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TISSUE-SPECIFIC PROMOTERS: THE IMPORTANCE AND POTENTIAL APPLICATION FOR GENETIC ENGINEERING IN OIL PALM

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ABSTRACT

Oil palm is the most prolific oil crop in the world with a productive life span of 20-30 years and this perenniality bestows significant advantages over other oil crops. However, the industry still faces a number of challenges and to ensure its future sustainability, efforts must be made to diversify applications to increase its economic value. Amongst potential strategies include the use of genetic engineering approaches to fulfil the needs. To ensure that expression of transgenes for the production of genetically engineered products is directed to targeted tissue(s), promoter sequences that are responsible to direct the expression of desired genes have been identified. In this review we discuss the isolation and characterisation of oil palm tissue-specific promoters from mesocarp and kernel, an inducible tissue-specific promoter from roots, and the utility of constitutive promoters. The tissue-specific and constitutive functions of these promoters were confirmed through transient expression studies in oil palm and some of the isolated promoters were further characterised using Arabidopsis *as a model system. We hope that these promoters can potentially be utilised to improve oil yield and quality, to fine tune the agronomic traits, and to generate high value-added products for the oil palm.*

Keywords: oil palm, promoter, transient assay.

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INTRODUCTION

Oil palm is the main commodity crop in Malaysia and has strongly contributed to the country's economic development. However, the oil palm industry is facing several challenges notably due to the decrease in land availability for further expansion, labour shortage and competition from other vegetable oils. Therefore, strategies should be put in place in order to meet the current challenges and to remain competitive in future. One potential area that can be explored for improvement of oil palm profitability is by developing new and value-added products through genetic engineering. In order to genetically engineer oil palm, a number of tools are first needed to be made available. One of the absolute prerequisites is the availability of promoter sequences to direct the expression of gene of interest in the desired tissues.

The oil palm genetic engineering programme in the Malaysian Palm Oil Board (MPOB) started in the late 1980s with the ultimate goal to produce transgenic palms containing high level of oleic acid (Cheah *et al*., 1995). Additionally, the programme is also aimed towards producing high value-added

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products such as industrial chemicals, nutraceuticals or even pharmaceuticals as well as novel oil/products (Parveez *et al*., 1999; 2015b; Siti Nor Akmar *et al*., 2001; Parveez, 2003). These improvements and products would be an attractive proposition for oil palm since it is the most productive oil crop in the world (Oil World, 2013). Successful genetic modifications of the oil palm, which depends on the integration of gene(s) of interest for the targeted products into the plant nuclear genome, depends on the availability of reproducible transformation methods such as microprojectile bombardment and *Agrobacterium*-mediated systems. While methods for the stable introduction of transgenes are being optimised (Parveez *et al*., 2000; Masli *et al*., 2009; Masani *et al*., 2014), expression of foreign genes in a particular plant species requires a constitutive or tissue-specific promoter to ensure that the gene(s) will be expressed in an appropriate spatial and temporal configuration that will enhance the targeted trait. Identification of strong and tissue-specific plant promoter will ensure a more focused control of transgene expression for reducing the potential deleterious impact on normal growth and development of the plant due to ectopic transgene expression (Schaart *et al*., 2002).

Spatial and temporal expression of the introduced gene(s) is necessary to ensure that the gene(s) is expressed in the target tissues (mesocarp or kernel) during oil synthesis. The work on leaf- and root-specific promoters is also important especially towards the production of biodegradable plastics, development of pest- and disease-resistant palms. Without these spatial and temporal specificities, the modification would likely result in the production of target products in a less optimised manner that might be detrimental to the growth and development of the plant. Finally, constitutive promoters are also required for driving reporter or selectable marker genes which are important for the production of transgenic oil palm..

