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Molecular Cloning and Characterization of *S*-adenosylmethionine Synthetase Isolated from Suspension Culture Cdna Library of Oil Palm (*Elaeis guineensis* Jacq.)

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Abstract: *S*-adenosylmethionine (AdoMet) synthetase (ATP: L-methionine *S*-adenosyltransferase EC 2.5.1.6) enzyme catalyzes the only known route for the biosynthesis of AdoMet, by the conversion of L-methionine and ATP. A cDNA clone encoding Adomet synthetase, designated as *OPADO-11A1* was isolated from oil palm suspension culture cDNA library. Southern blot analysis showed that more than one copy of *OPADO-11A1* genes might be present in the oil palm genome. We observed that the expression of *OPADO-11A1* was induced by wounding and UV irradiation. The mRNA transcript of *OPADO-11A1* was highly expressed after 2 h following wounding and no enzyme activity was detected from 9 to 24 hrs after wounding. For UV induction, the mRNA transcripts were detected at the highest levels at 15 to 20 min after induction and expression levels decreased after 20 min. However, no obvious changes of *OPADO-11A1* mRNA levels were observed after 2 hrs of ethephon treatment.

Key words: *S*-adenosylmethionine synthetase, Adomet synthetas, oil palm, *Elaeis guineensis* Jacq., suspension culture

Introduction

Plants respond to pathogen attack by activating a wide variety of defense reactions including transcriptional activation of defense-related genes, accumulation of antimicrobial phytoalexins (Dixon and Harrison, 1990), oxidative burst leading to the generation of reactive oxygen species (ROS) (Lamb and Dixon, 1997) and the deposition of lignin and other phenolic compounds in the cell wall (Bradley *et al*., 1992; Dixon and Paiva, 1995). According to Somssich and Hahlbrock (1998), many of the elicitor-responsive genes are involved in various biochemical pathways from both primary and secondary metabolism. However, secondary metabolism has been preferentially studied since it generates a wide range of products, which are involved in defense-related responses (Dixon and Paiva, 1995; Nicholson and Hammerschmidt, 1992).

AdoMet or sometimes referred to as SAM, transfers a methyl group to a methyl acceptor molecule, yielding *S*-adenosylhomosysteine which then hydrolyzed to adenosine and homocysteine, the later of which can be metabolized to methionine (Takusagawa *et al*., 1996). AdoMet synthetase cDNA clones have been isolated from several plant species (Izhaki *et al*., 1995). In all of the characterized systems, AdoMet is encoded by a small multigene family. It has been suggested that some of the AdoMet genes could be expressed constitutively, whereas others are specifically regulated by developmental or environmental factors, including fungal and bacterial elicitors (Kawalleck *et al*., 1992), salinity stress (Van de Löcht, 1990), exposure to ozone (Tuomainen *et al*., 1996) and mechanical stimuli (Espartero *et al*., 1994; Kim *et al*., 1994), all of which are also known to induce ethylene biosynthesis (Abeles *et al*., 1992). In addition, hormonal regulation of AdoMet synthetase has been reported in wheat embryos (Mathur *et al*., 1992) and in a dwarf mutant of pea (Mathur *et al*., 1993), whereby GA_3 treatment induced two additional isoenzymes of Adomet synthtase. Analysis of AdoMet synthetase genes expression during ovary senescence and fruit development showed that AdoMet synthetase transcript levels were up-regulated by auxins during fruit development setting and by ethylene during ovary senescence (Gomez-Gomez and Carrasco, 1996).

Here we reported the cloning of a novel cDNA from oil palm encoding a protein with 80-89% identities when compared to AdoMet synthetase I genes from rice, wheat and tomato.

