

ISOLATION AND CHARACTERIZATION OF THREE ETHYLENE PERCEPTION ELEMENTS AND THEIR EXPRESSIONS DURING LONGAN FRUIT DEVELOPMENT

JIAN-FEI KUANG^{1,2}, WANG-JIN LU², YUE-MING JIANG¹ AND JIAN-YE CHEN^{2*}

¹South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, P.R. China

²Guangdong Key Laboratory for Postharvest Science, College of Horticultural Science, South China Agricultural University, Guangzhou 510642, P.R. China

Abstract

Ethylene has long been regarded as the major regulator of fruit development. In the present work, three full-length cDNAs homologous to *Arabidopsis* ethylene perception element genes *ethylene response1* (*ETR1*), *ethylene response sensor1* (*ERS1*) and *constitutive triple response1* (*CTR1*), designated as *DIETR1*, *DIERS1* and *DICTR1*, respectively, were isolated and characterized from fruit of longan, a non-climacteric fruit. Homology analysis showed that *DIETR1* and *DIERS1* proteins contained three N-terminal membrane-spanning domains and the conserved histidine kinase domain while *DICTR1* protein possessed a conserved serine/threonine kinase domain, an ATP binding site and a serine/threonine kinase catalytic site. Northern blotting demonstrated that mRNA levels of *DIETR1* and *DIERS1* gradually decreased while *DICTR1* transcript increased steadily during fruit development. Furthermore, treatments with plant growth substances, abscisic acid (ABA) and ethrel, inhibited the accumulation of *DIERS1* transcript. In addition, ABA treatment suppressed the expression of *DICTR1*. Thus, *DIETR1* and *DIERS1* exhibited a different response to plant growth substances. It was suggested that *DIETR1* and *DIERS1* might play a role in the early stage of longan fruit development, whereas *DICTR1* was likely to be involved in fruit ripening.

Introduction

The plant hormone ethylene involves in a wide range of developmental and physiological responses including fruit growth and ripening (Guo & Ecker, 2004; Zhu & Guo, 2008; Mahmood *et al.*, 2008). The biosynthesis of ethylene in higher plants has been well studied. Ethylene is synthesized from S-adenosyl methionine, which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase and thence to ethylene by ACC oxidase (Yang & Hoffman, 1984; Shahroona *et al.*, 2006). However, the mechanism by which the ethylene signal is perceived and transduced to mediate phenotypic responses is not understood fully (Ezura & Owino, 2008).

Ethylene perception and signal transduction have been extensively studied at the biochemical and molecular genetic levels in *Arabidopsis thaliana* and other species including some climacteric fruits like tomato, pear, banana and kiwifruit (Bleecker & Schaller, 1996; Chang *et al.*, 1993; Lanahan *et al.*, 1994; Lashbrook *et al.*, 1998; Giovannoni, 2001; El-Sharkawy *et al.*, 2003; Yin *et al.*, 2008). To date, five *ETR1*-like genes, *At-ETR1* (Chang *et al.*, 1993), *At-ERS1* (Hua *et al.*, 1995), *At-ETR2* (Sakai *et al.*, 1998), *At-EIN4* and *At-ERS2* (Hua & Meyerowitz, 1998), have been identified in *Arabidopsis*. Functional *ETR1* homologues have been isolated from several plant species (Lanahan *et al.*, 1994; Sato-Nara *et al.*, 1999; Müller *et al.*, 2000). Some study indicates that ethylene receptor genes are differentially regulated throughout plant development in *Arabidopsis* and tomato (Hua & Meyerowitz, 1998; Lashbrook *et al.*, 1998). Importantly, *At-ERS1* (Hua & Meyerowitz, 1998) and *NR* (Wilkinson *et al.*, 1995; Payton *et al.*, 1996)

*Corresponding author E-mail: chenjianye@scau.edu.cn

Phone: (+ 86)-020-85280229; Fax: (+86)-020-85282107

are involved in the autoregulation of ethylene perception as their expression is up-regulated by the phytohormone. Ethylene receptor binding leading to a plant response depends on the ethylene signal transduction pathway. The *Arabidopsis* protein, At-CTR1, is one of the early elements of this pathway (Kieber *et al.*, 1993). It has been shown to be a Raf-like Ser/Thr protein kinase (MAPKK kinase) and, as the ethylene receptor proteins, is a negative regulator of the ethylene response. Mutant forms of *ctr1* confer a constitutive ethylene response in air (Kieber *et al.*, 1993). However, there is little information available on the isolation and characterization of the ethylene perception elements in non-climacteric fruit.

Longan (*Dimocarpus longan* Lour.), a non-climacteric fruit, is highly attractive with its commercial value in the international trade (Jiang *et al.*, 2002). Plant hormone ethylene is considered as the driving ripening process of climacteric fruit (Abbasi *et al.*, 2009) while the ethylene regulation involving in the ripening of non-climacteric fruit remains unknown. In non-climacteric citrus, fruit evolves very low amounts of ethylene during ripening but can respond to exogenous ethylene, as indicated by the changes in the ripening-related pigments and respiration (Goldschmidt *et al.*, 1993). Recent study has reported that young citrus fruitlets behave as climacteric fruits equipped with a system II-like ethylene biosynthesis activity, but the fruit loses its potential climacteric-like nature and retains system I activity during growth and maturation (Katz *et al.*, 2004). In addition, Trainotti *et al.*, (2005) has found that the expression of two type I ethylene receptor genes (i.e., *FaETR1* and *FaERS1*) shows a continuous increment during fruit ripening, suggesting that the ripening of strawberry is somewhat similar to climacteric fruits. These results indicate that the relationship between ethylene and ripening of non-climacteric fruit needs to be well documented.

In the present work, the isolation and characterization of three putative longan fruit ethylene perception response elements, including two ethylene receptors and a CTR1-like protein, were described. In addition, their expression profiles during longan fruit development in relation to their responses to plant growth regulators such as abscisic acid (ABA) and ethylene releaser (ethrel) treated at ripening stage were also investigated. This study can help understand and elucidate the mechanism of non-climacteric fruit development based on the point of ethylene perception.

Material and methods

Plant materials: Ten 5-year-old longan trees of 'Shijia' from a commercial orchard near Guangzhou, China were chosen for this experiment. Developing fruit located in different directions of each tree were collected weekly and were then sampled once a week for a period of 9 weeks, beginning at 14 days after anthesis (DAA) and ending at 77 DAA. Whole fruit tissues (including pericarp, aril and seed) before 35 DAA (14, 21, 28 and 35 DAA) while the separated aril tissues at 42 DAA and thereafter were collected, frozen in liquid nitrogen and then stored at -80°C until use.

