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Isolation and Characterization of *Myb*-Related Genes from Oil Palm (*Elaeis guineensis* Jacq.)

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Myb is a sequence-specific DNA-binding protein that can activate or inactivate promoters containing its binding site. Two different *Myb*-like genes were isolated from oil palm (*Elaeis guineensis*). The deduced amino acid sequence of *OpMyb24*, which was obtained from a zygotic embryo cDNA library, showed extensive homology to the snapdragon proteins Myb308 and Myb330, and to maize protein Zm38. Furthermore, the *OpMyb15* gene, from an oil palm suspension culture (OPSC) cDNA library, was very similar to MIXTA from *Antirrhinum majus*. *OpMyb15* was expressed in all tissues tested, while transcript of *OpMyb24* was detected everywhere except in three-month-old and young leaves. Although both transcripts accumulated to high levels in response to treatment with gibberellic acid, levels of *OpMyb24* decreased significantly after plants were either wounded or treated with heavy metals or UV.

Keywords: DNA binding domain, gibberellic acid, heavy metals, *Myb*-like transcription factor, UV, wounding

The *Myb* family of genes exists widely in both plants and animals. In the former, *R2R3-Myb* genes encode a group of functionally diverse transcriptional regulator proteins. These are characterized by two N-terminal conserved DNA-binding domains of approximately 50 amino acids, with constantly spaced tryptophan residues. As transcription factors, Myb-like proteins play different roles in regulating development and metabolism (Grotewold et al., 1998).

Plant transcription factors typically comprise a nuclear localization signal, an oligomerization site, a specific DNA-binding region, and a transcription regulation domain, although some may lack either of the two latter components (Washburn et al., 1997). The DNA binding domains are primarily basic. They contain amino acid residues that contact DNA bases at the *cis-acting* elements, thereby determining the specificity of the protein (Huang et al., 1996). The structural characteristic common to all known Myb proteins is that binding domain. These signature motifs of the transcription factor families are usually formed by two or three imperfect 51- or 52-residue repeats (R1, R2, and R3). Although most plant Myb-related proteins have two Myb domains, the potato MybSt1 (Baranowskij et al., 1994) and the *Arabidopsis* CCA1 (Wang et al., 1997) contain only one copy. Each repeat encodes three α helices, with the second and third helices forming a helix-turn-helix (HTH) structure when bound to DNA. This is similar to the motifs found in the λ repressor and homeodomain proteins (Lipsick, 1996). Myb proteins containing this domain have been shown to bind to DNA in a sequence-specific manner.

Myb-like transcription factors regulate a number of aspects of plant growth and development, e.g., secondary metabolism (Moyano et al., 1996), cellular morphogenesis (Noda et al., 1994), pathogen defense (Yang and Klessig, 1996), and the signal transduction pathways that respond to growth regulators (Iturriaga et al., 1996). Hartmann et al. (1998) have demonstrated that light-responsive units (LRUs), which consist of an ACGT-containing element (ACE) and a *Myb* recognition element (MRE), confer responsiveness to UV-emitting white light (Hartmann et al., 1998). Likewise, Urao et al. (1994) have reported that treatment with ABA induces the expression of *AtMyb2* in *Arabidopsis*. That *Myb* gene is also induced in response to dehydration or salt stress.

The objective of our study was to isolate *Myb* genes from screened cDNA libraries for the oil palm (*Elaeis guineensis*), and to compare their amino acid sequences with proteins from other species. In addition, we wished to determine what effect wounding or exposure to UV light, heavy metals, or gibberellic acid had on gene expression in various tissue types.

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MATERIALS AND METHODS

Plant Materials

Various tissues were harvested from one-month-old in-vitro seedlings of the oil palm (*E. guineensis*). These had been provided by Encik Zamzuri at the tissue culture laboratory of the Malaysian Palm Oil Board (MPOB).

cDNA Libraries

cDNA libraries for the oil palm zygotic embryo (OPZE) and suspension culture (OPSC) were obtained from Dr. Meilina Ong Abdullah (MPOB) and Ms. Ooi Siew Eng (University Putra Malaysia), respectively.

Genomic DNA Extraction and Southern Blot Analysis

Genomic DNA was extracted from young leaves, using the CTAB method described by Murray and Thompson (1980). Southern blot analysis was conducted to estimate the copy number of the *OpMyb15* and *OpMyb24* genes within the genome. To facilitate a comparison, different amounts (20 µg, 40 µg, or 60 µg) of genomic DNA were digested with restriction endonucleases EcoRI, BamHI, or KpnI (MBI Fermentas, USA). The digested genomic DNA was fractionated on a 0.8% (w/v) agarose gel and transferred to a Hybond N+ membrane (Amersham). The blotted membrane was then hybridized with either the ^{32}P-labeled OpMyb15 cDNA probe or a full-length OpMyb24 cDNA probe.

