Isolation and characterization of oil palm constitutive promoter derived from ubiquitin extension protein (uep1) gene

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The ubiquitin extension protein (uep1) gene was identified as a constitutively expressed gene in oil palm. We have isolated and characterized the 5' region of the oil palm uep1 gene, which contains an 828 bp sequence upstream of the uep1 translational start site. Construction of a pUEP1 transformation vector, which contains gusA reporter gene under the control of uep1 promoter, was carried out for functional analysis of the promoter through transient expression studies. It was found that the 5' region of uep1 functions as a constitutive promoter in oil palm and could drive GUS expression in all tissues tested, including embryogenic calli, embryoid, immature embryo, young leaflet from mature palm, green leaf, mesocarp and meristematic tissues (shoot tip). This promoter could also be used in dicot systems as it was demonstrated to be capable of driving gusA gene expression in tobacco.

Introduction
Oil palm has been identified as a renewable factory for the large-scale production of plant oil-derived chemicals in the future [1]. Introduction of useful genes through genetic engineering will enhance the oil palm yield and increase its agronomic traits. Constitutive expression of transgenes in the whole plant is required for certain traits such as vaccine and polymer production [2,3], disease resistance plant [4,5], tolerance to abiotic stresses [6,7] and herbicide and antibiotic resistance [8]. To meet the above requirement, the use of a strong constitutive promoter capable of driving a high expression of transgenes in most tissues is essential. The promoter is very important for producing the above traits as well as providing a good understanding toward regulation of transgene in transgenic plant.

A prominent example of a strong promoter that is commonly used for directing constitutive expression in transgenic plants is the CaMV35S promoter, which originated from the cauliflower mosaic virus [9]. Another promoter that is commonly used to drive a high transgene expression in monocots is maize polyubiquitin promoter. To date, the polyubiquitin promoters have been isolated from several monocot and dicot plants such as sunflower [10], tobacco [11], rice [12] and maize [13]. Many studies have indicated that the constitutive nature of ubiquitin genes accounts for the ability of their promoters to constitutively drive reporter gene expression in transformed cells and plants. The constitutive status of these genes is due to the presence of important sequences or motifs in the promoter region. In the maize ubi1 promoter region, two overlapping heat shock sequences were found at positions −214 and −204. The promoter did not contain GC boxes, but the sequence 5'-CACGGCA-3' (function unknown) occurred 4 times, at positions −236, −122, −96 and −91 [13]. Additionally, most studies indicated that constitutive promoters might contain multiple cis-acting elements, each of which interacts individually with cell- or tissue-specific trans-acting factors [14,15]. The combined activities of individual cis-acting elements confer constitutive gene expression in most tissue types. Furthermore, this synergy of multiple cis-acting elements has been found in CaMV35S [14] and rice actin 1 [15] constitutive promoters.

A promoter derived from another ubiquitin family, the ubiquitin extension protein (uep) gene, has been isolated from yeast [16] and several plants, including maize [17], tomato [18], barley [19],
followed by immediate cooling. Denatured total RNA was separated on a nylon membrane (Hybond N+ Amersham) was carried out using standard techniques [22].

Blotting experiments demonstrated that β-glucuronidase (GUS) expression driven by these promoters was constitutive in transgenic tobacco [21]. Similar results have also been acquired in potato [20]. However, the efficiency of promoters derived from this class of ubiquitin gene has not been tested in monocot systems. In the present study, an oil palm ubiquitin extension protein gene was constitutively expressed in all oil palm tissues tested. To further investigate the constitutive nature of the ubiquitin gene, its 5′ region was isolated and characterized. These outcomes of this work will facilitate genetic engineering program of oil palm. Additionally, such promoters may prove effective for the production of transgenic plants with agronomically beneficial traits in other monocot and dicot systems.