ABA and ethrel treatment: Six 5-year-old longan trees were used from the same orchard described above. About 400 fruits at 77 DAA located in different directions of each tree were tagged and dipped for 1 min in a solution containing 0 (control), 200 mg/L ABA or 500 mg/L ethrel. After 0, 6, 12, 24, 36 and 48 hours of each treatment, 60 randomly selected fruits were detached and excised, and the aril tissues were then frozen in liquid nitrogen and stored at -80°C prior to analysis.

RNA extraction and isolation of longan full length cDNAs encoding ethylene perception elements: Frozen tissues (10 g) were ground to a fine powder in a mortar using a pestle in the presence of liquid nitrogen. Total RNA was extracted using the hot borate method of Wan & Wilkins (1994). The extracted total RNA was used as templates for RT-PCR. The product (the first-strand cDNA) was subjected to PCR amplification. Degenerate primers of *ETRs* and *ERSs* (i.e., sense: 5'-GAGACG GGHAGRCATGTNAGRAT-3' and antisense: 5'-CATGGGMGTTCTCATTTCATGRITTCAT-3') and *CTRs* (i.e., sense: 5'-ATGGAGC AAGAYTTY CATGCTGAGCG-3' and antisense: 5'-ATCTCGMTKAACTTCNGGT GCCATCC-3') were designed with reference to the conserved amino acids sequences of *ETRs*, *ERSs* and *CTRs*, respectively. Reactions for the RT-PCR were subjected to one cycle of 94°C for 3 min, 35 cycles each at 94°C for 1 min, 45°C for 2 min and 72°C for 2 min, and then one cycle of 72°C for 10 min. PCR products of the predicted size were purified and cloned into pGEM-T easy vector (Promega, USA). The nucleotide sequences of the cDNA inserts were determined using the thermo sequenase dye terminator cycle sequencing kit and a 3730 DNA sequencer (PerkinElmer Applied Biosystems).

Consequently, 3'- or 5'- rapid amplification of cDNA ends (3'- or 5'-RACE-PCR) was performed using cDNA amplification kits (Takara, Shiga, Japan) according to the manufacturer's protocol. In order to amplify 3'-end and 5'-end fragments, the specific primers were designed based on the nucleotide sequences of the cDNA fragments already cloned by RT-PCR and are shown in Table 1. The 3'- and 5'- RACE-PCR products were cloned and sequenced as described above.

DNA sequence analysis, alignment and comparisons: Identification of nucleotide sequences from RT-PCR clones were established using the NCBI Blast program [<http://www.ncbi.nlm.nih.gov/BLAST>]. Alignment and comparison of sequence were made using the ClustalW program (<http://www.ebi.ac.uk/clustalw>). Open reading frame and protein prediction were made using NCBI ORF Finder [<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>]. The mass values for mature peptides were calculated using the PeptideMass program [<http://us.expasy.org/tools/peptide-mass.html>].

Northern blot analysis: Total RNA (10 µg) was separated on a 1.2% agarose-formaldehyde gel and blotted onto positively-charged nylon membrane (Biodyne[®]B, 0.45 µm, PALL Co. Sarasota, FL). The RNA was fixed to the membrane by baking for 2 h at 80°C and then cross-linked to the membranes using an ultraviolet cross linker (Amersham Biosciences, Piscataway, NJ). The membranes were prehybridized for more than 3 h in SDS buffer [50% deionized formamide (v/v), 5 × SSC, 7% SDS, 2% blocking reagent (Roche Diagnostics, Mannheim, Germany), 50 mM sodium-phosphate (pH 7.0) and 0.1% N-lauroylsarcosine (w/v)], and then hybridization was performed overnight in the same buffer containing the gene-specific digoxin (DIG)-labeled probes at 45°C. Probes were prepared with a DIG probe synthesis kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. All probes were synthesized from the 3'-untranslated regions of these genes. Following hybridization, membranes were washed twice for 10 min with 2 × SSC containing 0.1% SDS at 25°C, followed by washing twice for 30 min in 0.1 × SSC containing 0.1% SDS at 62°C. The signals were detected with chemiluminescence using CDP-Star[™] (Roche Diagnostics) as described by the manufacturer. The specific primers used for synthesis of DIG-labeled probes are listed in Table 2.

Table 1. The sequences of specific primers used for RACE of *DIETRI*, *DIERS1* and *DICTRI* genes.

Genes	Oligonucleotide sequences
<i>DIETRI</i> - 3RACE1	5'- CTGTGTCTCAATCACTGCCT -3'
<i>DIETRI</i> - 3RACE2	5'- GTCAAAGATTTCAGGAACAGG -3'
<i>DIERS1</i> - 3RACE1	5'- GATTCTCCCAACCAACAGTG -3'
<i>DIERS1</i> - 3RACE2	5'- TCTGGAAGAGTCTATGCGGG -3'
<i>DICTRI</i> - 3RACE1	5'-TCACTCAGCCCCCAAACCTTG-3'
<i>DICTRI</i> - 3RACE2	5'-GTGCGAGAGAGGTGTTGGAT-3'
<i>DIETRI</i> - 5RACE1	5'- ATCCACAAGGCACACTCTTCCAG -3'
<i>DIETRI</i> - 5RACE2	5'- GACACCACAGCAGTCAAAACCT -3'
<i>DIERS1</i> - 5RACE1	5'- CTCCCTGTCAAGTTCTTCGGTCT -3'
<i>DIERS1</i> - 5RACE2	5'- GTGAACAAGCAACAAAGCAGTCG -3'
<i>DICTRI</i> - 5RACE1	5'- CAAGTTTGGGGGCTGAGTGA -3'
<i>DICTRI</i> - 5RACE2	5'- ATCCAACACCTCTCTCGCAC -3'

Table 2. The sequences of specific primers used for the syntheses of DIG-labeled *DIETRI*, *DIERS1* and *DICTRI* probes.

Genes	DIG-For primers	DIG-Rev primers
<i>DIETRI</i>	5'-ACTGAGTTGACACCTGAGCA-3'	5'-CAAAGGCAGTGATTGAGACA-3'
<i>DIERS1</i>	5'-GAACCACGAAATGAGGACAC-3'	5'-GGGACACCACAGCCTGAATC-3'
<i>DICTRI</i>	5'-ATCTCCACACCTCAGCCAA-3'	5'-GCTCAAGTGATGTGCTATGC-3'

Results and Discussion

Isolation and characterization of *DIETRI* and *DIERS1*: In this study, two full-length cDNAs encoding ethylene receptors, designated as *DIETRI* and *DIERS1*, were obtained from longan fruit using a combination of RT-PCR and RACE. *DIETRI* cDNA (3230 bp) and *DIERS1* cDNA (2385 bp) consisted of a 5'-untranslated region of 671 bp, an open reading frame (ORF) of 2220 bp, and a 3'-untranslated region of 339 bp, a 5'-untranslated region of 171 bp, an ORF of 1908 bp and a 3'-untranslated region of 306 bp, respectively. They encoded the predicted polypeptides of 740 and 636 amino acids, with the predicted molecular weights of 94.7 and 70.9 kDa, respectively. A BLAST search of GenBank revealed that *DIETRI* shared a high homology with corresponding homologues from other fruit such as mango *MiETRI* (AAF61919, 90% identity) and pear *PcETRI* (AAL66202, 89% identity) while *DIERS1* was homologous to *CsERS1* (AAC99435, 78% identity) and *CpETRI* (AAG41977, 78% identity) isolated from citrus and papaya fruits, respectively, at the protein level.