Tissue-specific promoters play a vital role in targeting gene expression carrying the desired trait for the production of 'new' products to the desired tissue. In oil palm, studies on the gene expression regulation during the course of oil synthesis in both mesocarp and kernel tissues have served as background information to isolate their promoter for genetic manipulation. To date, mesocarp- and kernel-specific candidate genes encoding type 3 metallothionein-like (*MT3-A*) (Siti Nor Akmar and Zubaidah, 2009), lipase class 3 (Nurniwalis *et al*., 2015) and glutelin (Siti Nor Akmar *et al*., 2014) and their corresponding promoters (Siti Nor Akmar and Zubaidah, 2009; Nurniwalis *et al*., 2015; Siti Nor Akmar *et al*., 2014) have been isolated and characterised via transient assays using β-glucuronidase (GUS), and/or green fluorescence protein (GFP) as the reporter genes, as well as in the model plant (Zubaidah and Siti Nor Akmar, 2010; Parveez *et al*., 2010; Hanin *et al*., 2016) *Arabidopsis*. Similarly, isolation of constitutive promoters from the oil palm genes has also been reported (Masura *et al*., 2010; 2011).

MESOCARP-SPECIFIC PROMOTERS

For oil palm, the fruit is the most valuable part of the plam. Oil palm fruits produce two distinct types of oil, namely, from the mesocarp and kernel tissues. The oils derived from these tissues vary in their fatty acid composition and also the duration at which the oil accumulates during oil palm fruit development. Mesocarp and kernel are storage tissues, therefore, they are suitable targets for collecting genetically engineered products without detrimental effects on the plants. Controlling both tissues by channelling the substrates and intermediates for the production of storage oil protein specifically into the tissues would be expedient. In addition, this process can lead to an alteration in the levels of existing products or to the production of novel value-added products (Mohd Basri *et al*., 2005). Kernel tissue, which is separated from the mesocarp by the shell, could also be used to synthesise pharmaceutically important products such as vaccines or therapeutic proteins. This is a potentially practical solution as it is physically separated from mesocarp oil and could be extracted while the kernel is still liquid without affecting or contaminating the mesocarp. The isolation of mesocarp- and kernel-specific promoters from oil palm is elaborated in the following sections.

Type 3 *MT3-A* Promoter (MSP1)

The *MT3-A* gene was identified through subtractive hybridisation on mesocarp tissue between 5 WAA (weeks after anthesis) to 15 WAA. The promoter region of *MT3-A* (designated MSP1: GenBank Accession No. EU499363) was isolated from Dra I genome walker library (Siti Nor Akmar and Zubaidah, 2007; 2009) and several important motifs were found in the promoter sequence including an ethylene-responsive element (ERE-reverse) and two I-boxes (Zubaidah and Siti Nor Akmar, 2010) and the latter motif was also found in the fruit-specific promoter of strawberry (Agius *et al*., 2005) and other light-regulated genes (Terzaghi and Cashmore, 1995).

The *MT3-A* genomic sequence consists of the promoter sequence, 3 exons, 2 introns, 5'UTR and 3'UTR (*Figure 1A*). A vector construct containing the MSP1 promoter and GUS reporter gene was constructed (pMT3-A:GUS) and bombarded into mesocarp slices, root and leaf disks. As a control, we also generated a GUS construct driven by constitutive promoter (*CaMV 35S* promoter) to

