNANLRRMAP 600
Os-ERS2              AGKWNSGSGLGLALSRRFVSLMEGNIWLESEGVGKGCTAMFFVKLGMPEKPNANLRRMAP 600
                     ************************************************************

Os-ERS2-KDML105      HPLQPNQGAGGPDALSISIMDSNPRVPRVRYQSSV 635
Os-ERS2-Japonica     HPLQPNQGAGGPDALSISIMDSNPRVPRVRYQSSV 635
                     ***********************************
```

Figure 4 Amino acid sequence alignment of *Os-ERS2* from KDML105 variety rice compared with *Oryza sativa* japonica variety (Accession number AF460181) using ClustalW program.

ACKNOWLEDGMENTS

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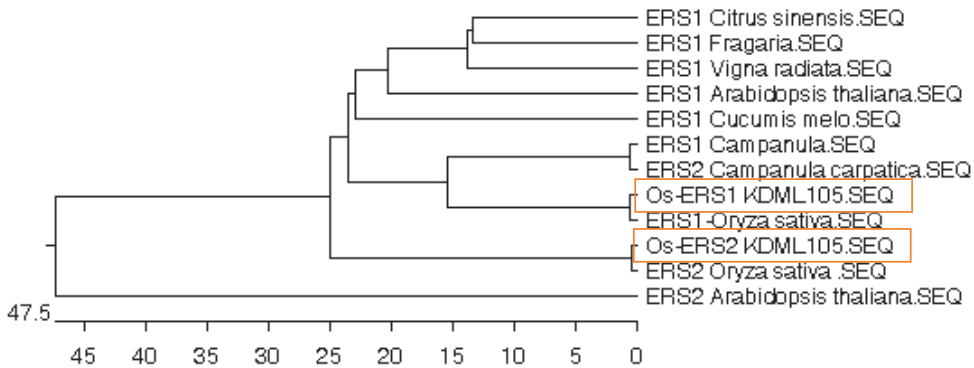

Figure 5 A phylogenetic tree generated with nucleotide sequence of ethylene receptor genes from various species. Cluster analysis was done using MegAlign program (Laser Gene). The accession number that appeared in Genbank database are listed below: *Oryza japonica* (AF013979 and AF460181), *Arabidopsis thaliana* (NM_129658 and NM_100312), *Cucumis melo* (AF037368), *Fragaria x ananassa* (AJ297512), *Citrus sinensis* (AF092088), *Vigna radiata* (AF098270) and *Campanula carpatica* (AF 413668 and AF413669).

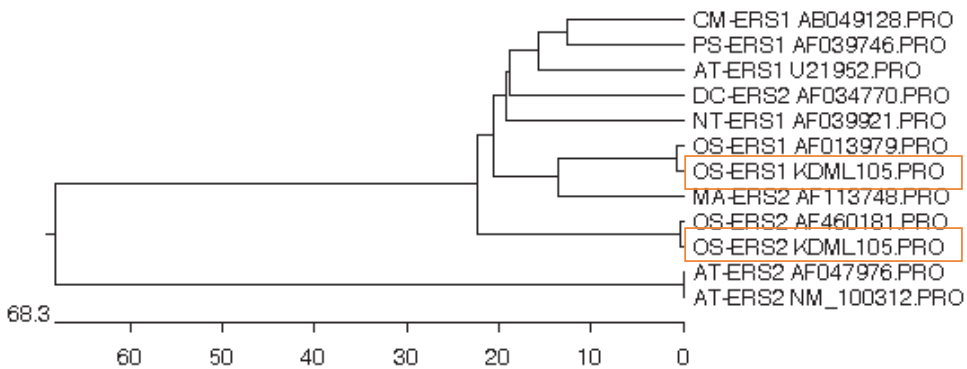

Figure 6 A phylogenetic tree generated with amino acid sequence of ethylene receptor genes from various species. The accession number that appeared in Genbank database are listed below:*Arabidopsis thaliana*: *AT-ERS1* (U21952), *AT-ERS2* (NM_100312), *AT-ERS2* (AF047976), *Oryza sativa*: *OS-ERS1* (AF013979), *OS-ERS2* (AF460181), *Dianthus caryophyllus*: *DC-ERS2* (AF034770), *Cucumis melo*: *CM-ERS* (AB049128), *Pisum sativum*: *PS-ERS1* (AF039746), *Musa acuminata*: *MA-ERS2* (AF113748), *Nicotiana tabacum*: *NT-ERS1* (AF039921).

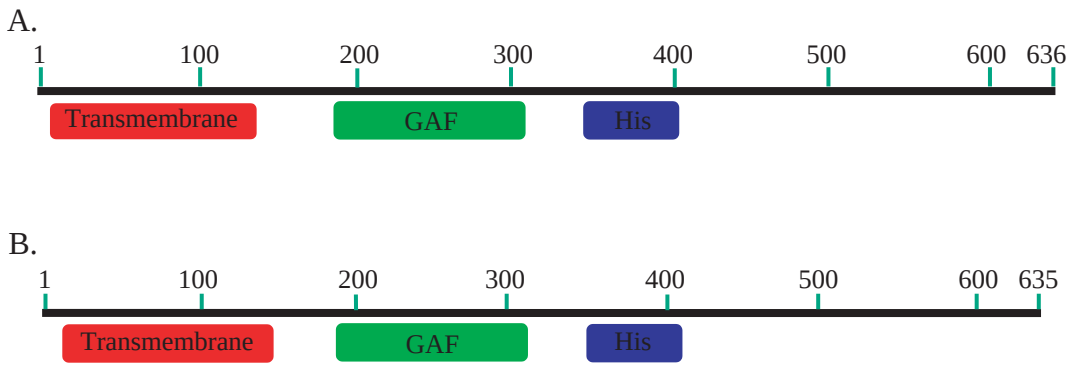

Figure 7 Diagram of conserved domain of *Os-ERS1* (A) and *Os-ERS2* (B) protein in *Oryza sativa indica* variety KDML105 using rpsblast. The black bar represent polypeptide chain of ethylene responsive sensor protein. The red, green and blue box refer to conserved domain within the amino peptide. Transmembrane domain (red box): these domain is high affinity to bind ethylene molecules. GAF domain (green box): these domain present in phytochromes and cGMP-specific phosphodiesterases. His KA (blue box): His Kinase A domain; these domains contain a conserved His residue and are activated via trans-autophosphorylation by the catalytic domain of the histidine kinase in two component signaling system. They subsequently transfer the phosphoryl group to the Asp acceptor residue of a response regulator protein.