Materials and Methods

Plant Materials: Plant and tissue materials were provided by Malaysian Palm Oil Board. In order to study the

expression of desired genes in response to stress induction, three different treatments were performed on one-month-old oil palm seedlings and three-month-old oil palm seedlings prior to total RNA extraction. Wound induction was carried out on leaf tissues of one-month-old seedlings by cutting the tissues with a sharp razor blade. The leaf tissue was cut into approximately 0.5 cm pieces and blotted on pre-wet Whatman no.3 paper at room temperature. Samples were collected and stored at –70°C at 2, 4 and 6 hr after wounding of one-month-old seedling. For UV irradiation, the whole one-month-old seedling was exposed under the UV light (454 nm) for 10, 15, 20 and 25 min. On the other hand, 3-month-old seedlings were sprayed until runoff with 0.1 mg ml^{-1}, 1.0 mg ml^{-1} and 5.0 mg ml^{-1} of ethephon (2-chloroethylphosphonic acid) and placed in a large transparent plastic bag for 2 and 48 hr until being harvested; Untreated leaves (control plant) were sprayed to runoff with water. Control plant tissues were used as a control in all of the induction studies in this experiment.

Extraction of Total RNA from Oil Palm: Total RNA from induced tissues were extracted by using a method developed by Schultz *et al*. (1994). Five grams of each tissue was used in the experiments. The quantity and purity of total RNA obtained were determined by using spectrophotometer.

Genomic DNA Extraction: Genomic DNA was extracted from oil palm young leaves by using the CTAB method.

Screening of Oil Palm Suspension Culture cDNA Library: Screening of the oil palm suspension culture cDNA library was performed with PCR-based screening technique, followed by plaque lift hybridization. The glycerol stock of the oil palm suspension culture cDNA library cloned in UniZAP™ XR vector (Stratagene) were kindly provided by Malaysian Palm Oil Board. Clone *OPADO-11A1* was obtained from the screening of oil palm suspension culture cDNA library by using gene specific primers constructed from partial cDNA of phenylalanine ammonia lyase (PAL) isolated from the earlier experiment (unpublished). PCR screening of cDNA library was performed based on the method described by Israel (1993) with minor modification. Gene specific primers D1RS (5' GCA GTT CTC CGA GCT CGT CAA 3') and D1RAS (5' ATC AAT GGC CTG GCA GAT TG 3') were used in the primary PCR amplification. The reaction mixture (25 μL) contained 10 mM Tris-HCl pH8.8, 50 mM KCl, 0.08% NP40, 1.5 mM $MgCl_2$, 0.2 mM dNTPs mix, 1.25 U Taq polymerase (Fermentas), 0.5 μM OP1-S and OP1-AS and 1 μL template. Denaturation of DNA template was performed at 94°C for 1 min for the first and the following cycles. Primer annealing was carried out at 60°C for 1 min and extension at 72°C for another 1 min. After a total of 35 cycles of amplification and final extension at 72°C for 7 min, PCR products were then electrophoresed on 1.0% (w v^{-1}) agarose gel and observed under UV. For secondary screening of the suspension culture cDNA library, phage sample from the positive well of primary PCR screening was plated on NZY agar with a concentration of approximately 500 pfu $plate^{-1}$. Plaque lift hybridization was then carried out according to the manufacturer's instruction by using the partial PAL cDNA as probe. After hybridization, the membranes were washed at the following conditions: 2 X 5 min in 2X SSC, 0.1% (v v^{-1}) SDS at room temperature; 2 X 10 min in 0.5X SSC, 0.1% SDS (v/v) at 55°C and 0.1X SSC, 0.1% SDS (v v^{-1}) at 60°C. The washed membranes were then covered with plastic wrap in a cassette containing an intensifying screen and exposed to Kodak X-ray film. The radioactive membranes were exposed for 1-2 weeks at –80°C.

Radiolabelling of DNA Probe: A radioactive DNA probe was prepared using High Prime labeling kit from Boehringer Mannheim. The purified double stranded partial PAL cDNA fragment (100 ng in 11 μL sterile dH_2O) derived from PCR amplification was heated for 10 min in boiling water. The denatured probe was immediately cooled on ice for 2 min. Later, 4 μL of High Prime solution (1 U μL Klenow polymerase, 0.125 mM dATP, 0.125 mM dGTP and 0.125 mM dTTP) and 5 μL of 50 μCi [α-^{32}P]dCTP were added and incubated at 37°C for 4 hr. The labeling reaction was terminated by adding 2 μL of 0.2 M EDTA, pH 8.0. Finally, the ^{32}P-labeled probe was purified using Chroma Spin™ Columns from Clontech to remove unincorporated dNTPs, salts and proteins.