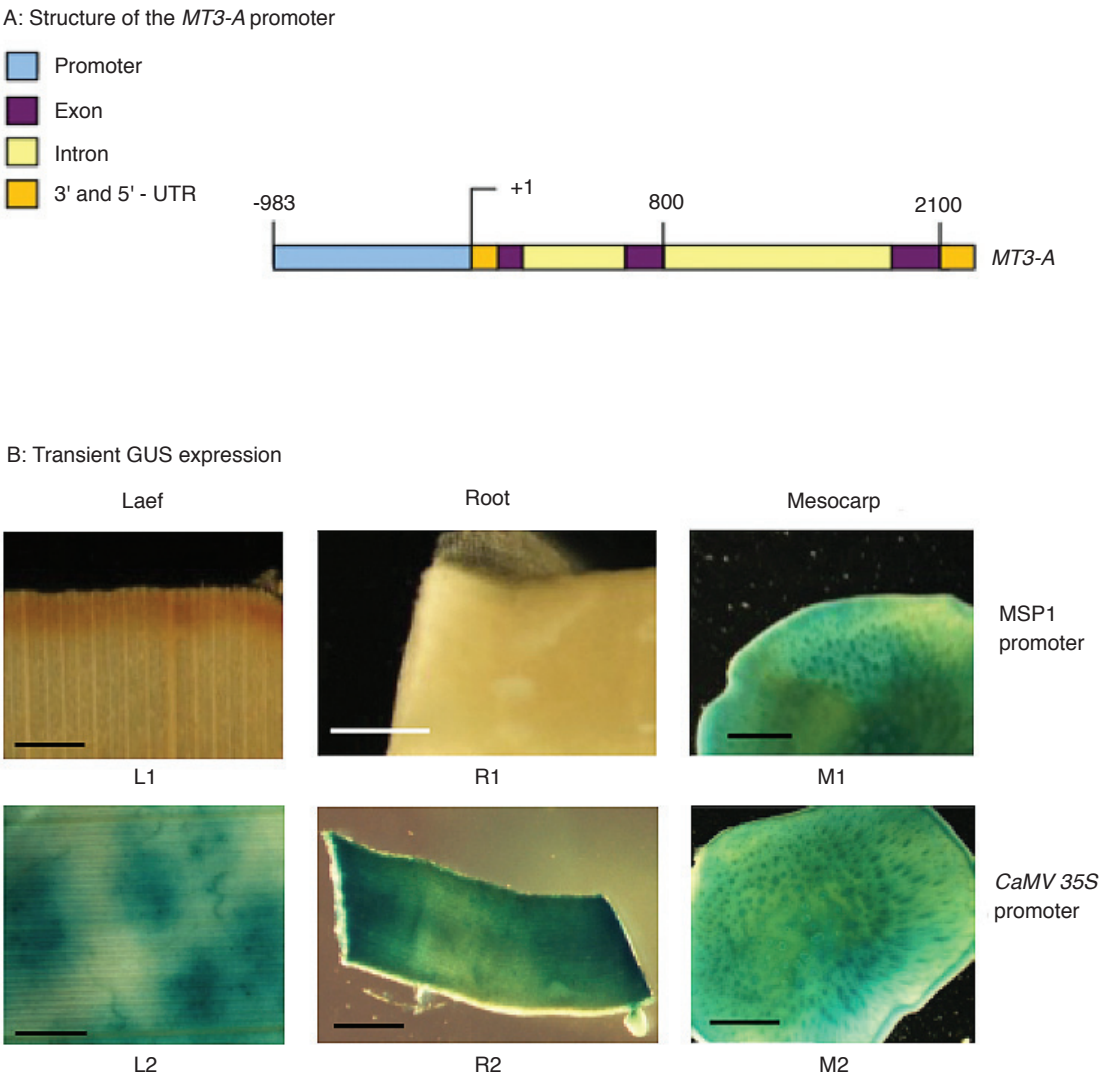

Figure 1. Characterisation and functional analysis of MSP1 (MT3-A) *promoter. (A) Structure of the oil palm* MT3-A *promoter. (B) Distribution of β-glucuronidase* (*GUS) activity in oil palm tissues bombarded with either* CaMV 35S *promoter*::GUS (*pCaMV35SGUS*) *or* MT3-*A promoter*::*GUS* (*pMT3-AGUS*). *Bar = 1 mm.*

yield pCaMV35SGUS plasmid. The MSP1 promoter showed mesocarp-specific activity based on the detected GUS expression (blue spots) only on bombarded mesocarp slices and not on bombarded leaf or root tissues (*Figure 1B*). These results provide support that activity of the MSP1 promoter is specific to mesocarp tissues.