PCR Amplification

Two sets of primers (WheS/WheAS and OsMybS/OsMybAS) were designed based on the conserved regions of the *Myb*-related genes in wheat and rice, respectively. The forward primers, designated WheS and OsMybS, were 5'-TGG GGA GGG CGC CGT GTT GCG A- 3' and 5'-GG(C/G) AAG AGC TGC CG(C/G) CT(C/G) CGG T-3', respectively. The reverse primers, WheAS and OsMybAS, were 5'-AGC AGG GAG TAG TAG CAG TGA- 3' and 5'-AGT TCC AGT AGT TCT TGA T(C/T)T C- 3', respectively. PCR amplification was performed in a thermal cycler (PTC-200; MJ Research, USA). The 25-µL reaction mixture contained 1× PCR buffer, 1.5 mM $MgCl_2$, a 0.2 mM dNTP mix, 1.25 U *Taq* DNA polymerase (MBI Fermentas), 0.8 µM forward primer, 0.8 µM reverse primer, and 2 µL of template. Amplification conditions comprised 35 cycles of denaturation at 95°C for 1 min, followed by primer annealing and extension reactions at 52°C and 72°C, respectively, for 1 min each. A final extension was conducted at 72°C for 7 min.

DNA Sequencing and Computer Analysis

After the PCR products were electrophoresed, the bands of interest were excised, purified, and cloned with the QIAquick Gel Extraction Kit (QIAGEN, USA) and the TOPO TA Cloning® Kit (Invitrogen, USA). Sequencing of putative clones was done using the ET terminator Sequencing Ready Reaction Kit (Amersham Pharmacia Biotech, USA) on an automated DNA sequencer (ABI 377-96 DNA Sequencer; Applied Biosystems, USA). Sequence analysis was carried out using BioEdit version 5.5 software. Homology was assessed at both the DNA and protein levels according to BLAST (Basic Local Alignment Search Tool) though the Internet (http://ingene2.upm.edu.my/Blast).z.

Plaque Lift Hybridization

About 3×10^4 pfu from each library was screened for OpMyb16. Two primers, OPS (5'-GGG AGG ACA GAC AAC GAG AT- 3') and OP16AS (5'-CAC CAT CTT CTC TCA GTC CAT A-3'), were designed and synthesized based on the partial oil palm *Myb*-like nucleotide sequence of clone Op16. This had been obtained in the earlier PCR amplification. These primers were used to amplify a 300-bp DNA fragment as a probe to screen the full-length *Myb*-related gene from the OPZE and OPSC cDNA libraries. The probe was labeled with [$\propto$-^{32}P] dCTP (3000 Ci mmol^{-1}), using a Random Priming Labeling kit (Boehringer Mannheim, Germany). Plaque hybridization was performed with the labeled probe. The positive clones were cored out from the NZY agar plates, randomly selected for secondary and tertiary screening, then excised in vivo to obtain plasmid pBluescript SK$^+$ from the UniZAP^{TM}XR vector.

Total RNA Extraction and Northern Blot Analysis for Stressed Seedlings

One-month-old in-vitro seedlings were used for testing the effects of various stresses on gene expression. For our wound-induction study, the seedlings were cut with a sharp razor blade into 0.4-cm pieces. The dissected tissues were then blotted for 2, 4, 6, or 12 h on Whatman 3 MM paper that was pre-moistened with tap water. For the UV light treatment, entire seedlings were exposed to radiation (1200 µJoules x 100) in the UV cross-linker (Stratagene, USA) for 10, 15, 20, or 25 min. To assess the influence of heavy metals, entire

seedlings were dipped in 50 mM $HgCl_2$ for 0.5, 1.0, or 2.0 h. Our hormone treatment involved incubating seedlings in test tubes containing an MS medium supplemented with 100 µM GA_3 for 3, 6, 12, 18, 24, or 48 h. After all these treatments, total RNA was extracted according to the method of Schultz et al. (1994). RNA was isolated from mature (28-cm) female and male flowers, immature (1- to 2-cm) female and male flowers, three-month-old leaves, meristems, and young leaves. RNA from the roots and mesocarps were isolated by the CsCl method (Davis et al., 1986). Northern blot analysis was then performed with the extracted RNA on a Hybond N+ membrane, followed by hybridization to the ^{32}P-labeled OpMyb24 probe.

Semi-Quantitative RT-PCR Analysis

Semi-quantitative RT-PCR was performed as described by Southerton et al. (1998), with minor modifications. Total RNA was isolated from tissues of in-vitro seedlings that had been incubated for various time intervals. We used a 25-µL reaction mixture containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 1.25 U *Taq* DNA polymerase, 0.5 µM forward primer, 0.5 µM reverse primer, and 25 ng of first-strand cDNA as the template. Denaturation was carried out at 95°C for 1 min, followed by primer annealing and extension reactions at 58°C and 72°C, respectively, for 1 min each. Primers used were Op15MybS (forward) (5′-GGG AGA GGA GGC TCC AAA CTG ATA T-3′), Op15MybAS (reverse) (5′-AGG AGC ATC GTC TAG TGC CAT GTC A-3′), Op24MybS (forward) (5′-ATG CTG CGA GAA AGC CCA CAC C-3′), and Op24MybAS (reverse) (5′-ATT TCC TCC CTG ATC TCC CTC TAA-3′).

