Gene expression of the oil palm transcription factor EgAP2-1 during fruit ripening and in response to ethylene and ABA treatments

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Abstract

A cDNA encoding an ERE-binding protein (EgAP2-1) was isolated from the oil palm fruit mesocarp treated with ethylene using yeast one-hybrid assay. EgAP2-1 belongs to the AP2 subfamily of the APETALA2/ethylene-responsive factor (AP2/ERF) proteins and contains two highly conserved AP2/EREBP DNA-binding domains (DNA-BD). Sequence comparison of EgAP2-1 with other AP2 proteins revealed high conservation of the two AP2/EREBP domains and linker region among these proteins. Its protein was localized to the nucleus of onion epidermis cells and showed ERE-specific binding, transcriptional activation, and transactivation properties in yeast and *in vitro*. Its mRNA was highly expressed in oil palm mesocarp with elevated levels in ripening fruits but not in leaves and roots. EgAP2-1 was induced in mesocarp in response to ethylene and abscisic acid but not other hormonal stimuli, including methyl jasmonate and salicylic acid, and abiotic stresses including drought, cold, and high-salinity. Our results demonstrate a link between the regulation of EgAP2-1 expression and ethylene- and/or ABA-coordinated control of the fruit ripening and suggest a regulatory role for EgAP2-1 during fruit ripening and development in oil palm.

Additional key words: abiotic stress, AP2/ERF, ethylene-responsive element, gene expression, yeast one-hybrid assay.

Introduction

The APETALA2/ethylene-responsive factor (AP2/ERF) superfamily of transcription factors play important regulatory roles in plant responses to various biotic and abiotic stresses as well as in plant growth and development (Yamaguchi and Shinozaki 2006). Completion of genome projects has led to the identification of 147, 167, 132, and 200 AP2/ERF members in *Arabidopsis*, rice, grapevine, and poplar genomes, respectively (Sakuma *et al.* 2002, Nakano *et al.* 2006, Riano-Pachon *et al.* 2007, Guo *et al.* 2008, Zhuang *et al.* 2008). The AP2/ERFs have been categorized into five subfamilies based on the number of AP2/EREBP DNA-BDs including ethylene-responsive factor (ERF), dehydration-responsive element binding protein/c-repeat binding factor (DREB/CBF), APETALA2 (AP2), ABI3/VP-related (RAV), and others (Nakano *et al.* 2006, Sharoni *et al.* 2011). The ERF and DREB/CBF subfamilies contain only one AP2/EREBP domain whereas the AP2 has two such domains. The RAV subfamily contains an additional B3 domain along with the AP2/EREBP domain. The AP2/EREBP domain consists of 60 - 70 amino acids and was firstly identified in the *Arabidopsis* AP2 protein (Jofuku *et al.* 1994) and later in ethylene responsive element binding proteins (EREBPs) from *Nicotiana tabaccum* (Ohme-Takagi and Shinshi 1995).

The ERFs bind to the ethylene response element (ERE), also called a GCC box, found in the promoter of many pathogenesis-related and other ethylene-regulated genes, such as 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase, and modulate the plant response to various biotic stresses and developmental processes (Tournier *et al.* 2003, Qin *et al.* 2006, El-Sharkawy *et al.* 2008). Some ERFs, such as the

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Abbreviations: ABA - abscisic acid; AD - activation domain; AP2/ERF - APETALA2/ethylene-responsive factor; BD - binding domain; CRT - c-repeat; CBF - CRT-binding factor; DRE - dehydration-responsive element; DREB - DRE-binding protein; EMSA - electrophoretic mobility shift assay; ERE - ethylene-responsive element; EREBP - ERE-binding protein; ERF - ethylene-responsive factor; GFP - green fluorescent protein; MJ - methyl jasmonate; SA - salicylic acid.

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tomato SlERF1 and apple MdERF1/2 have been reported to be involved in fruit ripening control (Li *et al.* 2007, Wang *et al.* 2007) whereas periwinkle ORCA1/2/3 and rice Sub1A/C regulate plant metabolism (Menke *et al.* 1999, Van der Fits and Memelink 2001, Fukao *et al.* 2006). The DREBs/CBFs bind to the dehydration response element/c-repeat (DRE/CRT) and activate the transcription of dehydration- and cold-responsive genes (Agarwal *et al.* 2010, Quan *et al.* 2010). Members of the RAV subfamily are involved in the ethylene response (Alonso *et al.* 2003), the brassino-steroid response (Hu *et al.* 2004), and biotic/abiotic stress responses in pepper (Sohn *et al.* 2006).