DNA sequencing and analysis: Sequencing was performed by using ABI PRISMS™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase and electrophorised on Applied Biosystems ABI 377-96 DNA Sequencer as described by the manufacturer. Sequence analysis was carried out using BioEdit v4.8.6 sequence alignment software and BLAST (Altschul *et al*., 1997).

Northern and Southern blot analysis: For Northern blot analysis, total RNA from various induced or non-induced tissues of oil palm were isolated, electrophoresed on 1% (w v^{-1}) formaldehyde denaturing gel and blotted on a Hybond N+ membrane. RNA blots were then hybridized to ^{32}P-labeled OPADO-11A1 probe.

Genomic DNA-blot analysis was conducted to estimate the copy number of the *OPADO-11A1* gene within the oil palm genome. To facilitate a comparison, different amounts (20, 40 or 60 μg) of genomic DNA were digested with *Dra*I. *Hind*III, *Rsa*I, *Sac*I and *Xba*I (MBI Fermentas, USA) restriction endonucleases. The digested genomic DNA was fractionated on 0.8% (w v^{-1}) agarose gel and transferred onto a Hybond N+ membrane. The blotted membrane was hybridized with ^{32}P-labeled OPADO-11A1 cDNA probe.

Rapid Amplification of cDNA Ends (RACE PCR): RACE PCR was carried out according to the Marathon™ cDNA PCR Amplification Kit instruction manual (Clontech). Gene specific primer, MET1 (5' CTCATCATTGGTAACAGTCTCATC 3') was generated from the 5' regions of clone 11A1 in order to amplify the 5' cDNA. As a starting material, 8.0 μg of total RNA extracted from young leaf tissues was used for the first strand cDNA synthesis. Adapter ligated DNA obtained was then diluted to 50X and used as template for PCR amplification. The PCR conditions were as same as that stated in the instruction manual except that the annealing temperature was 60°C. The resultant PCR product was then purified and sequenced.

Results and Discussions

Cloning and characterization of AdoMet Synthetase I cDNA: Clone *OPADO-11A1* encoding a cDNA fragment of AdoMet synthetase I was obtained from the screening of oil palm suspension culture cDNA library by using a partial PAL cDNA as probe. By using the combination method of PCR based screening and plaque lift hybridization, more than 20 positive signals were detected on the autoradiographs, which were also present on the replicas. About 15 positive clones were cored and *in vivo* excised from the λ ZAPII vector to the phagemid form. About 20 clones of different sizes were randomly selected for DNA sequencing and one of the clones, *OPADO-11A1*, showed very high similarities to cDNA encoding AdoMet synthetase I from various plant species. The 1428 bp nucleotide sequence of *OPADO-11A1* contig was produced from the alignment and joining of *OPADO-11A1* clone with the RACE-PCR product of approximately 557 bp. *OPADO-11A1* contained an ORF of 1182 bp predicted to encode 394 amino acids, with 282 bp of 3'UTR and 17 bp of poly(A)$^+$ tail. Sequence analysis showed that this fragment encodes a protein with 80-89% identities when compared to AdoMet synthetase I genes from *Oryza sativa*, *Hordeum vulgare* and *Lycopersicum esculentum*. The alignment of the deduced amino acid sequence of *OPADO-11A1* with sequences of AdoMet synthetase I from different plant species is shown in Fig. 1. According to the sequence alignments, *OPADO-11A1* was a near full-length clone, which was lacking about 20 amino acids from the 5' termini of the cDNA. It was observed that AdoMet synthetase I was highly conserved among different life forms including plant, animal and microorganism. Besides, very high similarities were also found between different isoforms of Adomet synthetase I, II and III (results not shown).