**Materials and methods**

**Reverse northern analyses**

Reverse northern analyses were performed according to the manufacturer’s instructions (Bio-Dot™ Microfiltration Apparatus, BIO-RAD). Wells of dot blots were rinsed with 300 μl 2× SSC (3 M NaCl, 300 mM tri-sodium citrate, pH 7.0). About 200 μg of PCR derived fragment was added to 0.4 N NaOH, then denatured by boiling for 10 min and immediately chilled on ice. About 100 μl of the prepared ampiclons was dot-botted onto a nylon membrane and asphered through the membrane under a vacuum. The wells were then rinsed twice with 300 μl 2× SSC, aspirated through the manifold under a vacuum, and briefly air-dried. The membrane was UV cross-linked and probed with [α-32P] cDNA. The cDNA was prepared from 3 μg of total RNA from oil palm tissues using the First Strand cDNA Synthesis Kit (manufacturer’s instructions (Bio-Dot™ Microfiltration Apparatus, BIO-RAD)).

**DNA sequencing for clone verification**

Plasmid DNA was prepared using the Plasmid Mini Preparation Kit (QIAGEN), according to the manufacturer’s protocol. Representative clones were sequenced using an automated DNA sequencer (ABI PRISM Model 377 Version 3.4), and DNA sequences were analyzed using VectorNTI software (Invitrogen). Following the removal of unreadable and vector sequences, the analysis was carried out to examine sequence alignment, ORF identification and contig analysis and assembly. DNA and protein homology searches against GenBank databases were performed using BLAST 2.0 [23].

**Construction of transformation vector**

The uep1 coding region, starting from the translation start site, was removed from the fragment to avoid undesired translation initiation from the uep1 gene, as well as to ensure that gusA expression is initiated using its own translational start codon. The promoter and its 5′ untranslated region were amplified from the plasmid pGWUEP1. The amplification also introduced a Spel site at the 5′ end and an XbaI site at the 3′ end. Purified PCR products were then ligated into pCRII-topo vector (TOPO TA Cloning Kit, Invitrogen Life Technologies) for further manipulation.

Four blunt-end digestions were performed using DraI, EcoRV, PruII and StrI. The digested DNA was purified and ligated to Genome Walker Adaptors. About 100 ng of each DNA genomic library was used as the DNA template for primary PCR reaction. PCR amplification was performed in reaction mixture containing 1× Advantage 2 PCR reaction buffer, 0.2 μl of each dNTP, 200 μM of each primer, and 0.5–1 unit Advantage 2 Polymerase Mix. The amplification was performed in 7 cycles of 94°C; 25 s, 72°C; 3 min, and followed by 32 cycles of 94°C; 25 s, 67°C; 3 min and 67°C; 7 min.

**Cloning the DNA fragment**

Purified PCR product was ligated into pCRII-topo vector (TOPO TA Cloning Kit, Invitrogen Life Technologies) for further manipulation. Ligation reactions consisted of 3 μl (about 30 ng) of purified PCR product, 1 μl of salt solution (1.2 M NaCl, 0.06 M MgCl2), and 1 μl (10 ng) of vector plasmid. Sterile water was added to a final volume of 6 μl. The mixture was incubated at room temperature for 5–10 min and then mobilized into One Shot® Chemically Competent E. coli, according to the manufacturer’s protocol.

**Northern analyses**

About 15 μg of total RNA from various oil palm tissues were denatured in RNA loading buffer (48% formamide, 6.4% formaldehyde, 1× MOPS buffer, 5.3% glycerol and 0.02% Bromophenol blue). The mixture was denatured by heating for 10 min at 65°C followed by immediate cooling. Denatured total RNA was separated on a 1% formaldehyde gel using 1× MOPS Buffer (20 mM Morpholinopropanesulfonic acid, 5 mM sodium acetate and 1 mM Na2EDTA pH 7.0) as the electrophoresis buffer. Transfer of RNA to a nylon membrane (Hybond N+ Amersham) was carried out using the capillary transfer method. The membrane was UV cross-linked and probed with [α-32P] DNA probe. The DNA was radiolabeled with [α-32P] according to the Megaprime™ DNA Labeling System manual (Amersham Life Science). Prehybridization and hybridization were carried out using standard techniques [22]. Blots were exposed to Kodak XAR-5 film for 12 hours to 3 days.