RESULTS AND DISCUSSION

PCR Cloning of Oil Palm *Myb*-Related cDNA

PCR amplification yielded two partial Myb clones: ~400-bp Op6 and ~50-bp Op16. BLASTX search results revealed that the partial sequences of Op6 and Op16 encoded proteins with 100% and 90% identities, respectively, when compared with AtMYB4 (GenBank Accession No. NC 003075), TMH27 (GenBank Accession No. S69189), GHMYB9 (GenBank Accession No. AF336286), and MYB308 (GenBank Accession No. JQ0960). The structural similarities detected between these two oil palm *Myb* gene fragments and the Myb proteins of other species included a second-half region of the R2 repeat with a third tryptophan residue, as well as a first-half region R3 repeat where the first tryptophan residue was replaced by phenylalanine (Fig. 1A).

Screening of Full-Length *Myb*-Related Genes from Oil Palm

Both amplified DNA fragments -- the 200-bp *OpMyb6* clone (primers used: OPS and OP6AS) and the 300-bp *OpMyb16* clone (primers OPS and OP16AS) -- were used to probe duplicate filters lifted from the two oil palm cDNA libraries. Unfortunately, we were unable to detect any signal from the shorter probe (OpMyb6), even after a prolonged exposure time.

When 3×10^4 pfu from each library was screened with the *OpMyb16* clone, 3 positive plaques were isolated from the primary screening of the OPZE cDNA library while 13 positive plaques were obtained from the OPSC cDNA library. Hybridization and washing were carried out at low stringency to allow the detection of weakly conserved members of the *Myb*-like gene family. After 24 positive plaques were selected from this secondary screening, they were in-vivo excised from the λZAP II vector. However, only 9 amplified products were obtained in the first batch of screening of 36 clones. These products showed two insert sizes -- 1100 bp and 800 bp.

A BLAST search and comparison revealed high homology with other plant *Myb* genes. Moreover, seven clones contained two distinct *Myb*-related genes. These were designated as *OpMyb15* (from the OPSC cDNA library) and *OpMyb24* (from the OPZE cDNA library). Clone *OpMyb24* carries a full-length coding region, with a 1140-bp cDNA insert and an ORF (open reading frame) of 675 bp encoding 225 amino acids. In contrast, clone *OpMyb15* is 1124-bp long, with a predicted ORF of 606 bp encoding 202 amino acids, and a partial *Myb*-related sequence that lacks the 5′ end. Amino acid sequences were deduced from the nucleotide sequences of both genes.

To establish the identity of the *OpMyb* genes, a protein BLASTX search was made. Like other plant Myb-related proteins, OpMyb24 and OpMyb15 have two imperfect repeats of 51 to 53 amino acids with conserved tryptophan residues. The Myb domains are localized in the N-terminal portions (Fig. 1B) and contain all the conserved amino acids, including regularly spaced tryptophan residues, which are essential to forming the domain structure. Apart from this N-terminal DNA-binding domain (DBD), however, no significant sequence similarity was found between OpMyb15 and OpMyb24 and other Myb proteins. Therefore, although these oil palm *Myb*-like genes share features of other *Myb* genes,