This promoter is currently being used in the oil palm genetic engineering programme to modify the mesocarp oil composition and also for the production of novel products in the mesocarp (Masani and Parveez, 2008; Masani *et al*., 2008; Kamaladini *et al*., 2011; Omidvar *et al*., 2010; Parveez *et al*., 2015a).

Lipase Class 3 (*FLL1*) Promoter (MSP2)

The full-length MSP2 gene was successfully isolated following its initial identification from a 17-week-old mesocarp cDNA library (Nurniwalis *et al*., 2008; 2015). A 756 bp genomic sequence of MSP2 which comprises the 671 bp of promoter sequence and 85 bp of 5′ UTR was amplified using Genome walking approach (*Figure 2A*). The MSP2 promoter contains sequences (TATA box, pyrimidine patch, and *cis*-acting regulatory elements) that make up the important components of the promoter.

Transient GUS expression was conducted to evaluate the effectiveness of the MSP2 promoter to direct transgene expression to the mesocarp. A vector construct containing the MSP2 promoter and GUS reporter gene was bombarded into mesocarp slices and leaf discs. The MSP2 promoter showed mesocarp-specific activity based on detected GUS expression only on the bombarded mesocarp slices (*Figure 2B*) especially in the vascular bundles, and not on bombarded leaf tissues. This promoter could direct expression of foreign genes to mesocarp