The AP2 proteins have been largely characterized from *Arabidopsis*. AP2 is involved in the specification of floral organ identity (Jofuku *et al.* 1994, Okamuro *et al.* 1997, Maes 1999) and maintenance of the stem cell niche of shoot meristem (Ohto *et al.* 2005, Würschum *et al.* 2006). AINTEGUMENTA (ANT) is required for integument initiation and promotion of growth within developing floral organs (Elliott *et al.* 1996, Klucher *et al.* 1996). Mutations in ANT has been reported to be associated with decrease in the numbers and size of the floral organs whereas its ectopic expression has resulted in the production of larger floral organs (Mizukami and Fischer 2000). BABY BOOM (BBM) has been implicated with a role in embryo development (Boutilier *et al.* 2002) and is evolutionary close to PLETHORA1 (PLT1) and PLETHORA2 (PLT2) which control the specification and maintenance of stem cells within the root apical meristem (Aida *et al.* 2004, Krizek 2009). Overexpression of EgAP2-1 in *Arabidopsis* has resulted in the formation of malformed leaves whereas its ectopic expression has improved *in vitro* regenerative capacity on shoot inducing medium (Morcillo *et al.* 2007).

Characterization of the AP2/ERFs from oil palm provides deeper understanding of the regulatory function of these transcription factors in different aspects of plant development and response to stresses. It also could provide a useful platform to study the ethylene-regulated fruit ripening genes for subsequent improvements of fruit quality and yield. In this study, an ERE-binding AP2 protein was isolated from the oil palm mesocarp treated with ethylene using yeast one-hybrid assay. The DNA-binding, transcription activation, subcellular localization, and transcriptional regulation of this gene was investigated at different developmental stages of mesocarp and in response to various hormones and abiotic stresses.

Materials and methods

Plants and treatments: Oil palm (*Elaeis guineensis* Jacq.; *E. dura* × *E. pisifera*) fruits at 8, 12, 15, 17, and 20 weeks after anthesis (WAA), leaves, and roots were collected from the Malaysian Palm Oil Board. For stress treatments, the mesocarp section of the fruits (8 to 20 WAA) was excised and placed on Murashige and Skoog (MS) media containing either 100 µM abscisic acid (ABA), 100 µM methyl jasmonate (MJ), 100 µM salicylic acid (SA), 20 % (m/v) polyethylene glycol (PEG 2000, drought induction), or 300 mM NaCl for 4 h. Ethylene treatment was performed using 2 cm^3 of 40 % (m/v) ethephon and 59.52 mM sodium bicarbonate for 6 h (Zhang *et al.* 2004). Cold treatment was performed by incubating the tissues at 4 °C for 4 h. Leaves and roots were also subjected to the same treatments.

Yeast one-hybrid assay was carried out using the *Matchmaker*™ library construction and screening kit (*Clontech*, Mountain View, CA, USA). Three tandem repeats of the ERE core sequence (GCC box: AGCCGCC) and its mutant (mERE: GACTACT) were synthesized and cloned upstream of the HIS3 minimal promoter in the pHIS2.1 vector. Total RNA was extracted from the ethylene-treated mesocarp (8 - 20 WAA) and pooled prior to the synthesis of double-stranded (ds) cDNAs. The pHIS2.1/3× ERE, pGADT7-Rec2, and ds cDNAs were co-transformed into the yeast strain Y187. The transformed clones were screened on synthetic defined (SD) media minus His/Leu/Trp plus 50 mM 3-amino-1,2,4-triazole (3-AT; a competitive inhibitor of the HIS3 gene product) and subjected to the yeast colony PCR and sequence analysis. Sequence alignment was carried out using the *ClustalW* program.

DNA-binding and transactivation assay of EgAP2-1 protein in yeast: The 1 434 bp coding region of the EgAP2-1 cDNA was amplified and fused to the yeast GAL4 activation domain (AD) in the pGADT7-Rec2 expression vector. DNA-binding was examined by co-transformation of the recombinant pGADT7-Rec2/ EgAP2-1 and pHIS2.1 vector carrying three tandem repeats of the ERE or mERE into the yeast strain Y187. The transformants expressing the HIS reporter gene were screened on SD/-His/-Leu/-Trp/ + 50 mM 3-AT media. Transactivation assay was performed by cloning the entire coding region of the EgAP2-1 cDNA downstream of the GAL4 DNA-BD in the pGBKT7 vector which carries the TRP reporter gene (*Clontech*). The recombinant plasmid was then transformed into the yeast strain Y187 harboring the *Lac*Z reporter gene. The cells were screened on selective SD/-Trp media and subjected to colony-lift filter β-galactosidase assay as described in the yeast protocols handbook (*Clontech*).