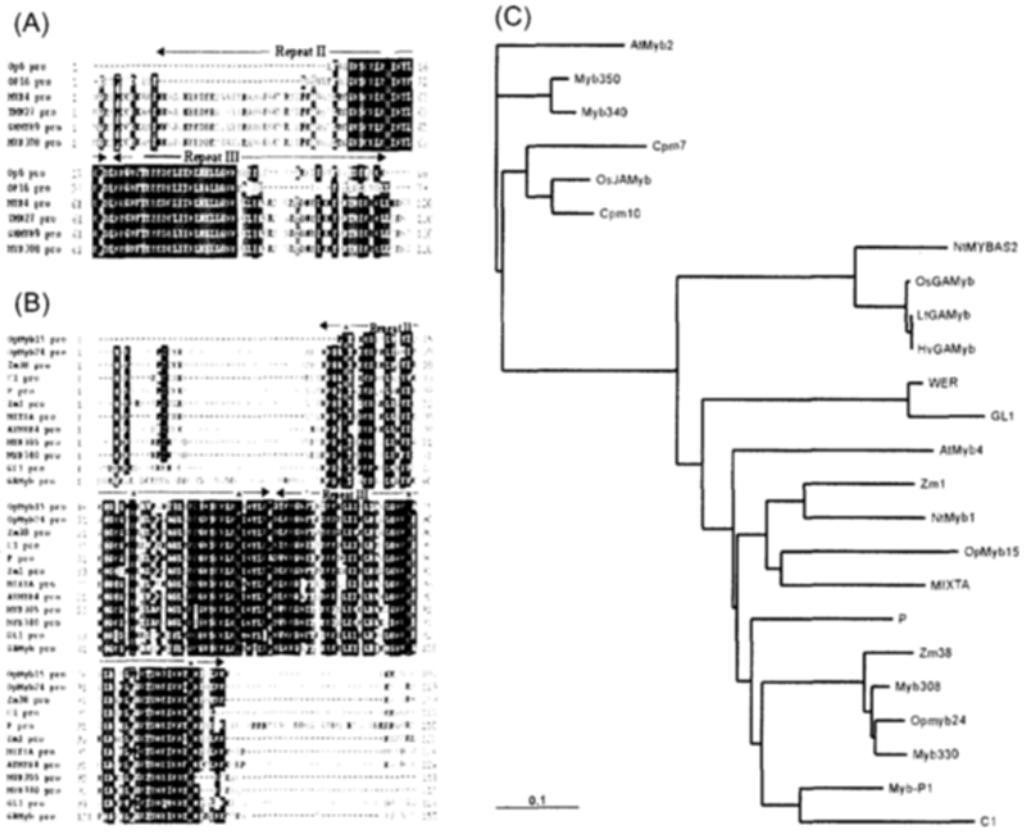

Figure 1. (A) Alignment of the deduced amino acid partial oil palm *Mybs* (Op6 and Op16) and other *Mybs* amino acid sequences, namely, MYB4 (GenBank Accession No. NP_195574), TMH27 (GenBank Accession No. S69189), GHMYB9 (GenBank Accession No. AAK19619), and MYB308 (GenBank Accession No. JQ0960). The third tryptophan residue present in repeat II is labeled with an asterisk (*). The first tryptophan residue present in repeat III of plant MYB proteins replaced by phenylalanine residue is marked with an arrowhead (▼). (B) Comparison of the deduced amino acid sequences of oil palm Mybs (OpMyb15 and OpMyb24) and other Myb-related proteins, namely, Zm38, C1, P, and Zm1 (*Zea mays* 227033, P10290, S26150, and 227032, respectively), MIXTA, MYB305, and MYB340 (*Antirrhinum majus* S45338, JQ0958, and JQ0959, respectively), ATMYB4 and GL1 (*Arabidopsis thaliana* NP 198018 and AAL01244, respectively), GAMYB (*Hordeum vulgare* X87690). Gaps (dashed lines) were inserted to optimize alignment. Sequence identity is boxed and shaded. Asterisks (*) represent conserved tryptophan residues. The first tryptophan residue present in repeat III of plant Myb proteins is replaced by another hydrophobic (phenylalanine, isoleucine, or leucine) residue, and is marked by (▼). (C) Alignment of amino acid sequences of Myb-related proteins. The dendogram was constructed following multiple sequence alignment of the two repeats of reported plant Myb proteins using BioEdit version 5.5 (UPGMA method).

little is known about the functions of their encoded proteins.

In many plant Myb-related proteins, including those from the oil palm, the first tryptophan residue of R3 is replaced by a phenylalanine residue. This substitution by a hydrophobic aromatic residue has no effect on the DNA binding activity of the animal c-MYB (Anton and Frampton, 1988). To illustrate the structural relationship between the two isolated oil palm Mybs and other plant R2R3-Myb factors, we aligned the DBDs to generate a dendrogram of 24 Mybs (Fig. 1C). Here, the two OpMybs were grouped into three distinct clusters. The degree of alignment among these sequences indicated that OpMyb15 and OpMyb24 are more similar to other plant Myb-related proteins than they are to one another. For example, the DBDs of OpMyb24 and snapdragon Myb308, snapdragon Myb330 and maize Zm38, exhibited 94%, 93%, and 90% similarity, respectively, whereas those regions of OpMyb24 and OpMyb15 were only 61% similar. Both the *Myb308* and *Myb330* genes play a role in phenylpropanoid and lignin biosynthesis (Tamagnone et al., 1998) while Zm38, as cloned from a leaf-specific cDNA library, has been implicated in the control of flavonoid production

(Marocco et al., 1989). OpMyb15 showed the greatest level of similarity (70%) to MIXTA of *Antirrhinum*, which controls the development of conical cells or trichomes, depending on the timing of its expression (Glover et al., 1998).

OpMyb24 and *OpMyb15* Expression in Various Tissues

The expression level of *OpMybs* mRNA was examined in nine plant organs, including mature and immature flowers. As our internal control, cyclophilin (*CyP*) was amplified using primer-pair CYS1 and CYAS1 (Gel photo not shown). *OpMyb15* was strongly expressed in 28-cm male flowers and meristems, and, to a lesser extent, in the 1- to 2-cm male flowers, 28-cm female flowers, and mesocarps. Its transcripts were also present, but at relatively lower levels, in the 1- to 2-cm female flowers, roots, three-month-old leaves, and young leaves (Fig. 2A). *OpMyb24* mRNA showed strong hybridization in the meristems, 28-cm male flowers, and 1- to 2-cm male flowers (Fig. 2B), and to a lesser extent in the 1- to 2-cm and the 28-cm female flowers. Its abundance was relatively low in the roots and mesocarp. In contrast to *OpMyb15*, however, transcripts of *OpMyb24* were barely detectable in the young leaves and the three-month-old leaves. Absolute expression levels of both transcripts varied among organ types. As expected, both hybridized to a 1.1-kb transcript. Although the *OpMyb15* sequence lacked the 5′ end, its expected full-length size was 1.1 kb to 1.2 kb, based on the alignment with *Myb*-related genes of other species.