Northern blot analysis and expression of OPADO-11A1 in response to wounding, UV irradiation and ethephon treatment: Wound induction was carried out for one-month-old and three-month-old seedlings. Total RNA was extracted after 2, 4 and 6 hrs after wounding for one-month-old seedlings. According to Fig. 2, *OPADO-11A1* transcripts were found to express at highest levels from 2 hrs after wounding for leaf tissue of one-month-old seedlings. A very low transcript level was detected after 4 hrs of wound induction and no activity was detected after 6 hrs. For UV irradiation, the whole one-month old seedlings were exposed to UV light (254 nm) for 10 to 25 min. Fig. 3 showed that *OPADO-11A1* transcript levels had increased in samples from 10 to 20 min after UV induction. Besides that, there was no significant increase of *OPADO-11A1* mRNA transcript levels for root tissues after 2 hrs of ethephon treatment at 0.1, 1.0 and 5.0 mg of ethephon (Fig. 4).

Southern blot analysis of OPADO-11A1: To elucidate the genomic organization of *OPADO-111A1*, Southern blot analysis was performed with the oil palm genomic DNA. Fig. 5 showed the autoradiograph for membrane hybridized with radiolabled clone *OPADO-11A1* as probe. Lanes 1 to 5 represented total DNA digested with *Dra*I, *Hin*dIII, *Rsa*I, *Sac*I and *Xba*I, respectively. Few distinct fragments of approximately 1.0 kb-4.0 kb were detected in all of the digested samples. The results suggested that more than one copy of *OPADO-11A1* might be present in the oil palm genome.

According to the results shown in the Northern blot analysis, temporal expression of *OPADO-11A1* was highly correlated to stress inductions such as wounding and UV irradiation. *OPADO-11A1* was found to express transiently and rapidly within few hours of induction and their expression levels decrease after a certain time frame. Transient and rapid expression of *OPADO-11A1* in response to wounding and UV irradiation might be considered as the plant defense reactions since they played a protective role in oil palm against further tissue disruption. In the natural environment, wounding might be caused by mechanical stress, insect or pathogen

	10	20	30	40
Rice(SAMI)	MAALDTFLFT	SESVNEGHPD	KLCDQVSDAV	LDACLAEDPD
Barley(SAMI)	MAAETFLFT	SESVNEGHPD	KLCDQVSDAV	LDACLAQDPD
Tomato(SAMI)	METFLFT	SESVNEGHPD	KLCDQISDAV	LDACLEQDPE
Kiwifruit(SAMI)	MDTFLFT	SESVNEGHPD	KLCDQVSDAI	LDACLKQDPE
Madagascar(SAMI)	METFLFT	SESVNEGHPD	KLCDQISDAV	LDACLEQDPD
Arabidopsis(SAMI)	METFLFT	SESVNEGHPD	KLCDQISDAV	LDACLEQDPD
11A1(Oil palm SAMI)	MDTFLFT	SESVNEGHPD	KLCDQISDAV	LDACLEQDPD
Consensus	MDTFLFT	SESVNEGHPD	KLCCDQ	SDA LDACL DP

	50	60	70	80
Rice(SAMI)	SKVACETCTK	TNMVMVFGEI	TTKANVDYEK	IVR-ETCRNI
Barley(SAMI)	SKVACETCTK	TNMVMVFGEI	TTKATVDYEK	IVR-DTCRDI
Tomato(SAMI)	SKVACETCTK	TNLVMVFGEI	TTKAIVDYEK	IVR-DTCRNI
Kiwifruit(SAMI)	SKVACESCTK	TNMVMVFGEI	TTKAQVNYEK	IVR-DTCRGI
Madagascar(SAMI)	SKVACETCTK	TNMVMVFGEI	TTKATVDYEK	IVR-DTCRSI
Arabidopsis(SAMI)	SKVACETCTK	TNMVMVFGEI	TTKATVDYEK	IVR-DTCRAI
11A1(Oil palm SAMI)	SKVACETCTK	TNMVMVFGEI	TTKANVDYEK	IVSRDTCRGI
Consensus	SKVACECTK	TN VMVFGEI	TTKA V YEK	IV TCR I