Electrophoretic mobility shift assay (EMSA) was carried out using the light shift chemiluminescent EMSA kit (*Pierce*, Rockford, IL, USA). Three tandem repeats of the ERE and mERE sequences were synthesized and labeled using the biotin 3′ end DNA labeling kit (*Pierce*). The crude protein was extracted from the transformed yeast cells as described in the yeast protocols handbook (*Clontech*) and used in a 0.02 cm^3 binding reaction containing 1× binding buffer, 2.5 % (v/v) glycerol, 5 mM $MgCl_2$, 50 μg cm^{-3} of poly(dI-dC), 0.05 % (v/v) NP-40, 0.003 cm^3 of protein extract, and 20 fmol of the biotin-labeled probe. Four picomoles of the unlabeled probe was used for competition with the labeled probe in EMSA. The binding reactions were separated on a 6 % (m/v) native polyacrylamide gel and blotted onto a nylon membrane. The DNA-protein complexes were detected using the chemiluminescent nucleic acid detection module (*Pierce*).

Subcellular localization of EgAP2-1 protein: The coding region of EgAP2-1 excluding the termination codon was cloned into the pDONR/zeo vector (*Invitrogen*, Carlsbad, CA, USA) and the resulted entry clone was used in a LR recombination reaction using the *Gateway BP* clonase enzyme mix system (*Invitrogen*) to subclone the EgAP2-1 sequence into N-terminal region of the GFP gene in the pMDC83 vector (*Invitrogen*). The recombinant construct encoding the EgAP2-1/GFP fusion protein driven by the CaMV35S promoter and the pMDC83 construct carrying CaMV35S:GFP (control) were introduced into onion epidermal cells using the *Agrobacterium*-mediated system as described by Zhang *et al*. (2004). Transformed cells were placed on MS medium, incubated at 26 °C in dark for 24 h, and the GFP fluorescence was visualized under a fluorescence microscope (*Nikon Eclips 90i*).

Reverse transcription (RT)-PCR: Total RNA was extracted from oil palm mesocarp, leaves, and roots using the RNeasy plant mini kit (*Qiagen*, Venlo, Netherlands) and subjected to RT-PCR analysis using the *Qiagen*® *OneStep* RT-PCR kit. Gene-specific primers were designed for EgAP2-1 (forward primer: 5′-CGGTCT CTTCTCCTCTGGTG-3′ and reverse primer: 5′-ACA TAGCTGGAGCCCTCGTA-3′) and the oil palm house-keeping *actin* gene (forward primer: 5′-CACTTCCTC ATGCCATCCTT-3′ and reverse primer: 5′-GCAGAC TCCAATTCCTGCTC-3′) as an internal control (GenBank acc. No. EU284857). RT-PCR reaction was set up in a 0.05 cm^3 reaction containing 1× *Qiagen OneStep* RT-PCR Buffer, 1 mM dNTP mix, 1 mM forward and reverse primers, 10 units of *Qiagen OneStep* RT-PCR enzyme mix, and 200 ng of total RNA. RT-PCR cycles were set as follow: reverse transcription at 50 °C for 30 min, initial PCR activation at 95 °C for 15 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. A final extension step was added at 72 °C for 10 min. Electrophoresis analysis was carried out on a 1.2 % (m/v) agarose gel, and RT-PCR products were subjected to sequence analysis to confirm the identity of the amplicons.

Results

Three tandem repeats of the ERE were used as a bait in the yeast one-hybrid assay to isolate the ERE-binding proteins from oil palm mesocarp treated with ethylene. Approximately 3 million transformed yeast clones were screened on the selective SD/-His/-Leu/-Trp/ + 50 mM 3-AT media. Two hundred clones were subjected to PCR analysis and 10 were selected based on the size of the cDNA and subjected to sequence analysis. A 1 673 bp cDNA clone was identified as EgAP2-1 (GenBank acc. No. AY691196) which was previously reported from the