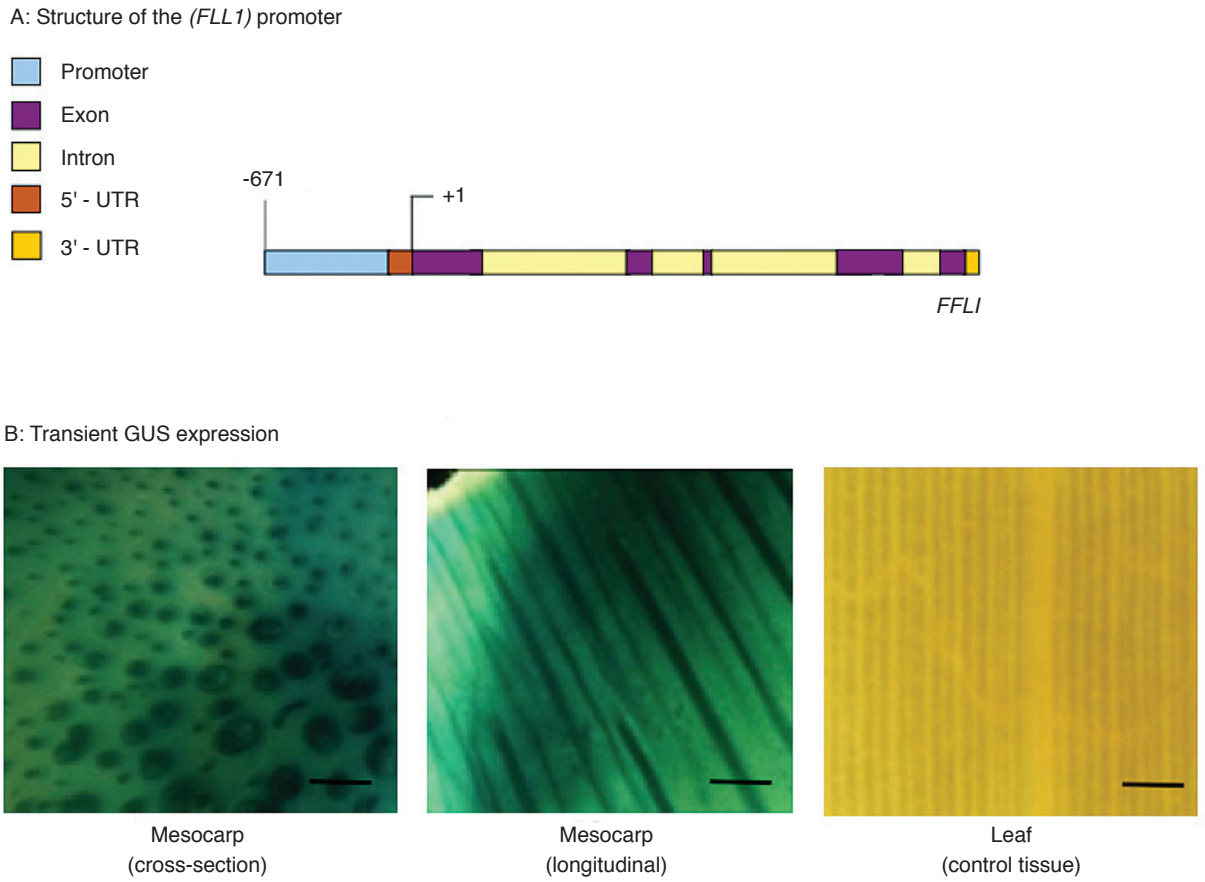

Figure 2. Characterisation and functional analysis of FLL1 *gene and promoter. (A) Structure of the oil palm* FLL1 *promoter. (B) Transient β-glucuronidase (GUS) expression of* FLL1 *promoter::GUS (pFLL1GUS) expression profile in the mesocarp and leaf tissues of oil palm. Bar = 500 µm.*

tissues and, can therefore potentially be used for genetic modification of oil palm for the focus product expressed in the mesocarp. These results are in agreement with northern blot and reverse transcription-polymerase chain reaction (RT-PCR) analysis where by the expression was detected throughout fruit development (Nurniwalis *et al.*, 2015). This promoter has great potential for the production of novel oils and products.

KERNEL-SPECIFIC PROMOTER

Seed Storage Protein Glutelin (*pOP-KT21*) Promoter

Following the isolation of the kernel-specific glutelin gene, the promoter region was isolated and fully characterised (Siti Nor Akmar *et al.*, 2007; 2014). Detailed *in silico* sequence analyses showed that the promoter contained a GluB-JB (rice glutelin gene) motif that is essential to regulate endosperm-specific expression. This promoter was then analysed via transient expression assay to evaluate the functionality of the promoter. Constructed plasmid containing the *pOP-KT21* promoter (GluP-EGFP) was bombarded into oil palm leaf discs, kernel and mesocarp tissue slices. GFP expression was detected in the kernel tissue slices bombarded with GluP-EGFP, but expression of GFP was not detected in the mesocarp and leaf tissues even though expression of co-bombarded *CaMV 35S*::*GUS* was detected (Siti Nor Akmar *et al.*, 2014). Therefore, we suggest that the oil palm *pOP-KT21* promoter is a functional promoter with enable expression in the kernel (Siti Nor Akmar *et al.*, 2007; 2014).

INDUCIBLE ROOT-SPECIFIC PROMOTER

Further efforts have been made in isolating root-specific promoters to be used in regulation of root-associated agronomic traits such as nutrient uptake, tolerance to drought, flood or tolerance to pathogens (Jeong and Jung, 2015). Root-specific promoters have been extensively isolated from other crop species such as rice, barley and tomato (Yamamoto *et al.*, 1991; Schünmann *et al.*, 2004; Sasaki *et al.*, 2012; Ueno *et al.*, 2010; Puig *et al.*, 2013; Kaur *et al.*, 2014) and functionally characterised. In oil palm, a root-specific promoter could be used as part of the

components to produce transgenic oil palm that is tolerant to *Ganoderma* or abiotic stresses such as flood and drought.