The optimal multiple sequence alignments of *DIETRI* and *DIERS1* proteins with other homologies are presented in Fig. 1A. It has been well documented that the *ETRI* and *ERS1* genes have three putative membrane-spanning subdomains in the N-terminus, and a histidine (His) kinase domain in C-terminal region (Chang *et al.*, 1993; Hua *et al.*, 1995). The three predicted transmembrane hydrophobic amino acid sequences at the N-terminus were ²⁶FFIALAYFSIPLLELIYFV⁴³, ⁵³WVLIQFGAFIVLCGATHLI⁷¹ and ⁸³VAIVMTVAKVSTAGVSCATALMLGHIIPDL¹¹² according to Hua *et al.*, (1998) and Huang *et al.*, (2007). As shown in Fig. 1, these three subdomains could be observed in the N-terminus of *DIETRI* and *DIERS1*. In addition, the conserved histidine kinase domain in the C-terminus was also found in both *DIETRI* and *DIERS1* genes. *DIETRI* and *DIERS1* were similar in their coding regions, except that *DIERS1* did not appear to contain a receiver domain (616–733 AA in *DIETRI*). These results indicated that these two ethylene receptor genes of longan fruit shared common features with *ETRs* and *ERSs* obtained from other plants.

AtETR1MEVCMC.IEPQWPADLLMKYQYISDFFIATAYFSIPLELIYFVKKSAWFPYRUVLVQFGAFIWL	65
LeETR1	MGSLLRMNRLSSIVESCNCIIDPOLPADLLMKYQYISDFFIATAYFSIPVLELIYFVKKSAWFPYRUVLVQFGAFIWL	80
DiETR1MESCMC.IESQWPADLLMKYQYISDFFIATAYFSIPLELIYFVKKSAWFPYRUVLVQFGAFIWL	65
AtERS1MESDCD.FETHVNQDPLLKRYQYISDVALALAYFSIPLELIYFVKKSAWFPYRUVLVQFGAFIWL	65
VrERS1NMESDCD.IDTQYPPDELLKRYQYISDVALALAYFSIPVLELIYFVKKSAWFPYRUVLVQFGAFIWL	66
DiERS1MESDCD.FDSQWPSEELVKRYQYISDVALALAYFSIPLELIYFVKKSAWFPYRUVLVQFGAFIWL	65
	Domain 1	Domain 2
AtETR1	GATHLINLWTFTHSRVTVALVMTAKVLTAVVSCATALMLVHIIPDLLSVKTRFLFLRNKAAELDREMGLIRTOEETGRH	145
LeETR1	GATHLINLWTFNMHSTRNVALVMTAKALTAIVVSCITALMLVHIIPDLLSVKTRFLFLRNKAAELDREMGLIRTOEETGRH	160
DiETR1	GATHLINLWTFMSHRTVAVVMITAKVLTAVVSCATALMLVHIIPDLLSVKTRFLFLRNKAAELDREMGLIRTOEETGRH	145
AtERS1	GATHFINLWTFMHSKAVAVVMTIAKVSCAIVVSCATALMLVHIIPDLLSVKTRFLFLRNKAAELDREMGLIRTOEETGRH	145
VrERS1	GATHFINLWTFSPHSKAVAVVMTIAKVSCAIVVSCATALMLVHIIPDLLSVKTRFLFLRNKAAELDREMGLIRTOEETGRH	146
DiERS1	GATHFINLWTFMSHKTVAVVMITAVKVSCAIVVSCATALLVHIIPDLLSVKTRFLFLRNKTEELDREMGLIRTOEETGRH	145
	Domain 3	
AtETR1	VRMLTHEIRSITLDRHTILKTTLVELGRTLALAEICALWMPTRTGLELQLSYTLRHQHPWEYTPIQLPVINQVFGTSRAVK	225
LeETR1	VRMLTHEIRSITLDRHTILKTTLVELGRTLALAEICALWMPTRTGLELQLSYTLRHQHPVGLTTPIQLPVINQVFGTNRVVK	240
DiETR1	VRMLTHEIRSITLDRHTILKTTLVELGRTLALAEICALWMPTRTGLELQLSYTLRQONPVGYTTPIQLPVINQVFSNRAMK	225
AtERS1	VRMLTHGIRITLDRHTILKTTLVELGRTLALAEICALWMPSSQSLYLQLSHTLSHKIQVGSSTP INLPIINELFNSAQAMH	225
VrERS1	VRMLTHEIRSITLDRHTILKTTLVELGRTLALAEICALWMPSSRNLNLQLSHTLTYHVQVGSSTQCTNPIVNEVFNSPRAMR	226
DiERS1	VRMLTHEIRSITLDRHTILKTTLVELGRTLALAEICALWMPSSRTGMLTLELSRSLTINQIKVGYTTP INLPIVNGVFNARSAMC	225
AtETR1	ISPNSPVARLRPVSQKYL..GEVVAVRVPPLLHLSNFCINDMPELSTRYALMVLMLPSDSARQWHVHELELVVWADQV	303
LeETR1	ISPNSPVARLRP.ACKYMP..GEVVAVRVPPLLHLSNFCINDMPELSTRYALMVLMLPSDSARQWHVHELELVVWADQV	317
DiETR1	ISPNCPVARLRPLACKYMETPGEVVAVRVPPLLHLSNFCINDMPELSTRYALMVLMLPSDSARQWHVHELELVVWADQV	305
AtERS1	IPHSCLAKIGPPVGRYSP..PEVVSVRVPPLLHLSNFCQSDSDLSGCGYAIMVLIPTDGARKRDRHELELVVWADQV	303
VrERS1	IPFTCLARIRPLVGRYVP..PEVVAVRVPPLLHLSNFCINDMPDISARNYAIMVLIPTDSVRRDRHELELVVWADQV	304
DiERS1	LPYSCLARVTPILGRYAP..PEVVAVRVPPLLHLSNFCINDMPDCSARIFAAILLILETNSARKQODHELELVVWADQV	303
AtETR1	AVALSHAAILEESMRARDLMEONVALDLARREAEATIRARNDFLAVMNHMERTPMHAIITLSSLLCETELTPEQRIMVE	383
LeETR1	AVALSHAAILEESMRARDLMEONVALDLARREAEAVRARNDFLAVMNHMERTPMHAIITLSSLLCETDLTPEQRIMVE	397