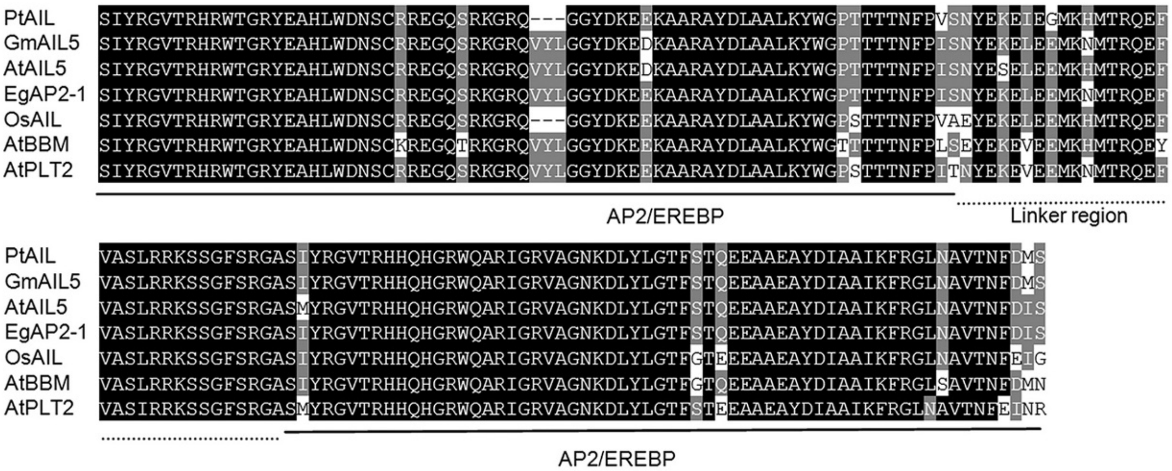

Fig. 1. Sequence alignment of EgAP2-1 with other AP2 proteins, including PtAIL from *Populus trichocarpa* (XP_002325046), GmAIL5 from *Glycine max* (APD37372), AtAIL5 from *Arabidopsis thaliana* (AED96895), OsAIL from *Oryza sativa* (AAL47210), AtBBM from *A. thaliana* (AAM33803), and AtPLT2 from *A. thaliana* (NP_175530). *Shading* indicates identities; the two conserved AP2/EREBP domains and linker regions are *underlined*.

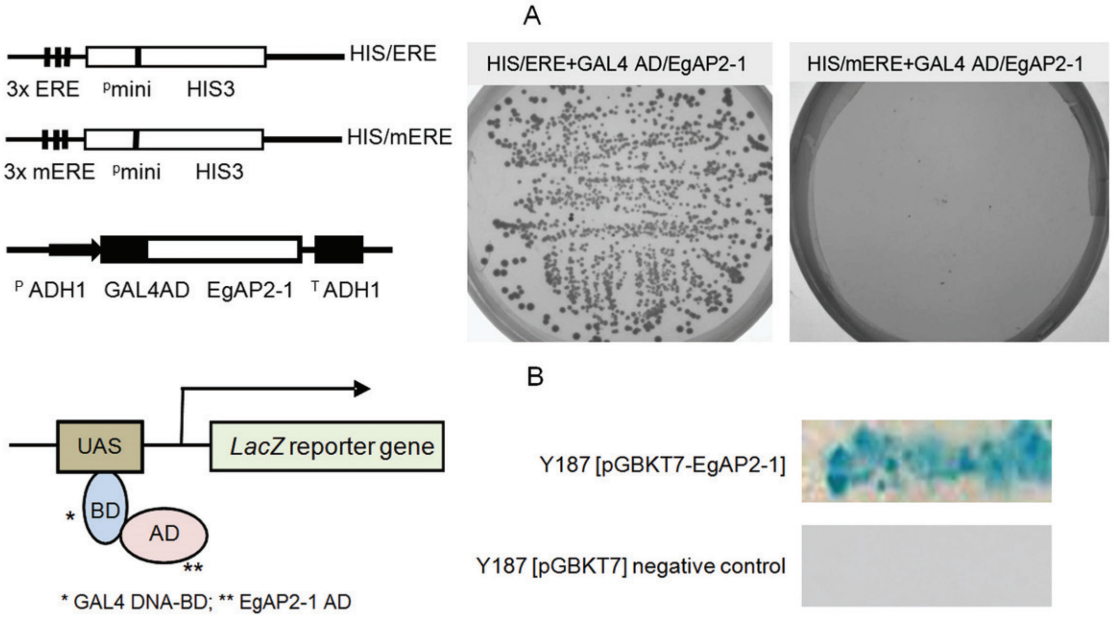

Fig. 2. ERE-binding and transactivation activity of EgAP2-1 protein. *A* - ERE-binding activity of EgAP2-1 in yeast. Sketch maps *on the left* show the vector constructs used in this experiment. Photograph *on the right* shows the growth of yeast transformants on SD/-His/-Trp/-Leu/ + 50 mM 3-AT plates. *B* - Transactivation analysis of EgAP2-1 protein. The protein was expressed as a fusion to the GAL4 DNA-BD in the pGBKT7 vector. The transformed yeast cells carrying the pGBKT7 vector alone were used as a control.