**Construction of transformation vector**

The uep1 coding region, starting from the translation start site, was removed from the fragment to avoid undesired translation initiation from the uep1 gene, as well as to ensure that gusA expression is initiated using its own translational start codon. The promoter and its 5′ untranslated region were amplified from the plasmid pGWUEP1. The amplification also introduced a Spel site at the 5′ end and an XbaI site at the 3′ end. Purified PCR products were then ligated into pCRII-topo vector (TOPO TA Cloning Kit, Invitrogen Life Technologies) to form pGWUEP2. pBI221 and uep1 promoter fragment (in pGWUEP2) were first digested with Spel and XbaI. Digestions were carried out in 100 μl reaction mixtures containing 20 μl DNA, 1× buffer and 5 μl (10 units) of each restriction enzyme and incubated overnight at 37°C. The mixtures were analyzed by agarose gel electrophoresis, and the fragments
were purified using the QIAquick Gel Extraction Kit (QIAGEN). The ligation reaction was carried out using 1 μl (10 ng) of purified pBI221 vector plasmid, 1× T4 ligation buffer, 1 unit of T4 ligase and 5 μl (50 ng) of purified DNA insert. Sterile water was added to a final volume of 20 μl, and the mixture was incubated overnight at 16°C. The mixture was then transformed into One Shot® Chemically Competent E. coli competent cells, according to the manufacturer’s protocol, and screened using restriction analysis. The resulted clone was designated as pUEP1. The construction of pUEP1 using pGWUEP2 is illustrated in Fig. 1.

Preparation of target materials for transformation

Oil palm tissues such as embryogenic calli, embryoid, immature embryo, young leaflet from mature palm, green leaves, stem, mesocarp nine weeks after anthesis (WAA) and tobacco green leaves were cultured on agar solidified medium containing Murashige and Skoog (MS) macro- and micronutrient supplemented with 1 mg/l naphthaleneacetic acid (NAA) and 30 g/l sucrose. Mesocarp tissues were sterilized in 20% bleach for 20 min and rinsed 3 times with sterile distilled water before being cultured. All explants except for embryoid were cut into 5 mm × 5 mm disks before

![Diagrammatical representation of the construction of pUEP1. The pGWUEP1 was digested with SphI and XbaI and uep1 promoter was cloned into pBI221 by replacing the CaMV35S to generate pUEP1. The arrows indicate the orientation of each DNA fragment assembled.](image-url)
being placed onto MS medium. All tissues were incubated in the dark for 24 hours at 28°C before bombardment.

**Bombardment of oil palm tissues**

Particle bombardment was conducted using the Bio-Rad PDS-1000 He biolistic particle delivery system (Bio-Rad, Hercules, CA, USA). To each aliquot of 100 μl of gold particles, 20 μg of DNA, 100 μl of 2.5 M CaCl₂, and 40 μl of 0.1 M spermidine were added sequentially, with continuous vortexing. Vortexing was continued for 3 min, followed by centrifugation at 10,000 rpm for 10 s. The supernatant was removed and the particles were washed twice with 500 μl of 100% ethanol, followed by centrifugation at 10,000 rpm for 60 s. Finally, DNA-coated gold particles were resuspended in 120 μl of absolute ethanol. For each bombardment, 6 μl of DNA-coated gold particles was dispensed onto the center of a macrocarrier and dried under sterile conditions. Target tissues were placed in the center of a petri dish containing agar. Transformation was carried out using the following parameters: bombardment pressure at 1100 psi; macrocarrier to stopping screen distance at 6 mm; target plate distance to stopping screen at 6 cm; chamber vacuum at 26 mmHg [20]. For oil palm green leaf and mesocarp, the tissues were bombarded at 1350 and 1550 psi and 4.5 and 7.5 cm distances between stopping plate to target tissues, respectively. Other parameters used were as optimized by Parveez (G.K.A. Parveez, Optimization of parameters involved in transformation of oil palm using the biolistic method, PhD thesis, Universiti Putra Malaysia, 1998). The bombarded tissues were then incubated in the dark for 48 hours at 28°C before GUS histochemical analysis.

For the experiments to evaluate the effect of auxin and ABA phytohormones on **uep1** promoter activity, auxin treatment was carried out by culturing the bombarded oil palm embryoid onto MS media supplemented with 5 mg/l NAA for 48 hours. For ABA response, the bombarded oil palm embryoids were placed on MS medium supplemented with different contents of ABA (0, 4 and 8 mg/l) for two days. The analysis was conducted with four replicates to increase its accuracy. The data were statistically analyzed using Duncan Multiple Range Test (DMRT).