Type 3 *MT3-B*: An Inducible Promoter for Root-specific Expression

The genomic clone of *MT3-B* gene contains the coding region, two introns and the promoter sequence (687 bp) as shown in *Figure 3A*. Amongst the important regulatory motifs (putative) found in the promoter sequence are a metal responsive element, a GCC-box and a root-specific element (Zubaidah and Siti Nor Akmar, 2010).

The functionality of the *MT3-B* promoter to direct expression of transgenes to oil palm tissues was determined using GUS transient assay. GUS expression was only observed in oil palm root tissues but not in the spear leaves and mesocarp at 12 WAA (*Figure 3B*). In contrast, all tissues (leaf, mesocarp and root) bombarded with plasmid containing constitutive *CaMV 35S* promoter (pCaMV35SGUS) showed GUS activity in all tissues tested (*Figure 3B*). These results are in agreement with the earlier findings by Siti Nor Akmar *et al*. (2002) using Northern blot analysis.

Functional characterisation of the *MT3-B* promoter was further carried out using *Arabidopsis* as a model system. GUS expression in all 12 transgenic plants driven by the *MT3-B* promoter was determined. Low GUS expression was observed in root and matured leaves and comparably higher expression was detected in cotyledon leaves (*Figure 3Ci*). No expression was detected in other tissues tested. Whereas GUS expression was detected in most tissues of transgenic *Arabidopsis* lines carrying constitutive *(CaMV 35S*) promoter driving GUS (*Figure 3Ci*).

Further characterisation was carried out to determine the effects of metal ions to the promoter activity in transgenic *Arabidopsis thaliana* driven by the *MT3-B* promoter. In this study, metal ions in the form of ($ZnSO_4$ and $FeSO_4$) were independently added to MS media and GUS expression was determined in transgenic *Arabidopsis thaliana* plants containing the *MT3-B* promoter:GUS fusion. Higher GUS expression was detected in transgenic *Arabidopsis thaliana* roots when the plant was treated with Zn^{2+} compared to untreated plants (*Figure 3Cii*). These findings were in line with results obtained from fluorometric assay (Zubaidah and Siti Nor Akmar, 2010) where we detected the increase of