DiETR1	AVALSHAAILEESMRARDLMEONVALDLARREAEATIRARNDFLAVMNHMERTPMHAIITLSSLLCETELTPEQRIMVE	385
AtERS1	AVALSHAAILEESMRARDLMEONVALDKARCEAEVARNDFLAVMNHMERTPMHAIITLSSLLLETETLSPQRVMIE	383
VrERS1	AVALSHAAILEESMRARDLMEONVALDLARREAEAMHARNDFLAVMNHMERTPMHAIITLSSLLLETETLTPQRVMIE	384
DiERS1	AVALSHAAILEESMRARONCLMEONVALDLARREAEKATHARNDFLAVMNHMERTPMHAIITLSSLLLETDLTPEQRIMTE	383
AtETR1	TILKSSNLLATLINDVLDLSRLEDGSLQELGTFNLHHTLFRVNLNIRPIAVVKKLPITLNLAPDLPEFVVGDEKRLMCI	463
LeETR1	TILKSSNLLATLINDVLDLSRLEDGSLQIDIGTFNLHHLFRVHSLIRPIASVKKLFTVLSLSSDLPYVIGDEKRLMCI	477
DiETR1	TILKSSNLLATLINDVLDLSRLEDGSLQELVTFNLHAAFRVNLNIRPIASVKKLMTLNLAPDLPEYAVGDEKRLMCI	465
AtERS1	TILKSSNLLATLINDVLDLSRLEDGSLLENEPFSLOAIFEEVVISLIRPIASVKKLSTNLILSADLPTAIGDEKRLMCI	463
VrERS1	TVLKSSNLLATLINDVLDLSRLEDGSLLEMGKFNHLHGVLGEIVELIRPIASVKKLPIITLILAPDLPTAIGDEKRLMCI	464
DiERS1	TVLKSSNLLATLINDVLDLSRLEDGSLNLDIGQFNLRVAVKQVMDLIRPIASVKKLSTNTSFLAPDLPTAIGDEKRLMCI	463
	Histidine kinase	
AtETR1	ILNVGNAVKFSKQGSISVTALVTRK...DTRAADFVVFVTSHFYLRVQVVDGSGACINPQDIPKIFTKFACQSLATR	539
LeETR1	LLNVGNAVKFSKQGNVSIATFVAKSDSLRDPRAPEFFAVPSENHFYLRVQVVDGSGACINPQDIPKIFTKFACQSLATR	557
DiETR1	LLNVGNAVKFSKQGSVSIATFVAKSRLRSDRAPDFAMPSENHFYLRVQVVDGSGACINPQDIPKIFTKFACQSLATR	545
AtERS1	ILNLMGNAVKFTKQGYVSIITIASIMPESLQELPSEFFPVLSDSHFYLRVQVVDGSGACINPQDIPKIFTKFACQSLATR	543
VrERS1	LLNVGNAVKFTKQGYVSIIRASVAKPESLQDWRPPEFYPTSSDGHFYLRVQVVDGSGACINPQDIPKIFTKFACQSGPAR	544
DiERS1	ILNVGNAVKFTKQGYVSIITATVAKPESRDRPPEFYPTSSDGHFYLRVQVVDGSGACINPQDIPKIFTKFACQSHSDNTR	543
AtETR1	SSGSGGLGLAISRFVNLMEGNIWIESDGLGKGTATFIDVVKLGISERSN.ESKQSGIPKVPPIPRHSNFTGLKVLVMDEN	618
LeETR1	NSGSGTGLGLAICRFVNLMEGHIWIESEGLGKGTATFIIKLGIPCRAN.ESKLPFVTKLPANHTQMSFGGLKVLVMDEN	636
DiETR1	NSGSGGLGLAICRFVNLMEGHIWIESEGLGKGTATFIVKLGIPHSN.ESKPAYGPKISGHG.QTNFGLKVLVMDEN	623
AtERS1	NHSGGGLGLAICRFVNLMEGNIWIESEGLEKGTATFIIIRLGCINGPS.....SSSGSMALH.....	601
VrERS1	PSSGAGLGLAICRFVNLMEGHIWIESEGLGKGTATFIVKLGICINPD.PSDHQATTRSQAYSGSGGLARFPFIKDED	623
DiERS1	KSGGAGLGLAISKFMNLMEGHIWIESEGLDKGTATFIVKLGVCNKPVGTF.AHQVAPKGRVNHGSDLTRFLVLDMDNG	623
AtETR1	GVSVMVTKGLLVHLGCEVTVVSSNEECLRVVSHHEKVVFMVDCMPGVENYQIALRIHEKFTKQRHQRPLLVALSNGTDRK	698
LeETR1	GVSVMVTKGLLVHLGCDVTVVGSRDECLRVVTHEKVVFMVDSMGGIDCYEVAVVIERHFGK.RHGRPLIVALTGNTDRV	715
DiETR1	AVRSVTKGLLVHLGCDVTVVSSNEECLRVVSEHEKVVFMVDCMPGIDGYDVAVVIERHFTTR.RHERPLIVALTGNTDKV	702
AtERS1	.LAAKSQTRFMNV.....	613
VrERS1	DSGFSTRNRQSF.....	636
DiERS1	EITSENPRYQSF.....	636
	Receiver domain	
AtETR1	TKEKMSFGLDGVLLKPVSLDNIRDVLSDLLEP...RVLYEG	737
LeETR1	TKENCMRVGMDGVILKPVSVYKMRSVLSLELLEH...FVVLLES	754
DiETR1	IKENCMRVGMDGVILKPVSLKMRSVLSDLELLEH...RVLFEA	741
AtERS1	613
VrERS1	636
DiERS1	636

Fig. 1. Alignments of *DiETR1* and *DiERS1* predicted proteins with *Arabidopsis thaliana* *AtETR1*, *AtERS1*, tomato *LeETR1* and mung bean *VrERS1* proteins. Black shading identified fully conserved residues by the six proteins. Conservative amino acid substitutions were represented by gray shading. Gaps were introduced to optimize alignment. Multiple alignments were made by CLUSTALW, then viewed with BOXSHADE program, and finally manually edited. Three transmembrane hydrophobic amino acid sequences were underlined. The histidine kinase domain and receiver domain were shown in boxes.

Isolation and characterization of *DICTR1*: *DIETR1* cDNA (3112 bp) consisted of a 5'-untranslated region of 127 bp, an open reading frame (ORF) of 2574 bp, and a 3'-untranslated region of 411 bp. The *DICTR1* gene encoded a putative protein of 858 amino acids with a predicted molecular weight of 83.04 kDa and an isoelectric point of 7.11.