**GUS histochemical assay**

GUS assay buffer (0.1 m NaPO₄ buffer pH 7.0, 0.5 mM K-ferricyanide, 0.5 mM K-ferrocyanide, 0.01 mM EDTA, 1 mg/ml X-gluc (5-Bromo-4-Chloro-3-Indolyl-β-D-glucuronide), 1 μl/ml Triton-X and 20% methanol (v/v)) [24] was filter-sterilized and stored at −20°C in the dark. Two days after bombardment, tissues were stained overnight (16 hours) at 37°C with GUS buffer. For green tissues, samples were subsequently soaked in 70% ethanol and incubated at 37°C for one hour. This procedure was repeated 5 times or until the plant tissues became light green or clear. The removal of chlorophyll improved the scoring of the blue staining of the plant tissues. Blue spots were scored optically using a Nikon UFX-DX microscope system.

**Results and discussion**

**Identification of a constitutively expressing gene from oil palm**

Reverse northern analysis was used to examine the expression pattern of 73 EST clones that were generated through a microarray.
approach (Fig. 2). The EST clones used in this study were provided by the Genomic Group of Malaysian Palm Oil Board (MPOB). These clones were shown to be expressed in all tissues tested, including inflorescence f18 (18 weeks after anthesis), embryoid and callus. This observation was a good indicator that these EST clones could encode constitutive genes in oil palm. However, further analyses were needed to confirm that they were expressed in other tissues.

In this study, the cDNA clone pOP-SFB1301, which encodes the ubiquitin extension protein gene uep1, was strongly expressed in all tissues tested. The analysis was carried out by normalizing the intensities of cDNA expression to the intensity of ribosomal DNA. The lowest value of cDNA expression obtained from the normalization in any specific tissue was set as 1-fold. The result indicated that the uep1 gene was expressed in all tissues tested with a range of 1.0–1.8-fold. The highest expression was detected in mesocarp at week 5 (Fig. 3). Detailed sequence analysis showed that uep1 encodes a polyprotein consisting of 76 amino acid residues of ubiquitin fused to a C-terminal extension. The extension was predicted to be 80 residues and identified as a small subunit ribosomal protein. Comparison of the pOP-SFB1301 cDNA sequence to entries in GenBank revealed that this gene had homologs in Arabidopsis, potato, barley, tomato, human and rat, and that the domains are highly conserved among plant and nonplant species.

In general, a ribosomal protein is any protein that conjugates with ribosomal RNA (rRNA) to make up the ribosomal subunits. In eukaryotes, the 40S subunit consists of 33 ribosomal proteins and an 18S rRNA. The assembly of rRNAs and ribosomal proteins to form 40S occurs within the nucleolus, a region of the nucleus specialized for this purpose [25]. Ubiquitin extension protein acts as ‘molecular chaperone’ that helps incorporate ribosomal proteins into the nascent ribosome [26]. In yeast, deleting the ubiquitin coding region from the ubiquitin extension protein gene resulted in phenotype deficiencies such as slow growth, abnormal RNA processing, and correspondingly low levels of 40S ribosomal subunits [26]. Although it is clear that uep1 is coding for a ribosomal protein involved in ribosome assembly, the precise function and mechanism of uep1 action are still unknown.

Northern analyses were utilized to study the transcript size and regulation of oil palm uep1. A transcript with a size of about 0.8 kb was detected in total RNA hybridization of various oil palm tissues including mesocarps, kernels, frond, young leaf, embryoids, root, flower and stem. The RNA transcript was found to be most abundant in young leaf, flower, root, stem and embryoids. High level of transcript was also detected in early stages of mesocarp development at 5–9 WAA. The level was slightly decreased at week 15–19 (Fig. 4). This slight variation could be caused by mRNA accumulation in the different stages of tissue development. A previous study showed that the gene is highly expressed in young tissues or tissues containing rapidly dividing cells than in more mature tissues [21]. This accumulation is required for active protein synthesis during the early stages of plant development. This pattern of expression has also been observed in tomato [18], barley [19], potato [20] and Arabidopsis [21]. However, uep1 expression in green mature leaves was slightly lower than that observed in young leaves. In potato, although the expression of GUS driven by an ubiquitin extension protein promoter was relatively low in mature leaves, the transcripts were increased in senescence leaves. This suggested that UEP may be involved in the synthesis of proteins required for senescence, or alternatively, that UEP may be required to supply sufficient ubiquitin for protein degradation [20,27]. Surprisingly, in kernels, both northern and reverse northern analyses revealed a low level of uep1 transcript in all stages tested. The low expression level of uep1 could be due to the lack of cellular division in kernels. However, further analyses must be carried out to investigate the authentic role of this gene in kernel development.