```
Os-ERS1-Japonica  ------------------------------MDG-CDCIEP-LWPTDELLIKYQYISDFF   27
Os-ERS1-KDML105   ------------------------------MDG-CDCIEP-LWPTDELLIKYQYISDFF   27
Os-ERS2-Japonica  ------------------------------MDGSCDCIEP-LWQADDLLVKYQYISDFF   28
Os-ERS2-KDML105   ------------------------------MDGSCDCIEP-LWQAGDLLVKYQYISDFF   28
At-ERS1           ------------------------------MES-CDCFET-HVNQDDLLVKYQYISDAL   27
At-ERS2           --MLKTLLVQWLVFFFFFLIGSVVTAAEDDGSLSLCNCDDEDSLFSYETILNSQKVGDFL   58
                                                IV

Os-ERS1-Japonica  IALAYFSIPLELIYFVKKS-SFFPYRWVLIQFGAFIVLCGATHLINLWTFTTHTKTVAMV   86
Os-ERS1-KDML105   IALAYFSIPLELIYFVKKS-SFFPYRWVLIQFGAFIVLCGATHLISLWTFTTHTKTVAMV   86
Os-ERS2-Japonica  IALAYFSIPLELIYFVKKS-AFFPYRWVLIQFGAFIVLCGATHLINLWTFAIYTKTIAVV   87
Os-ERS2-KDML105   IALAYFSIPLELIYFVKKS-AFFPYRWVLIQFGAFIVLCGATHLINLWTFAIYTKSIAVV   87
At-ERS1           IALAYFSIPLELIYFVQKS-AFFPYKWVLMQFGAFIILCGATHFINLWMFFMHSKAVAIV   86
At-ERS2           IAIAYFSIPIELVYFVSRTNVPSPYNWVVCEFIAFIVLCGMTHLLAGFTYGPHWPWVMTA  118
                            I                          II

Os-ERS1-Japonica  MTVAKVSTAVVSCATALMLVHIIPDLLSVKTRELFLKNKAEQLDREMGLIRTQEETGRHV   146
Os-ERS1-KDML105   MTVAKVSTAVVSCATALMLVHIIPDLLSVKTRELFLKNKAEQLDREMGLIRTQEETGRHV   146
At-ERS1           MTIAKVSCAVVSCATALMLVHIIPDLLSVKNRELFLKKKADELDREMGLILTQEETGRHV   146
Os-ERS2-Japonica  LTVAKAATAVVSCITALMLVHIIPDLLNVKLRERFLKDKADELDREMGIIRTQEETGRHV   147
Os-ERS2-KDML105   LTVAKAATAVVSCITALMLVHIIPDLLNVKLRERFLKDKADELDREMGIIRTQEETGRHV   147
At-ERS2           VTVFKMLTGIVSFLTALSLVTLLPLLLKAKVREFMLSKKTRELDREVGIIMKQTETSLHV   178
                                  III