A BLAST search of GenBank revealed that *DIETR1* shared the highest identity of 73% with that of *RhCTR1* (AAK40361) from rose and *PpCTR1* (ACR2362) from peach. Detailed analysis of the *DICTR1* proteins with other homologies revealed that the predicted *CTR1* protein was a serine/threonine protein kinase that was closely related to the Raf protein kinase family (Schenk & Jagalska, 1999; Kuroda *et al.*, 2003). As predicted, the *DICTR1* polypeptide exhibited a complete serine/threonine kinase domain spanning from 584-841 AA containing the ATP-binding site motif (590–611 AA), and the serine/threonine kinase catalytic motif (705–711 AA) (Fig. 2).

Accumulations of *DIETR1*, *DIERS1* and *DICTR1* mRNAs in aril tissues during longan fruit development: The development of longan fruit can be clearly divided into three stages according to the growth curve (data not shown). Stage I from 0 to 42 DAA showed a slow growth period and mainly maintained pericarp growth, while Stage II was a rapid growth phase from 42 to 70 DAA, in which the fruit primarily exhibited rapid aril growth whereas the pericarp and seed grew slowly, and Stage III from 70 to 77 DAA was mature stage and the fruit ceased growing almost (Feng *et al.*, 2008). Thus, the maturity of longan fruit can mainly be determined by the aril change (Jiang *et al.*, 2002). To understand the possible role of *DIETR1*, *DIERS1*, and *DICTR1* in fruit development, their expression patterns in aril tissues during fruit development were examined by northern blot analysis. As shown in Fig. 3, *DIETR1*, *DIERS1* and *DICTR1* exhibited different expression profiles during fruit development. *DIETR1* and *DIERS1* genes were highly expressed at the early stages of fruit development (from 14 to 35 DAA) (Fig. 3). In contrast, a slow increase in the *DICTR1* transcript levels was observed following fruit development (Fig. 3). A similar expression pattern during fruit development was also reported for other *CTR1* genes, including tomato *LeCTR1* (Adams-Phillips *et al.*, 2004), pear *PcCTR1* (El-Sharkawy *et al.*, 2003) and kiwifruit *AdCTR1* (Yin *et al.*, 2008). Thus, these results suggested that the expression of *DIETR1* and *DIERS1* might play an important role in the early fruit development while *DICTR1* might be involved in fruit ripening.

Regulation of *DIETR1*, *DIERS1* and *DICTR1* by ABA and Ethrel treatments: ABA and ethylene are related to fruit ripening. Ethylene plays a critical role in regulating ripening of climacteric fruits (Bondad *et al.*, 1970; Brady, 1987). ABA is considered to exhibit the key role in control ripening in some non-climacteric fruits (Coombe & Hale, 1973; Wang *et al.*, 2007). To examine the effect of ABA and ethylene, the accumulation patterns of *DIETR1*, *DIERS1* and *DICTR1* transcripts in aril tissues of longan fruit at 77 DAA were analyzed. As shown in Fig. 4, ABA and ethrel treatment exhibited little or no effect on the accumulation of *DIETR1* while the accumulation of *DIERS1* was obviously inhibited at 6, 12, 24 and 48 hours after ABA and ethrel treatments. Only ABA treatment suppressed the expression of *DICTR1* at 2, 6, 12, 24 and 48 hours. In *Arabidopsis*, *AtCTR1* was also repressed by ABA treatment (Arroyo *et al.*, 2003). These results suggested that *DIETR1*, *DIERS1*, and *DICTR1* genes in longan fruit responded differentially to ABA and ethylene treatments.