Although the uep1 levels varied slightly between different tissues, this molecular analysis verified the constitutive status of the uep1 gene. The results obtained from northern analyses also concurred with the reverse northern analyses. These results also suggested that expression of this gene is very important throughout the plant life cycle, coinciding with its role in ribosomal biogenesis.

**Isolation of a constitutive promoter from oil palm**

Genome walking procedure was employed to amplify the uep1 promoter region. A PCR product of about 1.1 kb, which included part of the uep1 coding region, was obtained. The DNA sequence of the fragment was analyzed for the identification of the promoter region. Based on the analyses (NCBI, Softberry, PlantCare, PLACE
DNA sequence and map of the oil palm upl1 genomic clone. The sequence is numbered from the 5’ PvuII site. The UPL1 coding region begins at residue 829 and extends to residue 1296. The predicted protein sequence is shown extending to the first stop codon 5’ to the initiating codon, methionine (M). Position of putative transcription start site (A) is indicated with large and bold font. The putative TATA box, CAAT box and other putative cis-elements are underlined and labeled.
databases and VNTI software), the 828 bp sequence upstream of the translational start site was identified as part of uep1 promoter. The region includes the $S'$ untranslated region of the gene. The putative transcription start site was predicted 100 nucleotides upstream of the translational initiation site (Fig. 5). A potential TATA box sequence was identified 30 bp upstream of the transcription start site. Additionally, computer analysis was used to identify other features in the gene architecture that could contribute to uep1 expression (Fig. 5). One of the important motifs identified was a sequence that closely matched the consensus sequence of upstream activation sites (UAS) in yeast ribosomal protein genes. This sequence function is to promote transcription [26] and was reported to be present in uep1 counterparts from other plants including barley, maize and tomato. However, such UAS sites have also been found within a few genes encoding nonribosomal protein [28]. The uep1 promoter region also contains T-rich stretches that represent a second sequence motif characteristic of ribosomal protein gene promoters [26] (Fig. 4). T-rich motifs contribute to the high transcriptional yields of various ribosomal protein gene promoters [29,30]. These TC-rich sequences were also found in yeast [16] and barley [19] ubiquitin extension protein promoters.

The putative promoter sequences were also analyzed using plant databases to find other important cis-acting regulatory elements. Examination of the nucleotide sequences upstream of the uep1 gene revealed that it contains multiple motifs as shown in Fig. 5. It was observed that oil palm uep1 promoter contains sequences associated with light-responsive elements (LRE) including GATA box and GT-1 like elements. The LRE motifs are highly conserved in photoregulated and generally required for high level, light-regulated and tissue-specific expression [31].

In addition, uep1 promoter also contains other interesting motifs such as ethylene- (ERE), abscisic acid- (ABRE), auxin- (AuXRE) and water stress-responsive elements (MYB). This indicated that UEP has a pivotal role in multiple hormonal signaling pathways and can be activated by both abiotic and some physical stresses. Garbarino and Belknap [20] reported that the ubiquitin extension protein gene in potato tubers was strongly induced by wounding [20]. The production of stress ethylene in wounded tissues resulted in a large increase in metabolic activity [32], which has been known to stimulate the biosynthesis of new ribosomal components [20,33]. This promoter also contains other elements that confer tissue- and cell-specific expression. Sequence analysis does demonstrate the presence of motifs similar to root-specific element, guard cell-specific expression and pollen-specific cis-acting elements. Another interesting element is AT-1 motif, which has also been found in barley, tomato and maize ubiquitin extension protein promoters, as well as photoregulated genes, nodulin- encoding genes and seed-protein encoding genes [17].