	90	100	110	120
Rice(SAMI)	GFVSADVGLD	ADHCKVLVNI	EQQSPDIAQG	VHGHFTKRPE
Barley(SAMI)	GFISDDVGLD	ADHCKVLVNI	EQQSPDIAQG	VHGHFTKRPE
Tomato(SAMI)	GFVSDDVGLD	ADNCKVLVYI	EQQSPDIAQG	VHGHLTKRPE
Kiwifruit(SAMI)	GFTSPDVGLD	ADHCKVLVNI	EQQSPDIAQG	VHGHLTKKPE
Madagascar(SAMI)	GFVSDDVGLD	ADNCKVLVNI	EQQSPDIAQG	VHGHLTKRPE
Arabidopsis(SAMI)	GFVSDDVGLD	ADKCKVLVNI	EQQSPDIAQG	VHGHFTKCPE
11A1(Oil palm SAMI)	GFTSDDVGLM	PDHCKVLVNI	EQQSPDIAPG	VHGHFTKRPE
Consensus	GF S DVGL	D CKVLV I	EQQSPDIA G	VHGH TK PE

	130	140	150	160
Rice(SAMI)	EIGAGDQGHM	FGYATDETPE	LMPLSHVLAT	KLGARLTEVR
Barley(SAMI)	EVGAGDQGIM	FGYATDETPE	LMPLTHMLAT	KLGARLTEVR
Tomato(SAMI)	EIGAGDQGHM	FGYATDETPE	LMPLSHVLAT	KLGARLTEVR
Kiwifruit(SAMI)	EIGAGDQGHM	FGYATDETPE	LMPLTHVLAT	KLGAKLTEVR
Madagascar(SAMI)	EIGAGDQGHM	FGYATDETPE	FMPLSHVLAT	KLGARLTEVR
Arabidopsis(SAMI)	DIGAGDQGHM	FGYATDETPE	LMPLSHVLAT	KLGARLTEVR
11A1(Oil palm SAMI)	EIGAGDQGHM	FGYATDETPE	FMPLSHVLAT	KLGARLTEVR
Consensus	GAGDQG M	FGYATDETPE	MPL H LAT	KLGA LTEVR

	170	180	190	200
Rice(SAMI)	KNGTCAWLRP	DGKTQVTVEY	RNESGARVPV	RVHTVLISTQ
Barley(SAMI)	KNGTCAWLRP	DGKTQVTIEY	LNEGGAMVPV	RVHTVLISTQ
Tomato(SAMI)	KNGTCAWLRP	DGKTQVTVEY	SNDNGAMVPI	RVHTVLISTQ
Kiwifruit(SAMI)	KNSTCPWLRP	DGKTQVTVEY	RNEGGAMVPI	RVHTVLISTQ
Madagascar(SAMI)	KNGTCPWLRP	DGKTQVTVEY	YNENGAMVPV	RVHTVVISTQ
Arabidopsis(SAMI)	KNGTCAWLRP	DGKTQVTVEY	YNDKGAMVPI	RVHTVLISTQ
11A1(Oil palm SAMI)	KNGTCPWLRP	DGKTQVTVEY	RNDHGAMVPI	RVHTVLISTQ
Consensus	KN TC WLRP	DGKTQVT EY	N GA VP	RVHTV ISTQ

	210	220	230	240
Rice(SAMI)	HDETVTNDEI	AADLKEHVIK	PVIPEQYLDE	KTIFHLNPSG
Barley(SAMI)	HDETVTNDEI	AADLKEHVIK	PVIPGKYLDE	NTIFHLNPSG
Tomato(SAMI)	HDETVTNDEI	ARDLKEHVIK	PVIPEKYLDE	NTIFHLNPSG
Kiwifruit(SAMI)	HDETVTNDQI	ANDLKKHVIK	PVVPAQYLDD	NTIFHLNPSG
Madagascar(SAMI)	HDETVTNDQI	AADLKEHVIK	PVIPEKYLDE	RTIFHLNPSG
Arabidopsis(SAMI)	HDETVTNDEI	ARDLKEHVIK	PVIPEKYLDE	KTIFHLNPSG
11A1(Oil palm SAMI)	HDETVTNDEI	AADLKEHVIK	PVVPEQYLDE	KTIFHLNPSG
Consensus	HDETVTND I	A DLK HVIK	PV P YLD	TIFHLNPSG