As stated previously, the OpMyb24 protein shows great similarity to *Antirrhinum* Myb308 and Myb330, both of which have increased expression as floral development progresses. This particular expression pattern suggests their involvement in the regulation of processes that occur in mature tissues rather than during earlier floral-organ differentiation. In our study, both *OpMyb15* and *OpMyb24* accumulated to high levels in the mature (28-cm) male flowers. This finding is consistent with that of Shirley (2001), who has reported that expression of the flavonoid biosynthetic genes increases as flower buds mature and color intensifies. The meristem- and organ-identity genes are the two main groups involved in flower development (Coen and Carpenter, 1993). The former are activated to promote inflorescence meristem development, thus inducing plants to flower. The fact that maximum expression of our two oil palm *Myb*-related genes occurred in the meristem and the mature male flowers suggests that these genes may be involved in either mediating a primary developmental event, e.g., floral induction, or regulating the processes taking place in mature flowers.

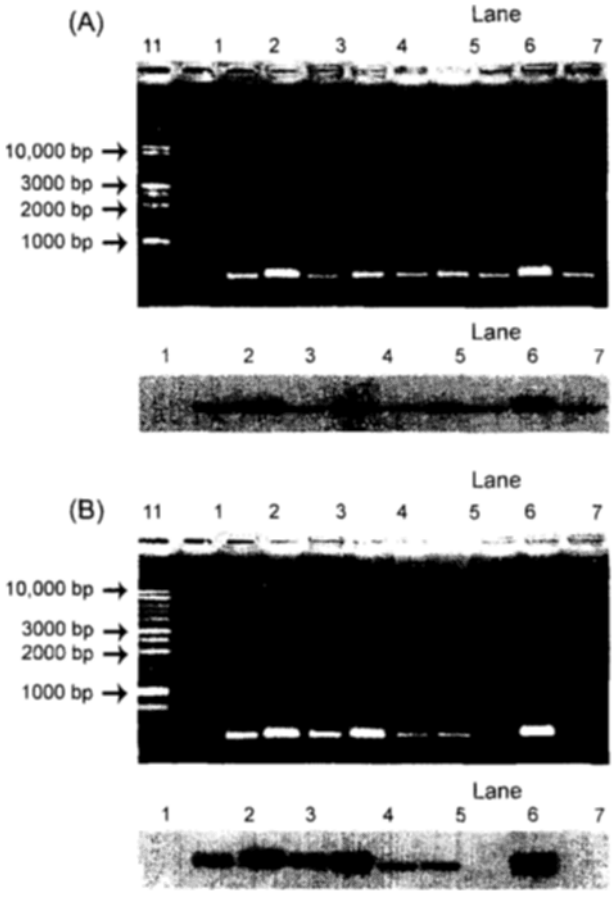

Figure 2. Expression levels of (A) *OpMyb15* and (B) *OpMyb24* in different tissues and confirmation of specificity of semi-quantitative RT-PCR analysis. Lane1, control without cDNA; Lane 2, 28-cm female flowers; Lane 3, 28-cm male flowers; Lane 4, 1- to 2-cm female flowers; Lane 5, 1-to 2-cm male flowers; Lane 6, roots; Lane 7, mesocarps; Lane 8, three-month-old leaves; Lane 9, meristems; Lane 10, young leaves; Lane 11, 1-kb DNA marker.

Expression of *OpMyb24* and *OpMyb15* in Response to Gibberellic Acid

Gibberellins are hormones important to the control of many aspects of plant development, including seed germination, shoot elongation, flower formation and development, fruit setting, seed development, and anthocyanin biosynthesis. Both *OpMyb15* and *OpMyb24* mRNA levels were affected by exogenous application of GA_3. *OpMyb15* transcripts increased in abundance 6 h after GA_3 treatment, then reached a maximum after 18 h, when levels were approximately two-fold higher