In conclusion, the data presented clearly indicate that the oil palm uep1 promoter is controlled by multiple cis-acting regulatory elements or motifs that confer constitutive expression. Studies on the regulation of the nominally constitutive CaMV35S promoter in transgenic plants have shown that constitutive promoters might contain multiple cis-acting elements, each of which interacts individually with cell- or tissue-specific trans-acting factors. The combined activities of individual cis-acting elements confer constitutive gene expression in most tissues [34]. Therefore, it is possible that the presence of multiple regulatory elements may enable uep1 expression to be maintained under various light influences, or if the abundance of specific trans-acting factors varies during development. Moreover, such a reiteration of genetic information may enable the gene to compensate for changing environmental conditions and developmental cues [35].

Evaluation of promoter activity

The activity of oil palm uep1 promoter was evaluated through transient expression study by bombarding the pUPE1 vector into different oil palm target tissues. For comparison, the oil palm tissues were also bombarded with pAH25 that carries gusA and bar genes driven by maize polyubiquitin promoter and the original pBI221 plasmid DNA that carries the gusA gene driven by CaMV3S promoter. pAH25 was used as the positive control as maize polyubiquitin promoter has been extensively and successfully used to express chimeric genes in monocot transformation studies [36]. More importantly, the promoter is capable of driving high expression of GUS reporter gene in oil palm tissues [37]. By contrast, CaMV35S promoter was also used as positive control as it is capable of driving expression of transgenes in monocot and dicot plant systems [38]. The constructs were bombarded in oil palm tissues and tobacco green leaf. These tissues were also bombarded with gold particles without DNA as an additional control. The GUS expression was detected in all tissues tested as indicated by the presence of blue spots. No GUS expression was detected in oil palm tissues bombarded with gold particles. This observation clearly indicated that the blue spots observed were due to introduced genes. GUS activity was determined by counting the GUS-positive spots optically. Each blue spot detected, whether in a single cell or a group of cell was considered as one expression unit as defined by Klein et al. [39]. The data collected were then summarized in the form of mean comparison and standard deviation as shown in Table 1. Histochemical GUS assay indicated that uep1 promoter was capable of driving GUS expression in all tissues tested including young leaflet

### Table 1: Comparison of promoter strength on transient gusA gene expression and GUS activity in oil palm tissues two days after bombardment

<table>
<thead>
<tr>
<th>Promoter/construct</th>
<th>Mean (standard error) of GUS foci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YMLP</td>
</tr>
<tr>
<td>Ubi1/pAH25</td>
<td>9166.6 ± 119.2</td>
</tr>
<tr>
<td>CaMV35S/pBI221</td>
<td>3504.2 ± 133.3</td>
</tr>
<tr>
<td>uep1/pUEP1</td>
<td>1812.0 ± 75.7</td>
</tr>
</tbody>
</table>

YMLP, young leaflet from mature palm; EC, embryogenic calli; EM, embryoid; ST, shoot tip (meristematic tissues); MS, mesocarp; TB, tobacco; IE, embryogenic calli.
FIGURE 6
Photographic representations of the comparison of transient histochemical assay in various oil palm tissues and tobacco bombarded with plasmid carrying gusA gene driven by different promoters. (a) None (bombarded without plasmid DNA), (b) pAHC25 (Ubi1), (c) pBI221 (CaMV35S) and (d) pUEP1 (uep1).
from mature palm, embryoid, green leaves (from plantlet), stem (from plantlet) and mesocarp (Fig. 6). The **uep1** promoter was also capable of driving *gusA* expression in all cross-section (vertically) part of immature embryo including the area containing meristemic tissue.

Overall, these experiments indicated that in oil palm, except for shoot tip, the highest GUS expression was obtained in tissues bombarded with constructs driven by maize *ubi1*, followed by the CaMV35S and *uep1* promoters. This result concurred with Chowdhury *et al.* [37], who reported that the activity of maize *ubi1* was superior to CaMV35S in all oil palm immature embryo, young leaf and embryogenic calli. Moreover, Callis *et al.* [21] found that expression of *Arabidopsis* ubiquitin extension protein (UBQ1 and UBQ6) promoters were also slightly lower than that of CaMV35S.

The activity of oil palm *uep1* promoter was also examined in a dicot system by bombarding this promoter construct into tobacco green leaves tissue (Fig. 6). As expected, high GUS expression was observed in tissues bombarded with CaMV35S promoter. This could be because the CaMV35S promoter is more effective in dicots than monocots. Interestingly, the *uep1* promoter could drive GUS expression in tobacco indicating that this promoter could also be used in dicot system. The activity of oil palm *uep1* was similar to *ubi1* promoter in terms of the number of blue spots.