oil palm by Morcillo *et al.* (2007) *via* library screening using the AP2 domain of the rice BBM-related gene OsBNM3lk (acc. No. Os01g67410) as a probe. EgAP2-1 was primarily studied with respect to its role in oil palm zygotic and somatic embryo development (Morcillo *et al.* 2007), however, in this study, the EgAP2-1 was isolated from the ethylene-treated oil palm mesocarp based on its interaction with the ERE in the yeast one-hybrid assay and subjected to a different array of analysis. EgAP2-1 contains an open reading frame (ORF) of 1 434 bp encoding a 478 amino acid protein with a predicted molecular mass of 51.89 kDa. Analysis of the amino acid sequence of EgAP2-1 showed the presence of two AP2/EREBP domains; however, it did not reveal the presence of any known nuclear localization signal (NLS) from this protein. Sequence alignment between EgAP2-1 and other related AP2 proteins using the *ClustalW* showed strong conservations of the two AP2/EREBP domains and linker region (Fig. 1) whereas little conservation was observed outside these regions. EgAP2-1 showed 57 and 53 % similarities to the AINTEGUMENTA-like (AIL) proteins from *Oryza sativa* and *Populus trichocarpa*, respectively. Earlier reports have indicated the presence of three additional motifs outside the AP2/EREBP domains of several AP2 proteins, such as PaAP2L1 from *Picea abies* (Vahala *et al.* 2001), but none was identified from EgAP2-1. These motifs were assumed to be important for either the transcription activation or the protein structure. Interestingly, a conserved KEDFLG amino acid motif was identified outside the AP2/EREBP domains at N-terminal region of the EgAP2-1 and AP2 proteins which has not been characterized so far.

EgAP2-1 was expressed as a fusion to the GAL4 AD to test if it could activate the HIS expression through the interaction with the ERE tandem repeats. The transformed yeast cells carrying the ERE and GAL4 AD/EgAP2-1 fusion protein (pHIS2.1/3× ERE + pGADT7-Rec2/EgAP2-1) expressed the HIS protein and formed colonies on SD/-His/-Trp/-Leu/ + 50 mM 3-AT media, whereby the transformed yeast cells carrying the mutant ERE and fusion protein (pHIS2.1/3× mERE + pGADT7-Rec2/EgAP2-1) did not grow on the same selective media (Fig. 2*A*). This observation indicated the ERE-specific binding and transcriptional activation ability of EgAP2-1 protein in yeast. Transactivation assay showed that the yeast cells harboring the pGBKT7/ EgAP2-1 were able to grow on a selective SD/-Trp media and developed blue color after β-galactosidase assay (Fig. 2*B*). However, the control yeast cells carrying the pGBKT7 vector alone could not express the TRP reporter gene due to the absence of the GAL4 AD in the pGBKT7 vector; therefore these cells did not grow on a Trp deficient SD media. These observations implicated the transactivation ability of EgAP2-1 protein in yeast.

EMSA showed a strong signal shift (Fig. 3*A*) which indicates a high affinity interaction between the labeled ERE probe and EgAP2-1 protein. No signal shift was observed when either the mERE or the endogenous protein extract from the control yeast was used. Stringency of the ERE/EgAP2-1 complex was further challenged using 200-fold excess of the unlabeled ERE probe in a competitive EMSA (Fig. 3*A*). It was shown that the signal shift could be inhibited by the competition from the excess amount of the unlabeled specific probe. This observation validated the ERE-specific binding activity of EgAP2-1 protein. To further investigate if the DRE could interact with EgAP2-1, the DRE sequence (TACCGACAT) was used as a bait in EMSA (Fig. 3*B*). No signal shift was observed corresponding to the DRE/EgAP2-1 complex which indicated that EgAP2-1 could not recognize and interact with the DRE.

Subcellular localization of the EgAP2-1 protein was investigated by examining the expression of EgAP2-1/GFP fusion protein in transiently transformed onion epidermis cells. The EgAP2-1/GFP fusion protein was localized exclusively in the nucleus whereas the fluorescence of the GFP protein (control) was observed in the whole cell driven by the CaMV35S promoter (Fig. 4). This observation clearly demonstrated that EgAP2-1 is a nuclear-localized protein.