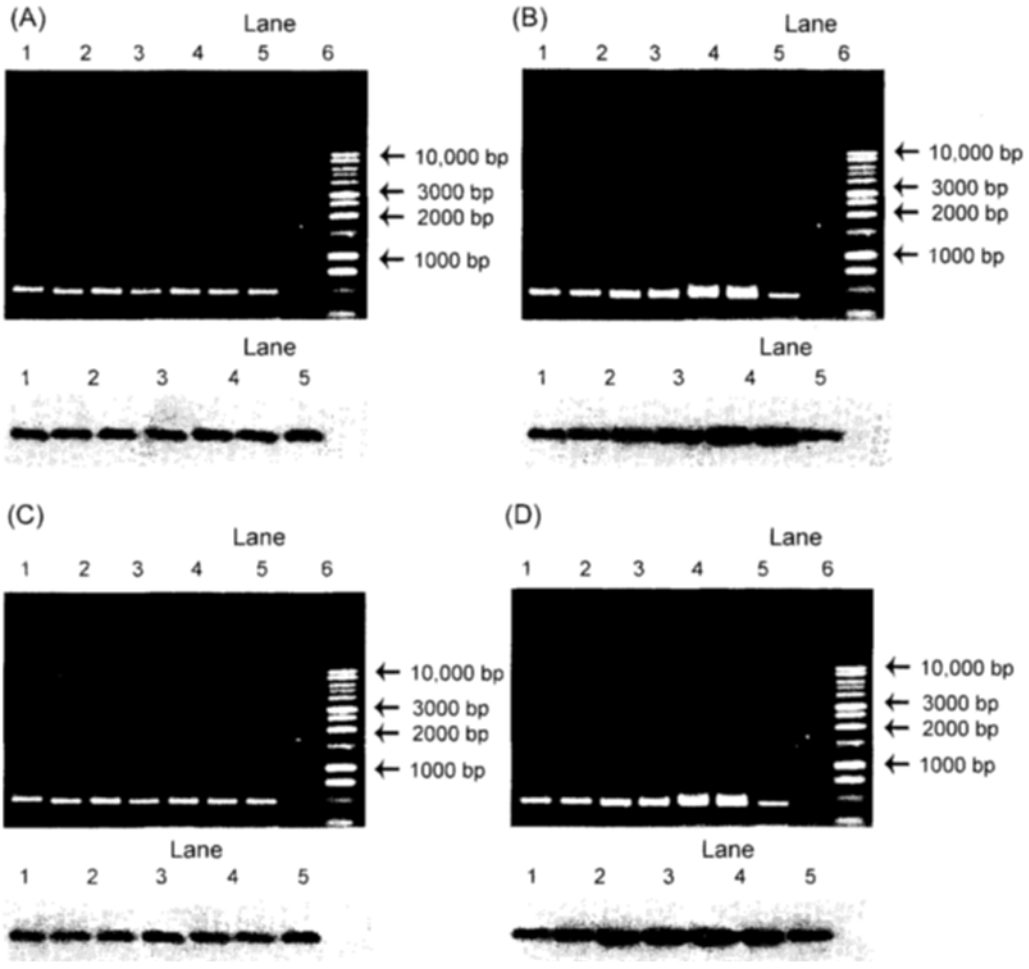

Figure 3. Expression levels of *OpMyb15* in (A) untreated and (B) in-vitro seedlings treated with 100 μM GA_3, and of *OpMyb24* in (C) untreated and (D) in-vitro seedlings treated with 100 μM GA_3, as well as confirmation of specificity of semi-quantitative RT-PCR analysis. Each lane indicates hours after exposure to treatment. Lane1, 0 h; Lane 2, 3 h; Lane 3, 6 h; Lane 4, 12 h; Lane 5, 18 h; Lane 6, 24 h; Lane 7, 48 h; Lane 8, control within cDNA; Lane 9, 1-kb DNA marker.

compared with the untreated control. The *OpMyb15* mRNA then decreased to a basal level 48 h after treatment (Fig. 3). Likewise, mRNA levels of *OpMyb24* rose after 3 h of GA_3 treatment, reaching a maximum at 6 h (Fig. 3), then declining to a basal level after 48 h. GA signaling stimulates the expression of GAMYB, which then induces the α-*Amy1* gene. Gubler et al. (1999) have demonstrated that GAMYB can transactivate the expression of GUS fused to promoter fragments of three other GA-related genes -- an α-*Amy2* gene, an E11 (1-3, 1-4)-β-glucanase gene, and a cathepsin B-like protease gene. This suggests that *OpMyb15* and *OpMyb24* may be involved in GA_3-induced processes even though our maximum increase of mRNA levels was only two fold. Nevertheless, their possible involvement in stimulating the expression of other GA-regulated genes contributes to our understanding of oil palm MYB proteins.

OpMyb24 Expression in Response to Wounding

Wounding by mechanical injury, pathogens, or herbivore attack is one of the most severe environmental stresses, prompting plants to respond by inducing the expression of certain defensive genes. Here, we examined the change in expression of *OpMyb24* in response to wounding. RNA gel blots prepared from one-month-old leaf segments were incubated on premoistened filter paper for 2, 4, 6, or 12 h, then probed with the *OpMyb24* cDNA fragment. Compared with the *CyP* loading control, expression of our gene was of lower abundance. Levels of *OpMyb24* decreased sig-