Os-ERS1-Japonica  RMLTHEIRSTLDRHTILKTTLVELGGTLGLEECALWMPSRSGSSLQLSHTLRHQIT----   200
Os-ERS1-KDML105   RMLTHEIRSTLDRHTILKTTLVELGGTLGLEECALWMPSRSGSSLQLSHTLRHQIT----   200
At-ERS1           RMLTHGIRRTLDRHTILRTTLVELGKTLCLEECALWMPSQSGLYLQLSHTLSHKIQ----   200
Os-ERS2-Japonica  HMLTHEIRSTLDRHTILRTTLVELGRTLALAECALWMPTRSGSALQLSHTIYNSAA----   201
Os-ERS2-KDML105   HMLTHEIRSTLDRHTILRTTLVELGRTLALAECALWMPTRSGSALQLSHTIYNSAA----   201
At-ERS2           RMLTTKIRTSLDRHTILYTTLVELSKTLGLKNCAVWIPNEIKTEMNLTHELRPRIDDENE   238
```

Figure 8 Amino acid sequence alignment of putative transmembrane domain (amino terminal ethylene binding domains) (Os-ERS1 and Os-ERS2) between *Oryza sativa* and *Arabidopsis thaliana*. The four hydrophobic segments (I to IV) are underlined.

```
                                H
                                □♦HE□*-PL
Os-ERS1-KDML105   NDFLAVMNHEMRTPMNAIIALSSLLLETELTPE-QRLMVETVLKSSNLLATLINDVLDLS  403
Os-ERS1-Japonica  GEFLANVSHELRTPLTAIRGYLELLEEELLDDEEQREYLERILEEAERLLRLINDLLDLS   64
At-ERS1           NDFLAVMNHEMRTPMHAIISLSSLLLETELSPE-QRVMIETILKSSNLVATLISDVLDLS  403
Os-ERS2-KDML105   NDFLAVMNHEMRTPMRAIVSLSSLLLETNLSAEQRLMVETILKSSDLLATLTNDVLDVSK  405
Os-ERS2-Japonica  SEFLANLSHELRTPLTAIRGYLELLLDTELSEEQREYLETILREAERLLRLINDLLDLSR   62
At-ERS2           AAFEQMMSDAMRCPVRSILGLLPLILQDGKLPENQTVIVDAMRRTSELLVQLVNNAGDIN  443

                          N
                          □+Q□□□N□□+NA                                  □+D
Os-ERS1-KDML105   LMQTILNISGNAVKFTKE--GHITLVASVVKADSLREFRTPDFHPTASDDNFYLKVQIKD  517
Os-ERS1-Japonica  LMQTILNISGNAVKFTKE--GHITLVASVVKADSLREFRTPDFHPTASDDNFYLKVQIKD  517
At-ERS1           LMQTILNIMGNAVKFTKE-GYISIIASIMKPESLQELPSPEFFPVLSDSHFYLCV-QVKD  517
Os-ERS2-KDML105   LMQIILNVAGNSIKFTKEGHVSITASMARPDALRGPHEPDYHPVVSDGFFYLA--VQVKD  520
Os-ERS2-Japonica  LMQIILNVAGNSIKFTKEGHVSITASMARPDALRGPHEPDYHPVVSDGFFYLA--VQVKD  520
At-ERS2           VFQAILHMLGVLMNRKIKGNVTFWVFPESGNSDVSERKDIQEAVWRHCYSKEYMEVRF--