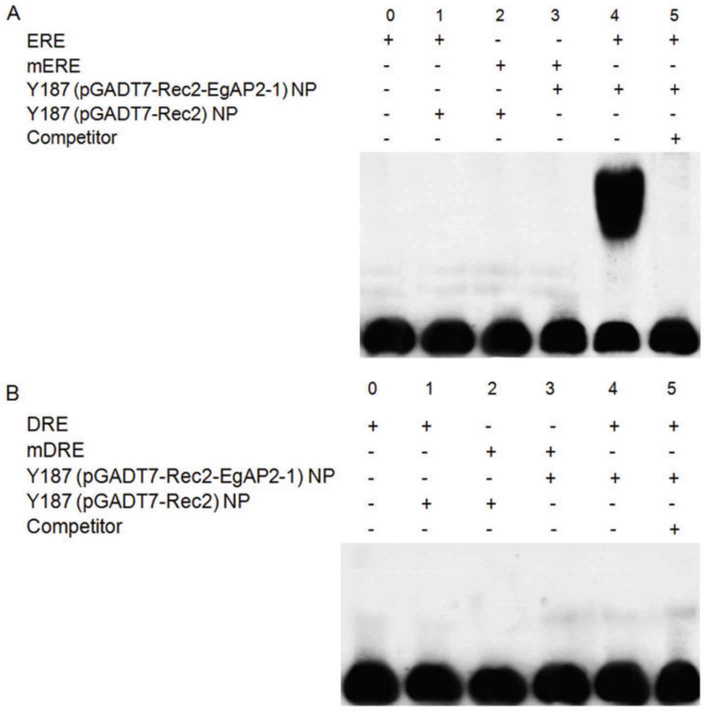

Fig. 3. EMSA showing the interaction of EgAP2-1 protein with the ERE and DRE probes. *A* - Interaction of EgAP2-1 with the ERE probe; *lane* 0: position of the free ERE probe; *lanes* 1 and 2 (controls): the ERE and mERE probes with nuclear protein extract from untransformed yeast, respectively; *lane* 3 (control): the mERE probe with EgAP2-1 protein; *lane* 4: the ERE probe with EgAP2-1 protein; *lane* 5: the ERE probe with 200-fold molar excess of the competitor. *B* - Interaction of EgAP2-1 with the DRE probe; *lane* 0: position of the free DRE probe; *lanes* 1 and 2 (controls): the DRE and mDRE probes with nuclear protein extract from untransformed yeast, respectively; *lane* 3 (control): the mDRE probe with EgAP2-1 protein; *lane* 4: the DRE probe with EgAP2-1 protein; *lane* 5: the DRE probe with 200-fold molar excess of the competitor.

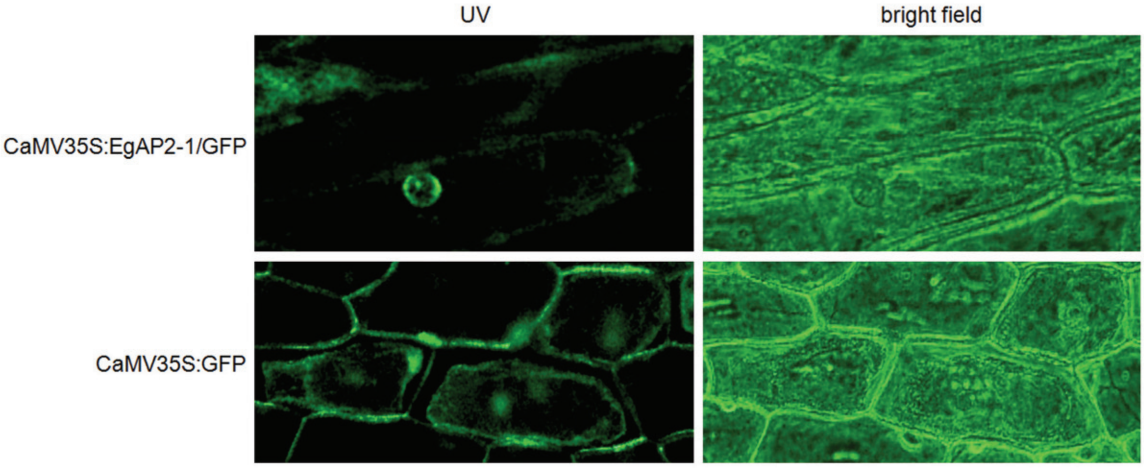

Fig. 4. Subcellular localization of EgAP2-1 protein in onion epidermis cells. The photographs were taken in the dark field under UV radiation for green fluorescence and in the bright irradiance for the morphology of the cells.

A semi quantitative RT-PCR was used to look at the expression profile of EgAP2-1 gene at different developmental stages of oil palm mesocarp, leaves, and roots, and in response to different hormones and abiotic stresses. Sequence analysis confirmed that all the amplicons were the same and derived from EgAP2-1 transcript. High level of the EgAP2-1 mRNA transcripts was primarily detected in untreated mesocarp but not in leaves and roots (Fig. 5*A*). The expression of EgAP2-1 in untreated mesocarp was found to be developmentally up-regulated during the ripening. Interestingly, the ethylene treatment increased the expression of EgAP2-1 even further in mesocarp but not in leaves and roots. The expression of EgAP2-1 was further studied in mesocarp at 17 WAA in response to ethylene, ABA, MJ, SA, drought, cold, and NaCl treatments. Both ethylene and ABA treatments resulted in the similar response profile when EgAP2-1 transcripts increased after almost 4 h of each treatment and reached a maximum level after 12 - 24 h (Fig. 5*B*,*C*). This observation indicated the positive regulatory function of this gene in ethylene and ABA signaling pathways. The expression of EgAP2-1 remained rather unaffected in response to MJ, SA, drought, cold, and high NaCl concentration (Fig. 5*D*-*H*).