AtCTR1	MEMPGRRSNYTLLESCFSDDQVSVSVTGAPPPHYDSLSSSENRSNHNSGNTGKAKAERGGFDWDPSSGGGGDHLRNMNPVRV	80
LeCTR1	..MSGRSSYTLLESCFSDDQVSVSVTGAPPPHYDSLSSSENRSNHNSGNTGKAKAERGGFDWDPSSGGGGDHLRNMNPVRV	66
RhCTR1	MEMPGRRSNYTLLESCFSDDQVSVSVTGAPPPHYDSLSSSENRSNHNSGNTGKAKAERGGFDWDPSSGGGGDHLRNMNPVRV	65
D1CTR1	MEMPGRRSNYTLLESCFSDDQVSVSVTGAPPPHYDSLSSSENRSNHNSGNTGKAKAERGGFDWDPSSGGGGDHLRNMNPVRV	68
AtCTR1	GNNMYASSLGLQ...SSGSFSGESSLSDGYMPTLS.AAANEIESVGFPODDGFRLGFGGGGDLRIQMAAD...SAGG	152
LeCTR1	GSFRVPGSICSORCSSEGSFSGSSLSGENYVGTFS...GHKNEGCG.....	109
RhCTR1	G.NVYSS.VGLQRCSSGSFSGESSLSDGYMPTLS.AAANEIESVGFPODDGFRLGFGGGGDLRIQMAAD...SAGG	141
D1CTR1	G.SLFSSSLGLQRCSSGSFSGSSLSGENYVGTFS...GHKNEGCG.....	131
AtCTR1	SSSGKSWAQOOTESYQLQLALALRLSSEATCADDPNFLDPVPDESALRTSP..SSAETVSHRFVWNGCLSYFDKVPDGFY	230
LeCTR1	SSVARSWAQOOTESYQLQLALALRLSSEATCADDPNFLDPVTVLARSDDSTASAVTMSHRLWINGCMSYFDKVPDGFY	189
RhCTR1	SSSGKSWAQOOTESYQLQLALALRLSSEATCADDPNFLDPVPDESALRLS...SSADAVSHRFVWNGCLSYFDKVPDGFY	218
D1CTR1	SSSGKSWAQOOTESYQLQLALALRLSSEATCADDPNFLDPVPDESALRLS...ASSAEVSHRFVWNGCLSYFDKVPDGFY	210
AtCTR1	MMNGLDPYITWTLCDLHESGRIPSIESLRAVDSGVDSLEAIVDRRSDFAFKELHNRVHDISCSCTITTKAVDQDLAKLI	310
LeCTR1	MIYGMDFYVWALCSVVOESGRIPSIESLRAVDSKAPKAPVEVILIDRCNDLSLKELONRVHSISPSCTITTKAVDQDLAKLV	269
RhCTR1	LIHGLDSYVWMSCTDVOESGRIPSIESLRAVDSGVDSLEAIVDRRSDFAFKELHNRVHDISCSCTITTKAVDQDLAKLV	298
D1CTR1	LVNGLDPYITWTLCDLHESGRIPSIESLRAVDSGVDSLEAIVDRRSDFAFKELHNRVHDISCSCTITTKAVDQDLAKLV	290
AtCTR1	CNRMGPGVIMGEDELVPMWKECIDGLKEIF.KVVVPIGSLSVGLCRHRALLFKVLADITDLPRIAAGCKYCNRDDASSC	389
LeCTR1	CDHMCGAAPAGEEELVSMKGCNSDLKDFRGTIVLPIGSLSVGLCRHRALLFKVLADITDLPRIAAGCKYCNRDDASSC	349
RhCTR1	CSRMGGSASVGEAEFFSIWRESSDDLKDCGLGSVWVPIGSLSVGLCRHRALLFKVLADITDLPRIAAGCKYCNRDDASSC	378
D1CTR1	CNHMCCSASAGEDDVLPIWKECSDIKDCLGSLVWVPIGSLSVGLCRHRALLFKVLADITDLPRIAAGCKYCNRYDASSC	370
AtCTR1	LVRFGLDREYLVLDLIGKPGCHLCEPDSLLNGPSSISISSPLRFPKPEVPAVDPRLLAKQYFSDSOSLNLVDFD.....	463
LeCTR1	LVRFEHREYLVLDLIGKPGCHLCEPDSLLNGPSSISISSPLRFPKPEVPAVDPRLLAKQYFSDSOSLNLVDFD.....	423
RhCTR1	LVRFGIDRELLVLDLIGKPGCHLCEPDSLLNGPSSISISSPLRFPKPEVPAVDPRLLAKQYFSDSOSLNLVDFD.....	458
D1CTR1	LVRFGLDREYLVLDLIGKPGCHLCEPDSLLNGPSSISISSPLRFPKPEVPAVDPRLLAKQYFSDSOSLNLVDFD.....	450
AtCTR1	.ASDDMGFSMFHRQYDNPGGENDALAENGGGSLPPS.....ANMPPQNMMRASNOIEAAP..M	518
LeCTR1	.SSAGAAADGDAGCSDRSCIDRNNVVSSSNRDEISQLPLPLNAWKGRDKESQLSKMYP.RSMLNPVNMDEDQVLVK	501
RhCTR1	GDEDNKGFSMYPRK...KFTDGNMLFLVSG.LGDDTSMHVD.....DRNPQLKRSFNPSQNIHVQQTVLKQDIPLK	525
D1CTR1	VDEGDGTFKSMYPRK...KFTDGNMLFLVSG.LGDDTSMHVD.....DRNPQLKRSFNPSQNIHVQQTVLKQDIPLK	530
AtCTR1	NAPF.....ISQVFNANRELGLDGGDDMIPWCDLNIKREKIGAGSFGTVHRAEWHGSDVA	574
LeCTR1	HVVP.FREDAQSPMTRPDTVMNDRFLAGGGHVVSAIPSEELDLDVEEFNIPWCDLNIKREKIGAGSFGTVHRAEWHGSDVA	580
RhCTR1	RIPF...IGHRDISRLDTSKDSRFGE.GLQVWPSKPNKELTLDVDDLDIPWCDLNIKREKIGAGSFGTVHRAEWHGSDVA	600
D1CTR1	HIEGVQPCCLKLTDQRDLTSKDVRYAG.SGOLWPSNASKELSLDVEDLDIPWCDLNIKREKIGAGSFGTVHRAEWHGSDVA	609
#####		
ATP-binding site motif		
AtCTR1	VKILMEQDFHAERWNEFLREVAIMKRLRHPNIVLFMGAVTQPPNLSIVTEYLSRGSLYRLLHRSGAREQLDERRRLSMAY	654
LeCTR1	VKILMEQDFHAERLKEFLREVAIMKRLRHPNIVLFMGAVITQPPNLSIVTEYLSRGSLYRLLHRSGAREVLDERRRLSMAY	660
RhCTR1	VKILMEQDFHAERWNEFLREVAIMKRLRHPNIVLFMGAVTQPPNLSIVTEYLSRGSLYRLLHRSGAREVLDERRRLSMAY	678
D1CTR1	VKILMEQDFHAERWNEFLREVAIMKRLRHPNIVLFMGAVTQPPNLSIVTEYLSRGSLYRLLHRSGAREVLDERRRLSMAY	689
~~*~*~*~*~*		
Serine/threonine kinase domain		
AtCTR1	DVAAGMNYLHRRNPPIVHRDLKSPNLLVDKKYTVKVCDFGLSRKANTFLSSKSAAGTPEWMAPEVLRDEPSNEKSDVYS	734
LeCTR1	DVAAGMNYLHRRNPPIVHRDLKSPNLLVDKKYTVKICDFGLSRKANTFLSSKSAAGTPEWMAPEVLRDEPSNEKSDVYS	740
RhCTR1	DVAAGMNYLHRRNPPIVHRDLKSPNLLVDKKYTVKVCDFGLSRKANTFLSSKSAAGTPEWMAPEVLRDEPSNEKSDVYS	758
D1CTR1	DVAAGMNYLHRRNPPIVHRDLKSPNLLVDKKYTVKVCDFGLSRKANTFLSSKSAAGTPEWMAPEVLRDEPSNEKSDVYS	769
Serine/threonine kinase catalytic motif		
AtCTR1	FGVILWELATLQQPWGNLNPAQVVAAVGFKCRKLEIPRDLNPOVAAIIEGCTWNEPWKRPSFATIMDLRRLKSAVPPP	814
LeCTR1	FGVILWELATLQQPWGNLNPAQVVAAVGFKCRKLEIPRDLNPOVAAIIEGCTWNEPWKRPSFATIMDLRRLKSAVPPP	820
RhCTR1	FGVILWELATLQQPWGNLNPAQVVAAVGFKCRKLEIPRDLNPOVAAIIEGCTWNEPWKRPSFATIMDLRRLKSAVPPP	838
D1CTR1	FGVILWELATLQQPWGNLNPAQVVAAVGFKCRKLEIPRDLNPOVAAIIEGCTWNEPWKRPSFATIMDLRRLKSAVPPP	849
AtCTR1	NRSDL...	819
LeCTR1	GHTDMQLL	828
RhCTR1	SHADMPIL	846
D1CTR1	SRADMQLL	857

Fig. 2. Alignment of *D1CTR1* predicted protein with *Arabidopsis thaliana* *AtCTR1*, tomato *LeCTR1*, and rose *RhCTR1* proteins. Black shading identified fully conserved residues by the four proteins. Conservative amino acid substitutions were represented by gray shading. Gaps were introduced to optimize alignment. Multiple alignments were done by CLUSTALW, then viewed with BOXSHADE program, and finally manually edited. The serine/threonine kinase domain in the C-terminal region was boxed. The ATP-binding site motif (GAGSFGTV) and the serine/threonine kinase catalytic motif (HRDLKSPN) were marked with '#' and '*', respectively.