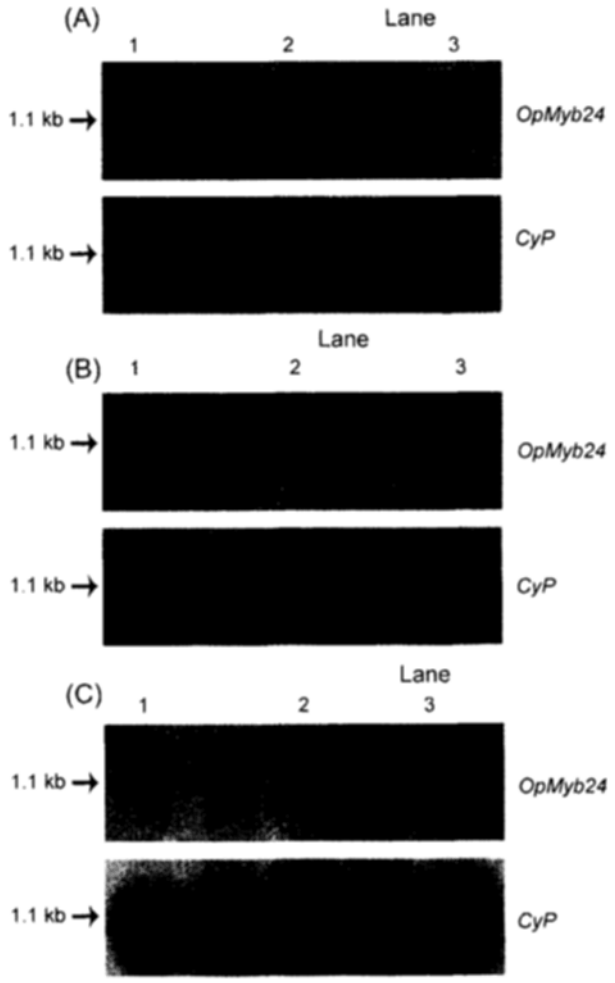

Figure 4. Northern analysis of *OpMyb24* gene in one-month-old oil palm in-vitro seedlings treated for different periods by wounding, $HgCl_2$, or UV light. Autoradiographs of RNA blot containing about 20 µg of total RNA isolated from (A) non-wounded seedlings (Lane 1) or seedlings wounded for 2 h (Lane 2), 4 h (Lane 3), 6 h (Lane 4), or 12 h (Lane 5); (B) untreated seedlings (Lane 1) or seedlings treated with $HgCl_2$ for 0.5 h (Lane 2), 1 h (Lane 3), or 2 h (Lane 4); (C) seedlings exposed to UV light (1200 µJoules x 100) for 10 min (Lane 2), 15 min (Lane 3), 20 min (Lane 4), or 25 min (Lane 5). RNA blots were hybridized with a ^{32}P-labeled *OpMyb24* cDNA fragment, then re-probed with cyclophilin (*CyP*) as a loading control.

nificantly after the leaves were wounded, with a very small amount being accumulated after 4, 6, or 12 h. Moreover, hardly any transcript was detected after 2 h compared with the non-wounded leaves (Fig. 4). In contrast, levels of the *CyP* transcript were not affected by wounding and showed constitutive mRNA amounts over the entire experimental period.

Sugimoto et al. (2000) have reported that, in tobacco protoplasts, wounding induces *NtMYB2* RNA. *NtMYB2* activates expression from a promoter with a 13-bp motif and from a promoter of the phenylalanine ammonia lyase gene (*Pv-PAL2*). Overexpression in transgenic tobacco plants also induces the expression of a tobacco retrotransposon, *Tto1*, as well as a *PAL* gene. Ectopic overexpression of *AtMYB13* can lead to a change in florescence architecture (Kirik et al., 1998). Although in the current study, *OpMyb24* responded to injury, no specific data delineates its exact role in wound signaling. Hence, further analysis of *OpMyb24* will aid in determining the molecular mechanism involved in activating retrotransposons and phenylpropanoid biosynthetic genes in response to stress and, particularly, wounding.

$HgCl_2$ Treatment

When plants and plant cells are exposed to high levels of heavy metals, protein denaturation inhibits further growth. For example, Radtke et al. (1995) have cloned a zinc finger factor (MTF-1) that binds specifically to heavy metal-responsive DNA sequence elements in metallothionein promoters. They have also shown that this transcription factor is essential for basal and heavy metal-induced transcription. However, neither the *Myb* transcription factor nor *PAL* genes, which typically respond to stresses such as UV, wounding, and fungal infection, have been identified as being involved in the defense against heavy-metal stresses. In our analysis of *OpMyb24* gene activity in $HgCl_2$-treated seedlings, its expression pattern was similar to that found with the wounded seedlings. In particular, *OpMyb24* activity did not increase but, rather, was downregulated. No significant fluctuations in mRNA levels were observed in the control experiment (Fig. 4).

In plants, the detoxification of heavy metals involves the use of small Cys-rich ligands called phytochelatins. These ligands, synthesized from GSH, are able to bind heavy metals, and are stored in the vacuoles. When cells are exposed to the metals, phytochelatins are synthesized and the GSH level drops immediately (Zenk, 1996). Thus, the repression of *OpMyb24* by $HgCl_2$ might be an indirect effect mediated either by depletion of the GSH pool or as a consequence of phytochelatin synthesis. In addition, de-repression may be an important mechanism for acclimatizing oil palm to $HgCl_2$. Alternatively, *OpMyb24* may not be involved after all in a biochemical response to $HgCl_2$. Further analysis is needed of plant molecular responses to trace metals.