Discussion

In this study, the EgAP2-1 was isolated from oil palm mesocarp based on its interaction with the ERE sequence. This gene was previously reported from oil palm *via* genomic approach (Morcillo *et al.* 2007). EgAP2-1 belongs to the AP2 subfamily of the AP2/ERFs and contains two AP2/EREBP DNA-BDs separated by a linker region. Comparison of the amino acid sequences of EgAP2-1 and other related AP2 proteins revealed strong conservations of the two AP2/EREBP domains and linker region. In addition, a conserved KEDFLG motif was identified at N-terminal regions of the EgAP2-1 and AP2 proteins outside the AP2/EREBP domain. Generally, regions outside the DNA-binding domain of transcription factors contain functionally important domains involved in transcriptional activity, protein interactions, and nuclear localization (Nakano *et al.* 2006). Analysis of the EgAP2-1 amino acid sequence did not reveal the presence of any NLS similar to those identified in other proteins; however, a subcellular localization experiment showed that the EgAP2-1 protein was constitutively localized in the nucleus which is the typical characteristic of most transcription factors. The actual NLS remained to be identified experimentally in this protein.

Some ERFs, such as TINY, TERF1, JcERF, Tsi1, and Pti4 link the ERE- and DRE-derived gene expression pathways through the interaction with both ERE and DRE sequences (Park *et al.* 2001, Tang *et al.* 2007, Sun *et al.* 2008, Zhang *et al.* 2008). *In vitro* analysis revealed that EgAP2-1 could specifically bind to the ERE but not the DRE. This observation implicated the ERE-mediated regulatory function of this protein. Transactivation assay showed that EgAP2-1 could activate the *lac*Z expression in yeast.

The AP2 proteins have been primarily characterized with respect to their roles in plant growth and development but not stress-related functions. The EgAP2-1 transcripts were found abundantly in untreated oil palm mesocarp with an increasing content during fruit ripening but not in leaves and roots. Other AP2 proteins, such as BBM, PLT, and AIL5/6/7 have also been reported to have differential expression patterns (Boutilier *et al.* 2002, Aida *et al.* 2004, Nole-Wilson *et al.* 2005). Morcillo *et al.* (2007) studied the transcriptional regulation of EgAP2-1 in oil palm and found that the EgAP2-1 transcripts were mainly accumulated in

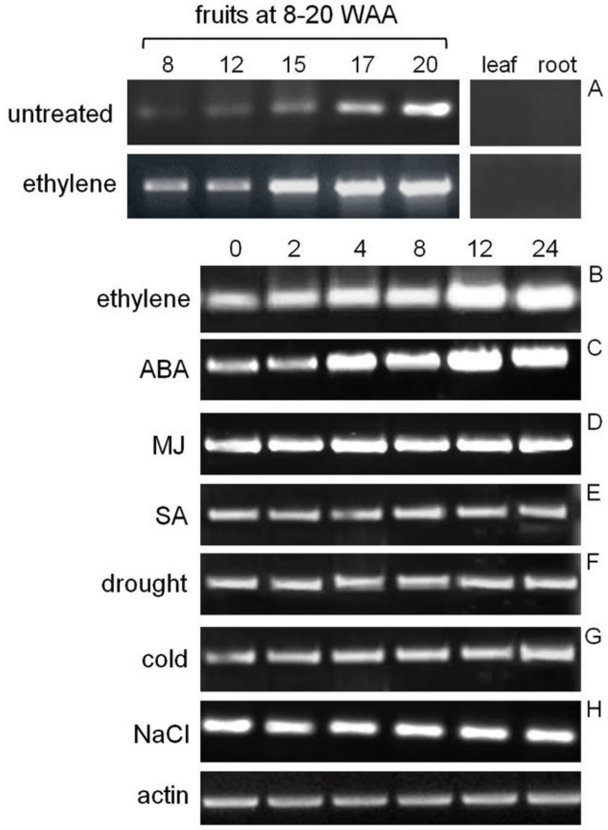

Fig. 5. Transcriptional analysis of EgAP2-1 gene at different developmental stages of oil palm mesocarp, leaves, and roots, and under various treatments. *A* - Expression pattern of EgAP2-1 in mesocarp at different ages (8 - 20 WAA), leaves, and roots before (untreated) and after treatment with ethylene. *B, C, D, E, F, G, H* - Expression pattern of EgAP2-1 in mesocarp in response to ethylene, ABA, MJ, SA, drought, cold, and NaCl, respectively. Time intervals for all the treatments were the same and RT-PCR was carried out at 0, 2, 4, 8, 12, and 24 h of each treatment. The *actin* housekeeping gene was used as an internal control RNA. The expression pattern of *actin* was similar in all the tissues and treatments.