Generally, results showed that the strength of oil palm *uep1* promoter is slightly lower than the other promoters used, particularly to pAHCS5. The differences in the promoter activities could be due to the presence of an intron region located adjacent to the maize *ubi1* promoter. Numerous studies have shown that the high expression capacity of constitutive promoters in monocots is usually caused by the presence of an intron located in the 5’ untranslated region [40]. It has been suggested that the 5’ intron may be required in *in vivo* for efficient mRNA splicing [41]. However, *uep1* does not contain a native intron for this purpose. Therefore, it could be suggested that the relatively low strength of the *uep1* promoter may be due to the lack of intron in the *uep1* gene, particularly in the pUEP1 transformation vector. Sivamani and Qu [42] reported that when the rice polyubiquitin intron was placed behind the rice *Act1* promoter (without its own 5’ UTR), the promoter activity was enhanced by 8–9-fold. Thus, the efficiency of the *uep1* promoter could potentially be increased by the insertion of an intron into the construct.

The activity of the *uep1* promoter could also be significantly enhanced if the *gusA* coding sequence was fused in-frame to its ubiquitin monomer coding sequence. This strategy slightly increases the activity of ubiquitin promoters in both dicots and monocots. It has been reported that, when fused to a 76-amino-acid ubiquitin monomer sequence, potato *uep*-driven GUS expression was 5–10 times higher than constructs that did not contain fused ubiquitin monomer [20]. Moreover, the activity of a rice polyubiquitin promoter was significantly enhanced when the reporter gene was fused to both its 5’UTR and a nine-amino-acid coding region of ubiquitin monomer [42]. The inclusion of the ubiquitin monomer coding region is thought to increase either transcription of the reporter gene or message stability [20]. The ubiquitin moiety is subsequently removed by ubiquitin-C-terminal hydrolases or de-ubiquitinat-

**Activity of the** *uep1* **promoter in response to exogenous auxin and abscisic acid**

Auxin and ABA phytohormones play a pivotal role in many physiological and developmental processes in plants. As *uep1* consists of cis-acting elements associated with auxin (AuXRE) located at −580 and abscisic acid (−ABRE) at −604 upstream of the promoter region, further study was carried out to investigate whether or not the promoter transient activity could be elevated by the hormone induction. An increased level of *gusA* expression was observed in both treatments, indicating an induction of promoter activity by the hormones (Fig. 7). However, the increment of *gusA* activity by hormone treatments was not significantly different (Fig. 6). This could be because the TGTCTC auxin response (AuxRE) and ABRE responsive elements were only present in a single copy in oil palm *uep1* promoter region. Therefore, it is possibly not sufficient for increasing auxin- or ABA-mediated induction of *gus* transcription. The GH3 auxin-regulated genes were found to consist of at least three auxin response elements (AuxREs) which function independently to one another to confer auxin inducibility [45]. Studies have also indicated that to stimulate ABA responsiveness, a minimal ABA-responsive complex (ABRC) which contains multiple ABREs or the combination of

**FIGURE 7**

GUS activity induced by (a) auxin and (b) abscisic acid in oil palm embryoid bombarded with pUEP1 construct. Means with the same letter are not significantly different at *P* < 0.05 according to Duncan’s Multiple Range Test. Bars represent standard error.
an ABRE with coupling element (CE) was necessary in minimal promoter region [46,47]. By contrast, the nonsignificant difference in transient activity after hormone treatment may also be due to short exposure duration. Testing the hormone effects on stably transformed model plants, such as Arabidopsis or tobacco may be more practical and useful.

Conclusion
In this study, uep1 promoter was capable of directing the expression of a readily detectable level of GUS in all tissues tested. The result demonstrated its role as a constitutive promoter in oil palm. This study has shown that the endogenous uep1 promoter could be used as a crucial biotechnology tool for producing transgenic oil palm, whereby constitutive and high level expression of transgenes may be required. Although the strength of the promoter was relatively lower than positive controls, modification of uep1 promoter by the addition of intron and ubiquitin monomer could potentially increase the strength of the oil palm uep1 promoter.

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