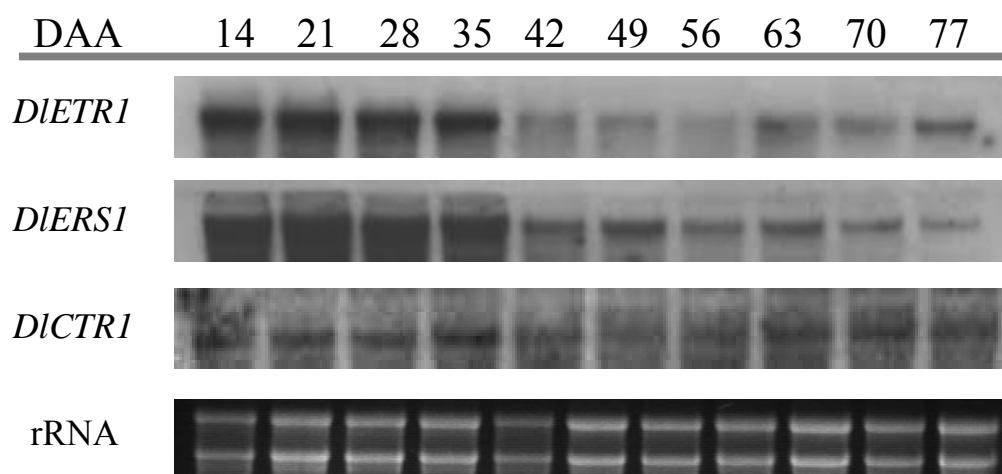


Fig. 3. Differential expression patterns of *DIETRI*, *DIERS1* and *DICTRI* in aril tissues of longan fruit at different developmental stages. Total RNA (10 μ g per lane) was used for northern blot analysis and hybridized with gene-specific DIG-labeled probes. Ethidium bromide-stained rRNA was shown as the loading control.

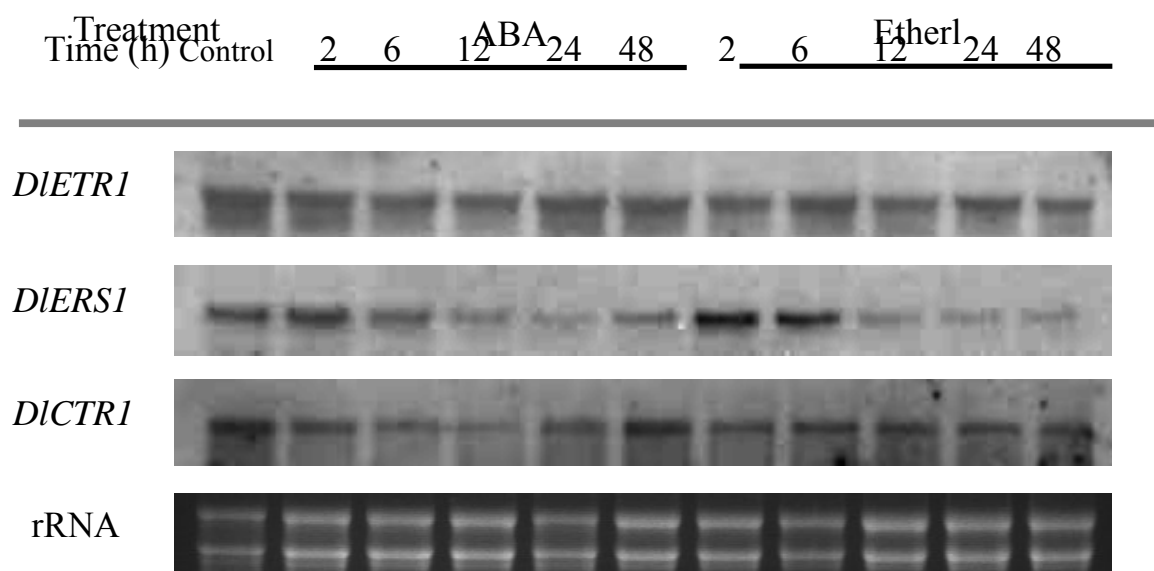


Fig. 4. Differential regulations of *DIETRI*, *DIERS1* and *DICTRI* in aril tissues of longan fruit after ABA and ethrel treatments at 77 DAA. Fruit were dipped for 1 min in a solution containing 0 (control), 200 mg/L ABA or 500 mg/L ethrel. Total RNA (10 μ g per lane) was used for northern blot analysis and hybridized with DIG-labeled probes. Ethidium bromide-stained rRNA was shown as the loading control.

Acknowledgements

This work was supported in part by the Guangdong Modern Agro-industry Technology Research System (grant No. 2009-356), the National Natural Science Foundation of China (grant NO U0631004) and Guangdong Science Foundation (grant No. 06200670).