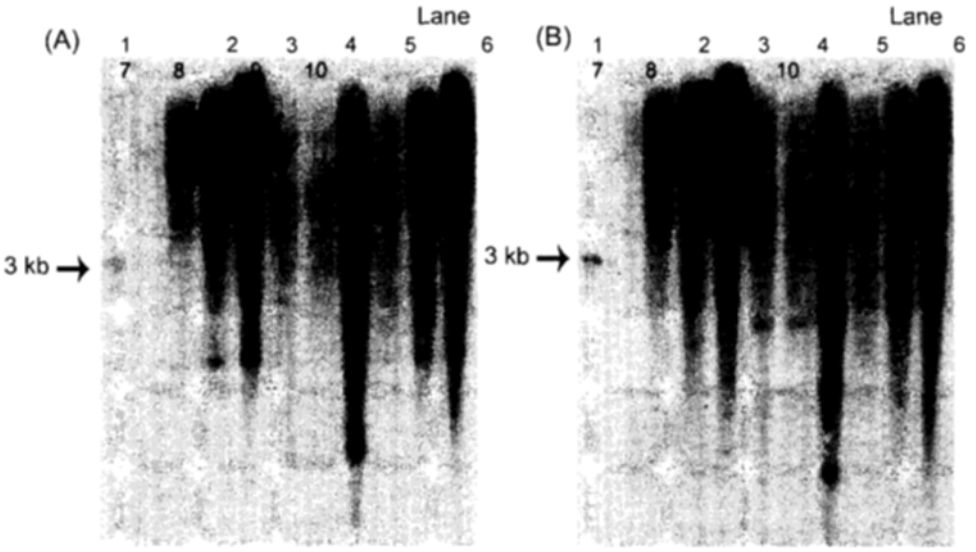

Figure 5. DNA gel blot analysis of the *OpMyb* genes in oil palm genomic DNA. EcoRI was used to digest 20 μg genomic DNA (Lane 2), 40 μg (Lane 3), or 60 μg (Lane 4); BamHI was used to digest 20 μg genomic DNA (Lane 5), 40 μg (Lane 6), or 60 μg (Lane 7); KpnI was used to digest 20 μg genomic DNA (Lane 8), 40 μg (Lane 9), or 60 μg (Lane 10). Blot was hybridized with (A) *OpMyb15* cDNA probe or (B) a full-length *OpMyb24* cDNA probe. Lane 1: 1-kb DNA marker.

UV Treatment

R2R3-type MYB-related proteins may play a role in the response to UV. To examine whether UV light induced expression of the *OpMyb24* gene, mRNA levels were measured after in-vitro seedlings were exposed to various periods of UV light. Cyclophilin was used as a loading control in the quantitative analysis. Although levels of total RNA blotted did not differ among the UV-treated seedlings, the level of RNA from the non-treated seedlings was approximately half the probed amount. For all UV treatments, *OpMyb24* expression was highest in seedlings exposed for 15 min, with mRNA levels decreasing after 20 min, and reaching a minimum after 25 min of exposure (Fig. 4). Bieza and Lois (2001) have reported that an *Arabidopsis* mutant, *uvt1*, is tolerant of lethal doses of UV-B. Therefore, our results may help us in further study of the exact role of UV-absorbing, protective pigments in oil palm, as well as the relative contribution of other mechanisms to the overall tolerance to UV radiation in that species.

Genomic Southern Analysis

At least three strong bands were found in each lane containing 60 μg of the digested genomic DNA that had hybridized to either probe (Fig. 5, A and B). The same results were noted for the 60 μg of genomic DNA digested with BamHI and KpnI (Fig. 5B). No clear differences were seen between the lanes containing either 20 or 40 μg of BamHI-digested DNA. In addition, when 60 μg of DNA was digested with EcoRI, i.e., an enzyme that cuts within the *OpMyb15* cDNA sequence, three prominent and two fainter bands were produced (Fig. 5A).

Because our two probes (*OpMyb15* and *OpMyb24*) included the conserved R2/R3 repeat region of an *Myb*-related gene, a large number of hybridizing loci were detected. However, no internal restriction site existed except for the EcoRI site within *OpMyb15*. Our results may roughly estimate the actual number of genes, which suggests that both *OpMyb15* and *OpMyb24* are encoded by a small gene family.

CONCLUSION

We have isolated a full-length cDNA clone (*OpMyb24*) and a partial cDNA clone (*OpMyb15*) that encode two distinct *Myb*-related genes. The structural similarity between our Myb proteins (deduced from oil palm) and those of other plant species includes a typical R2/R3 repeat and a tryptophan hydrophobic core. OpMyb15 and OpMyb24 are also more similar to other plant Myb-related proteins than to each other. Results of semi-quantitative RT-PCR revealed that the 1.1-kb *OpMyb15* and *OpMyb24* genes were expressed in every tissue

type, although the latter was barely detectable in three-month-old leaves and young leaves at the PCR cycle chosen. Transcripts of both genes were most abundant in the mature male flowers and in meristems. When in-vitro seedlings were treated with GA_3, transcript levels of both *OpMyb* genes increased approximately two fold. This suggests that *OpMybs* may be involved either in GA_3-induced processes or in mediating GA signaling during growth and flowering responses. Its potential regulatory role will contribute to understanding the function of the oil palm Myb proteins.

We prepared RNA gel blots to determine the expression levels of *OpMyb24* gene in response to UV radiation, mechanical wounding, or $HgCl_2$ treatment. Compared with untreated seedlings, the mRNA level of *OpMyb24* was dramatically higher after 15 min of exposure to UV light. This finding may provide us with an important tool for better understanding the protective role of UV-absorbing pigments and other tolerance mechanisms in the oil palm. In our wounded or $HgCl_2$-treated seedlings, *OpMyb24* expression decreased significantly or was hardly detectable compared with the controls. This may indicate that de-repression is an important mechanism for acclimation of oil palm to injuries and heavy metals. Finally, further characterization by Southern analysis demonstrated that *OpMyb15* and *OpMyb24* exist as a small gene family.

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