                    G1          F              G2
                    -G♦G□♦      □F-PF          G♦GLGL
Os-ERS1-KDML105   TGCGISPQDLPQVFTKFPQSQPGGNRGYSGSGLGLAICKRFVTLMGGHIWLDSEGTGRGC  577
Os-ERS1-Japonica  NGPGIPEEDLERIFERF--SDGSRSRKGGGTGLGLSIVKKLVELHGGRIEVESE-PGGGT   97
At-ERS1           TGCGIHTQDIPLLFTKFVQPRTGTQRNHSGGGLGLALCKRFVGLMGGYMWIESEGLEKGC  577
Os-ERS2-KDML105   TGCGISPQDMPHTFRKFAHPENAGKWNS-GSGLGLALSRRFVSLMEGNIWLESEGVGKGC  580
Os-ERS2-Japonica  NGPGIPEEDLERIFERFSDGSRSRK-GG-GTGLGLSIVKKLVELHGGRIEVESE-PGGGT  100
At-ERS2           G------FEVTAEGEESSSSSSGSNLEEEEENPSLNACQNIVKYMQGNIRVVEDGLGLVK  612
```

Figure 9 Amino acid sequence alignment of putative histidine protein kinase domains (*Os-ERS1* and Os-ERS2). The five consensus motifs (H, N, G1, F, and G2) found in bacterial histidine protein kinases are indicated on the top of the sequences. Open triangle symbol are indicate nonpolar residues, filled diamonds indicate polar residues. The asterisk signs indicate basic residues, minus signs indicate acidic residues and plus sign indicate the position with more than 50% conservation in bacterial histidine kinase.

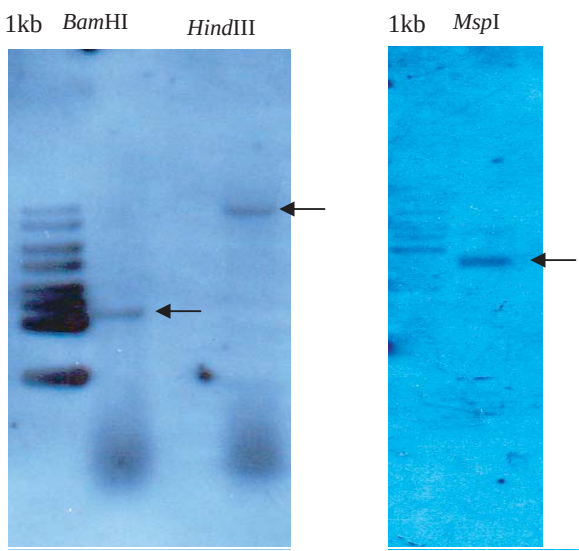

Figure 10 Southern analysis of *Os-ERS1* and *Os-ERS2* genes in *Oryza sativa* indica, Khao Dok Mali 105 variety. (a) Genomic DNA of KDML105 rice was digested with *Bam*HI and *Hind*III restriction enzyme and probe with *Os-ERS1* gene specific probe. (b) Genomic DNA of KDML105 rice was digested with *Msp*I restriction enzyme and probe with *Os-ERS2* gene specific probe.

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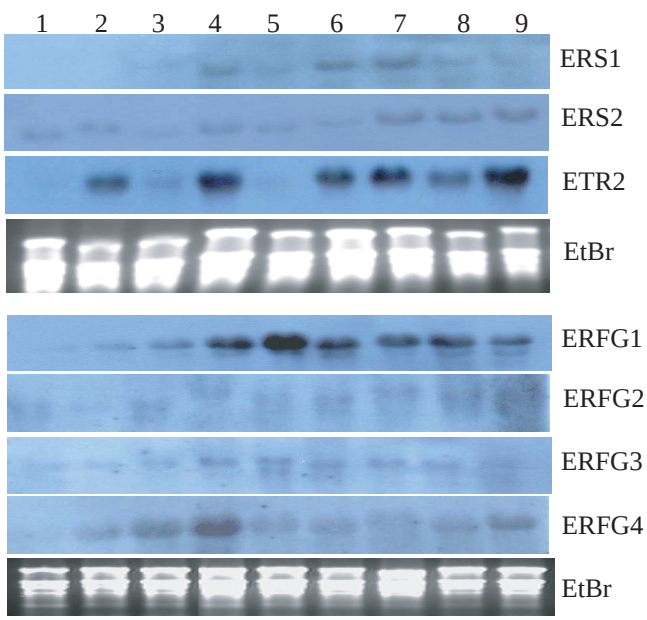

Figure 11 Expression levels of ethylene signal transduction genes in Khaw Dok Mali 105 rice during RTBV infection. Lane 1: Non-infected rice (Control), Lanes 2 and 3: Rice at 4 and 10 days after injection with dH_2O, respectively, Lanes 4 and 5: Rice at 4 and 10 days after inoculation with *Agrobacterium*, respectively. Lanes 6, 7, 8 and 9: Rice at 2, 4, 6, 10 days after inoculation with infectious clone of RTBVCN, respectively.

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