References

- Abbasi, N.A., Z. Iqbal, M. Maqbool and I.A. Hafiz. 2009. Postharvest quality of mango (*Mangifera indica* L.) fruit as affected by chitosan coating. *Pak. J. Bot.*, 41(1): 343-357.
- Adams-Phillips, L., C. Barry, P. Kannan, J. Leclercq, M. Bouzayen and J. Giovannoni. 2004. Evidence that CTR1-mediated ethylene signal transduction in tomato is encoded by a multigene family whose members display distinct regulatory features. *Plant Mol Biol.*, 54(3): 387-404.
- Arroyo A., F. Bossi, R.R. Finkelstein and P. Leon. 2003. Three Genes that affect sugar sensing (Abscisic Acid Insensitive 4, Abscisic Acid Insensitive 5, and Constitutive Triple Response 1) are differentially regulated by glucose in Arabidopsis. *Plant Physiol.*, 133:231-242.
- Bleecker A.B. and G.E. Schaller. 1996. The mechanism of ethylene perception. *Plant Physiol.*, 111(3): 653-660.
- Bondad N.D., Er.B. Pantastico and D.B. Mendoza. 1970. Ethrel, a new ripening stimulant for banana. *Agr Los Banos*, 10(2): 7-16.
- Brady C.J. 1987. Fruit ripening. *Ann Rev Plant Physiol.*, 38: 155-178.
- Chang C., S.F. Kwok, A.B. Bleecker and E.M. Meyerowitz. 1993. *Arabidopsis* ethylene-response gene ETR1: similarity of product to two-component regulators. *Science*, 262(5133): 539-544.
- Coombe, B.G. and H.Z. Hale. 1973. The hormone content of ripening grape berries and the effects of growth substance treatments. *Plant Physiol.*, 51(4): 629-634.
- El-Sharkawy I., B. Jones, Z.G. Li, J.M. Lelievre, J.C. Pech and A. Latche. 2003. Isolation and characterization of four ethylene perception elements and their expression during ripening in pears (*Pyrus communis* L.) with/without cold requirement. *J Exp Bot.*, 54(387): 1615-1625.
- Ezura H. and W.O. Owino. 2008. Melon, an alternative model plant for elucidating fruit ripening. *Plant Sci.*, 175(1-2): 121-129.
- Feng, H.L., Y.X. Zhong, H. Xie, J.Y. Chen, J.G. Li and W.J. Lu. 2008. Differential expression and regulation of longan *XET* genes in relation to fruit growth. *Plant Sci.*, 174(1): 32-37.
- Giovannoni, J. 2001. Molecular biology of fruit maturation and ripening. *Ann Rev Plant Physiol Plant Mol. Biol.*, 52: 725-749.
- Goldschmidt, E.E., M. Huberman and R. Goren. 1993. Probing the role of endogenous ethylene in the degreening of citrus fruit with ethylene antagonists. *Plant Growth Regul.*, 12(3): 325-329.
- Guo, H. and J.R. Ecker. 2003. The ethylene signaling pathway: new insights. *Curr Opin Plant Biol.*, 7(1): 40-49.
- Hua, J., C. Chang, Q. Sun and E.M. Meyerowitz. 1995. Ethylene insensitivity conferred by *Arabidopsis* *ERS* gene. *Science*, 269(5231): 1712-1714.
- Hua, J. and E.M. Meyerowitz. 1998. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell*, 94(2): 261-271.
- Hua, J., H. Sakai, S. Nourizadeh, Q.G. Chen, A.B. Bleecker, J.R. Ecker and E.M. Meyerowitz. 1998. *EIN4* and *ERS2* are members of the putative ethylene receptor gene family in *Arabidopsis*. *Plant Cell*, 10(8): 1321-1332.
- Huang, W.F., P.L. Huang and Y.Y. Do. 2007. Ethylene receptor transcript accumulation patterns during flower senescence in *Oncidium* 'Gower Ramsey' as affected by exogenous ethylene and pollinia cap dislodgement. *Postharvest Biol. Technol.*, 44(2): 87-94.
- Jiang, Y.M., Z.Q. Zhang, D.C. Joyce and S. Ketsa. 2002. Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). *Postharvest Biol. Technol.*, 26(3): 241-252.
- Katz, E., P.M. Lagunes, J.R.D. Weiss and E.E. Goldschmidt. 2004. Molecular and physiological evidence suggests the existence of a system II-like pathway of ethylene production in non-climacteric Citrus fruit. *Planta*, 219(2): 243-252.
- Kieber, J., M. Rothenberg, G. Roman, K. Feldmann and J. Ecker. 1993. CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. *Cell*, 72(3): 427-441.
- Kuroda, S., M. Hakata, Y. Hirose, M. Shiraishi and S. Abe. 2003. Ethylene production and enhanced transcription of an ethylene receptor gene, *ERS1*, in *Delphinium* during abscission of florets. *Plant Physiol Biochem.*, 41(9): 812-820.

- Lanahan, M.B., H.C. Yen, J.J. Giovannoni and H.J. Klee. 1994. The Never ripe mutation blocks ethylene perception in tomato. *Plant Cell*, 6(4): 521-530.
- Lashbrook, C.C., D.M. Tieman and H.J. Klee. 1998. Differential regulation of the tomato ETR gene family throughout plant development. *The Plant J.*, 15(2): 243-252.
- Mahmood, M.H., A. Khalid, M. Khalid and M. Arshad. 2008. Response of etiolated pea seedlings and cotton to ethylene produced from L-methionine by soil microorganisms. *Pak. J. Bot.*, 40(2): 859-866.
- Müller, R., B.M. Stummann and M. Serek. 2000. Characterization of an ethylene receptor family with differential expression in rose (*Rosa hybrida* L.) flowers. *Plant Cell Rep.*, 19(12): 1232-1239.
- Sakai, H., J. Hua, Q.G. Chen, C. Chang, L.J. Medrano, A.B. Bleecker and E.M. Meyerowitz. 1998. *ETR2* is an *ETR1* like gene involved in ethylene signaling in *Arabidopsis*. *Proc Natl Acad Sci USA.*, 95(10): 5812-5817.
- Sato-Nara K., K.L. Yuhashi, K. Higashi, K. Hosoya, M. Kubota and H. Ezura. 1999. Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiol.*, 120(1): 321-330.
- Schenk, P.W. and B.E. Snaar-Jagalska. 1999. Signal perception and transduction: the role of protein kinases. *Biochim Biophys Acta.*, 1449(1): 1-24.
- Shaharoon, B., R. Bibi, M. Arshad, Z.A. Zahir and Zia-ul-Hassan. 2006. 1-Aminocyclopropane-1-carboxylate (ACC)-deaminase rhizobacteria extenuates ACC-induced classical triple response in etiolated pea seedlings. *Pak. J. Bot.*, 38(5): 1491-1499.
- Trainotti L., A. Pavanello and G. Casadoro. 2005. Different ethylene receptors show an increased expression during the ripening of strawberries: does such an increment imply a role for ethylene in the ripening of these non-climacteric fruits? *J. Exp. Bot.*, 56(418): 2037-2046.
- Payton S., R.G. Fray, S. Brown and D. Grierson. 1996. Ethylene receptor expression is regulated during fruit ripening, flower senescence and abscission. *Plant Mol Biol.*, 31(6): 1227-1231.
- Wan, C.Y. and T.A. Wilkins. 1994. A modified hot borate method significantly enhances the yield of high quality RNA from cotton (*Gossypium hirsutum* L.). *Anal Biochem.*, 223(1): 7-12.
- Wang, H., H. Huang and X. Huang. 2007. Differential effects of abscisic acid and ethylene on the fruit maturation of *Litchi chinensis* Sonn.. *Plant Growth Regul.*, 52(3): 189-198.
- Wilkinson, J.Q., M.B. Lanahan, H.C. Yen, J.J. Giovannoni and H.J. Klee. 1995. An ethylene-inducible component of signal transduction encoded by never-ripe. *Science*, 270(5243): 1807-1809.
- Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annu Rev Plant Physiol.*, 35: 155-189.
- Yin, X., K. Chen, A.C. Allan, R. Wu, B. Zhang, N. Lallu and I.B. Ferguson. 2008. Ethylene-induced modulation of genes associated with the ethylene signalling pathway in ripening kiwifruit. *J Exp Bot.*, 59(8): 2097-2108.
- Zhu, Z. and H. Guo. 2008. Genetic basis of ethylene perception and signal transduction in *Arabidopsis*. *J. Integr. Plant Biol.*, 50(7): 808-815.

(Received for publication 30_December 2009)