	250	260	270	280
Rice(SAMI)	RFVIGGPHGD	AGLTGRKIII	DTYGGWGAHG	GGAFSGKDPT
Barley(SAMI)	RFVIGGPHGD	AGLTARKIII	DTYGGWGAHG	GGAFSGKDPT
Tomato(SAMI)	RFVIGGPHGD	AGLTGRKIII	DTYGGWGAHG	GGAFSGKDPT
Kiwifruit(SAMI)	RFVIGGPHGD	AGLTGRKIII	DTYGGWGAHG	GGAFSGKDPT
Madagascar(SAMI)	RFVIGGPHGD	AGLTGRKIII	DTYGGWGAHG	GGAFSGKDPT
Arabidopsis(SAMI)	RFVIGGPHGD	AGLTGRKIII	DTYGGWGAHG	GGAFSGKDPT
11A1(Oil palm SAMI)	RFVIGGPHGD	AGLTGRKIII	DTYGGWGAHG	GGAFSGKDPT
Consensus	RFVIGGPHGD	AGLTRKIII	DTYGGWGAHG	GGAFSGKDPT

	290	300	310	320
Rice(SAMI)	KVDRSGAYVA	RQAAKSIVAS	GLARRCIVQV	SYAIGVPEPL
Barley(SAMI)	KVDRSGAYIA	RQAAKSIIAS	GLARRCIVQI	SYAIGVPEPL
Tomato(SAMI)	KVDRSGAYIV	RQAAKSIVAS	GLARRCIVQV	SYAIGVPEPL
Kiwifruit(SAMI)	KVDRSGAYIV	RQAAKSVVAS	GLARRCLVQV	SYAIGVAEPL
Madagascar(SAMI)	KVDRSGAYIV	RQAAKSIVAN	GLARRCIVQV	SYAIGVPEPL
Arabidopsis(SAMI)	KVDRSGAYIV	RQAAKSVVAN	GMARRALVQV	SYAIGVPEPL
11A1(Oil palm SAMI)	QVDRSGGYIA	RQAAKSIVAN	GLARRCIVQV	SYAIGVPEPL
Consensus	VDRSG Y	RQAAKS A	G ARR VQ	SYAIGV EPL

	330	340	350	360
Rice(SAMI)	SVFVDTYGTG	RIPDKEILKI	VKENFDFRPG	MIIINLDLKK
Barley(SAMI)	SVFVDSYGTG	KIPDREILKL	VKENFDFRPG	MITINLDLKK
Tomato(SAMI)	SVFVDTYGTG	KIPDREILKI	VKENFDFRPG	MMSINLDLKR
Kiwifruit(SAMI)	SVFVDTYKTG	KIADKDILAL	IKENFDFRPG	MIAINLDLKR
Madagascar(SAMI)	SVFVDTYGTG	KIPDKEILKI	VKENFDFRPG	MIAINLDLKR
Arabidopsis(SAMI)	SVFVDTYETG	LIPDKEILKI	VKESFDFRPG	MMTINLDLKR
11A1(Oil palm SAMI)	SVFVDTYGTG	KIPDKEILKI	VKENFDFRPG	MIIINLDLKR
Consensus	SVFVD Y TG	I D IL	KE FDFRPG	M INLDLK

	370	380	390	
Rice(SAMI)	GGNGRYLKTA	AYGHFGRDDP	DFTWEVVKPL	KWEKPSA
Barley(SAMI)	GGN-RFIKTA	AYGHFGRDDA	DFTWEVVKPL	KFDKASA
Tomato(SAMI)	GGNRRFLKTA	AYGHFGRDDP	DFTWEVVKPL	KWEKPQD
Kiwifruit(SAMI)	GGNLRYQKTA	AYGHFGRDDP	DFTWETVKIL	KPKA---
Madagascar(SAMI)	GGSGRFLKTA	AYGHFGRDDP	DFTWEVVKPL	KWEKAAN
Arabidopsis(SAMI)	GGNGRFLKTA	AYGHFGRDDP	DFTWEVVKPL	KWDKPQA
11A1(Oil palm SAMI)	GGNGRFLKTA	AYGHFGRDDT	DFTWEVVKPL	KGEKPAA
Consensus	GG R KTA	AYGHFGRDD	DFTWE VK L	K