developing zygotic embryo and roots but not in leaves, inflorescence, and vegetative shoot apex. This suggested the down-regulation of this gene during post-embryonic development. We performed data mining from two large transcriptomic datasets from oil palm (Bourgis *et al.* 2011, Tranbarger *et al.* 2011) and interestingly found no evidence for the expression of EgAP2-1 in mesocarp; however, our present study clearly showed the expression of this gene in mesocarp. Differential expression of EgAP2-1 in oil palm could be a result of different genetic backgrounds used in the above mentioned and our study, as different oil palm varieties/cultivars have often distinct gene expression patterns. The EgAP2-1 expression was induced in mesocarp in response to ethylene and ABA but not MJ, SA, drought, cold, and high NaCl concentration. These observations indicated that the transcription of EgAP2-1 was positively regulated through the ethylene and/or ABA signaling pathways. The ABA is involved in abiotic stress signaling whereas ethylene, MJ, and SA are part of the biotic stress response (Fukao *et al*. 2006). Ethylene and ABA not only play a regulatory role in plant response to stress but also are involved in fruit ripening control. To date, many researchers have focused on the physiological aspects of the interaction between ethylene and ABA during fruit ripening. In tomato, ABA content peaks up just prior to that of ethylene during ripening (Zhang *et al.* 2009). By contrast, studies of oil palm fruits have shown a simultaneous increase in both ethylene and ABA content during fruit ripening which suggests a regulatory function and/or interaction of these two hormones in fruit ripening control (Tranbarger *et al.* 2011). In the present study, EgAP2-1 transcripts increased in mesocarp at the late stages of fruit ripening which was in concert with the increase in the content of ethylene and ABA in fruits. Based on the response of EgAP2-1 mRNA to various stimuli, it seems that EgAP2-1 putatively occurred in mesocarp during oil palm fruit ripening in an ethylene- and/or ABA-dependent manners. However, the finding that EgAP2-1 protein could only bind to the ERE but not the DRE suggested a possible regulatory role for this protein during ripening rather than an abiotic stress-related function. Recently, there has been a growing interest in understanding the regulatory functions of AP2/ERFs in fruit ripening. The AP2 proteins play divergent roles in plant developmental process, however, minimal data are available detailing the function of these proteins in fruit ripening. Recently, an AP2 protein from tomato SlAP2a was reported to be involved in fruit ripening control (Chung *et al.* 2010). In contrast, a large number of ERFs have been identified with regulatory functions in fruit ripening control. SlERF1 and SlERF2 were found to be involved in the regulation of fruit ripening and softening tomato fruits (Pirrello *et al.* 2006, Li *et al.* 2007). Wang *et al.* (2007) identified two ERFs, MdERF1 and MdERF2, with a role in apple fruit ripening control. Two fruit ripening-related transcription factor genes, RIN and CNR, have been used for commercial production of tomato cultivars showing slow ripening and extended shelf-life (Moore *et al.* 2002, Manning *et al.* 2006). Tranbarger *et al.* (2011) studied the regulatory mechanisms underlying the oil palm fruit maturation and ripening and identified a number of ethylene-related transcripts similar to those ERFs which have been implicated in the ripening tomato, kiwi, apple, and plum (Wang *et al.* 2007, El-Sharkawy *et al.* 2009, Sharma *et al.* 2010, Yin *et al.* 2010). These transcripts had expression profiles associated with the increase of ethylene in ripening fruits.

The availability of well characterized genetic mutants in fleshy berries, such as tomato and grape, has provided considerable information on fruit development and ripening. In contrast, the molecular basis of oil palm fruit development, maturation, and ripening has received very little attention. Identification of ethylene- and/or ABA-regulated transcription factors during fruit ripening in oil palm could provide a potential tool for metabolic engineering and control of fruit ripening in order to improve the quality and yield of the fruits. EgAP2-1 was previously reported to be involved in oil palm embryo development (Morcillo *et al.* 2007). Our data suggest a possible regulatory role for EgAP2-1 in ethylene- and/or ABA-mediated control of fruit ripening in oil palm, however, further studies are required to fully comprehend the function of this gene.

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