Fig. 1: Aligned amino acid sequences of clone *OPADO-11A1* with the sequences of Adomet synthetase I from rice (*Oryza sativa*, Acc No. P46611), barley (*Hordeum vulgare*, Acc No. P50299), tomato (*Lycopersicum esculentum*, Acc No. P43280), kiwi fruit (*Actinidia chinensis*, Acc No. P50301), madagascar periwinkle (*Catharanthus roseus*, Acc No. Q96551) and Arabidopsis (*Arabidopsis thaliana*, Acc No. P23686)

attacks. The sites of wounding would create an opening in the plant surface, which further exposes the inner part of plant tissues that are susceptible to pathogen invasion. It had been well documented that the production of plant secondary metabolites such as antimicrobial phytoalexins and aromatic polymer lignin played an important role in plant defense responses. For instance, lignin and suberin could serve as a physical barrier in response to stress condition or pathogen infection. Hence, AdoMet synthetase which is transcriptionally activated during the plant defense response probably to provide the activated methyl groups for both ethylene production and numerous methylation steps involved in secondary product formation (Kawalleck *et al.*, 1992).

On the other hand, it was found that Adomet synthetase I transcript signals were more intense as compared to PAL in ethephon treated root tissues (Fig. 4). The differences between AdoMet synthetase I and PAL transcript levels in ethephon treated tissues suggested that there was a close relationship between AdoMet synthetase I with ethephon induced reactions in the plant. This was mainly due to the fact that AdoMet synthetase was involved in the biosynthesis of AdoMet, which acted as a precursor for ethylene production in plants. Since ethephon was known to induce the production of ethylene, it might also induce the accumulation of Adomet synthetase transcripts to a higher level in order to support the production of ethylene in induced tissues. The transient and rapid expression of *OPADO-11A1* upon wound

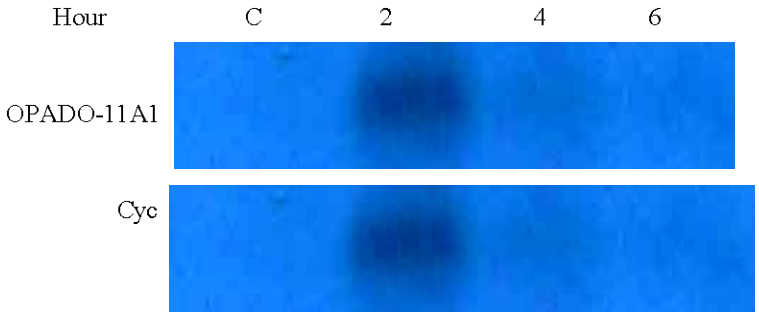

Fig. 2: RNA gel blot analyses of *OPADO-11A1* and cyclophilin in response to wounding of one-month-old leaf tissue of oil palm *in vitro* seedlings. Lanes C-4 represents control of unwounded tissues and 2, 4 and 6 hrs after wounding of leaf tissue, respectively. The blot was also hybridized with cyclophilin (Cyc) as a control for RNA loading

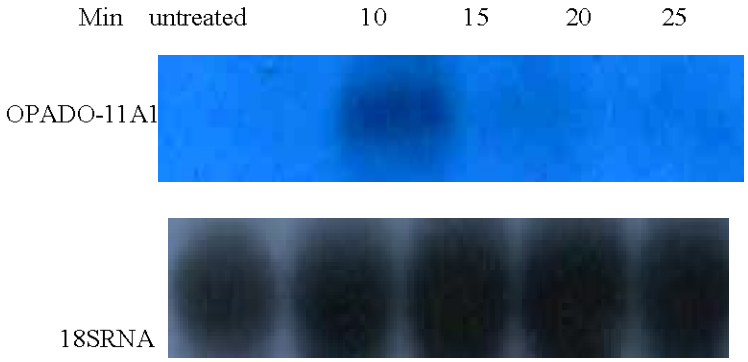

Fig. 3: RNA gel blot analyses of OPADO-11A1 and 18S RNA in response to UV irradiation of one-month-old oil palm *in vitro* seedlings. Lanes 1-5 represents control of untreated tissue, 10, 15, 20 and 25 min after UV irradiation, respectively. The blot was also hybridized with and 18S RNA as a control for RNA loading

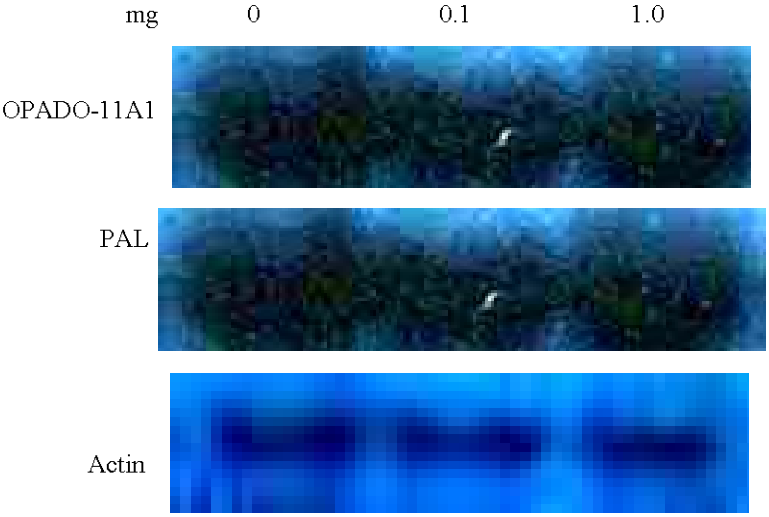

Fig. 4: RNA gel blot analyses of PAL, OPADO-11AI and actin in response to 0.1 and 1.0 mg of ethephon treatments of root tissues of 3-month-old oil palm seedlings after 2 hrs. Lanes 1-3 represent control of untreated tissue, 0.1 and 1.0 mg of ethephon treated tissues, respectively. Blot was also hybridized with actin as control of RNA loading

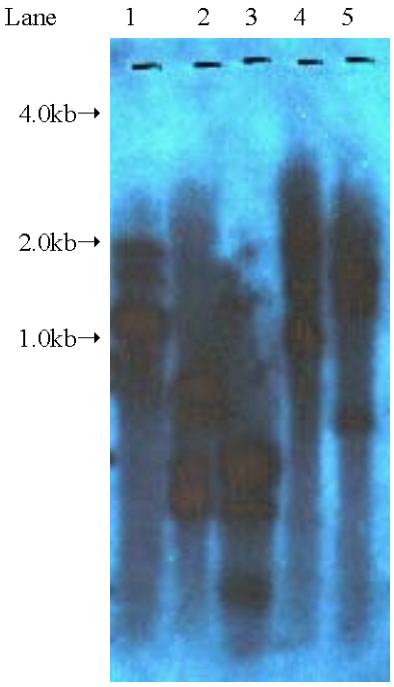

Fig. 5: Southern blot analysis of clone OPADO-11A1 for oil palm total DNA. Lane 1-5 represent DNA digested with *Dra*I, *Hin*dIII, *Rsa*I, *Sac*I and *Xba*I, respectively

induction in this experiment suggested that they might participate in the production of secondary metabolites such as lignin monomers at the wound sites. It was also observed that the expression of *OPADO-11A1* corresponded to the expression of PAL in most of the experiments (results not shown), which suggested that both genes might involved in inter-related pathways during the plant defense response.

Based on the published reports to date, the knowledge of molecular biology of defense and stress responses in oil palm is still limited. In this study, oil palm defense response genes have been cloned and studied from the molecular aspect in order to provide a better understanding of the molecular mechanisms involved in oil palm defense reactions. The fact that genes that have been studied in this experiment are involved in defense or stress responses are supported by various literatures published.

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