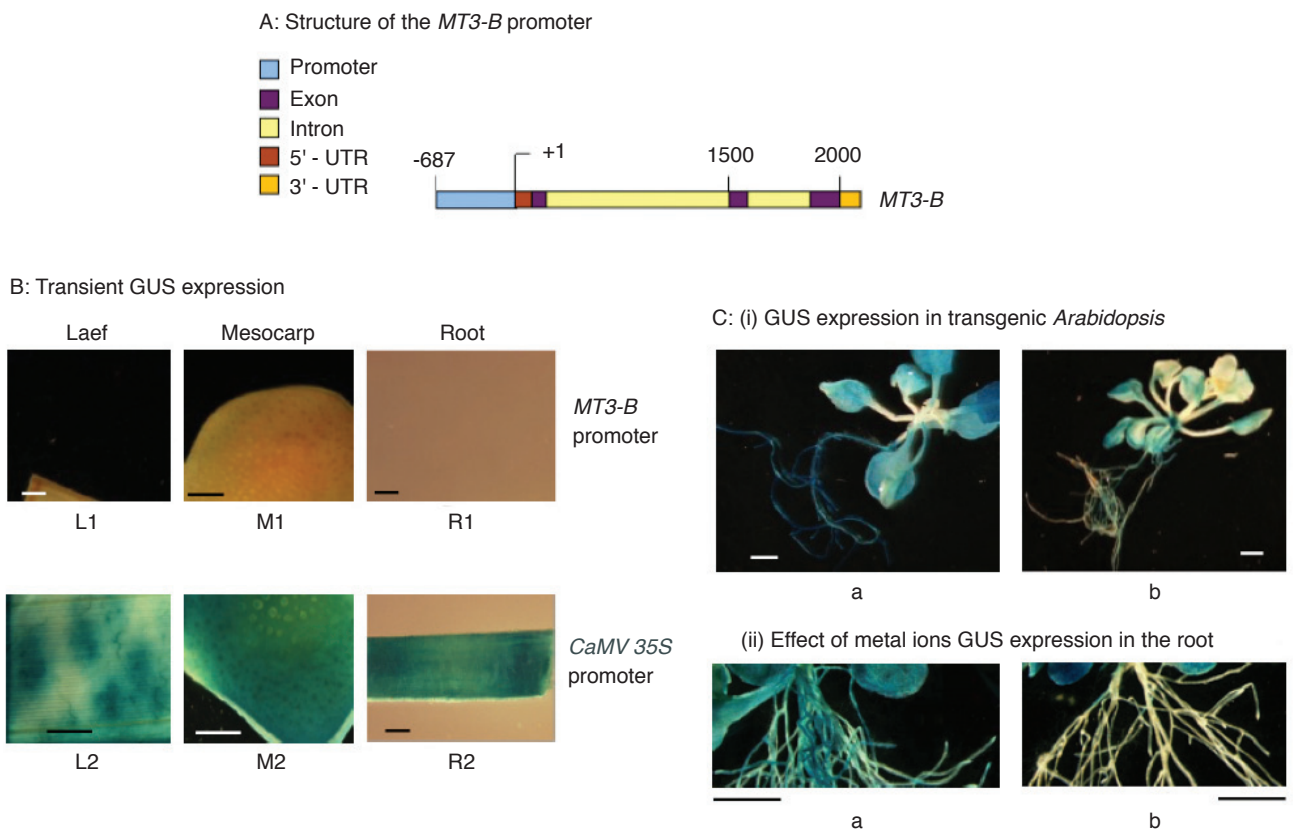

Figure 3. Characterisation and functional analysis of MT3-B *promoter. A: Structure of the oil palm* MT3-B *promoter. B: Distribution of β-gluchronidase (GUS) activity in oil palm tissues bombarded with either* CaMV 35S *promoter (Li, M1 and R2) or* MT3-B *promoter (L2, M2 and R2). C (i): Comparison of GUS expression between transgenic* Arabidopsis *plants containing the constitutive* CaMV 35S *promoter (a) and the oil palm* MT3-B *promoter (b). C(ii): The effect of metal ion (ZnSO4) on the expression of the GUS gene in transgenic* Arabidopsis *root containing the oil palm* MT3-B *gene promoter(a) and the transgenic plant cultured on MS media without metal ions (b). Bar = 1 mm.*

GUS expression (2.5- and 5-fold) in the roots with the presence of 80 μM of Fe^{2+} and Zn^{2+} respectively. These observations suggest the hypothesis that the activity of the oil palm *MT3-B* promoter is stimulated by Zn^{2+} and Fe^{2+} in root tissues.

LEAF-SPECIFIC PROMOTER

Oil palm leaves have also been identified as a potential site for targeting the expression of novel metabolites that are impossible to produce via conventional breeding. The novel features of the leaf-specific promoter will prevent the accumulation of novel metabolites in the commodity oil which is extracted from mesocarp and kernel tissues. Furthermore, leaves are present throughout the plant life cycle thus this will enable early harvesting and continuous supply of novel metabolites (Chan, 2003). Manipulation of metabolic pathway in the leaf tissues for production of novel metabolites has been demonstrated previously such as production of biodegradable plastic or polyhydroxybutyrate (PHB) in *Arabidopsis* leaves (Poirier *et al*., 1992; Nawrath *et al*., 1994; Mittendorf *et al*., 1998).

Light-harvesting Chlorophyll A/B Binding Protein Gene (*LS01*) Promoter

The *LS01* promoter was isolated using genome walking approach and the size of the isolated promoter was 932 bp (*Figure 4A*) (Chan and Siti Nor Akmar, 2009; 2012; 2014). The transcription start site is located at 32±7 bp upstream of the promoter sequence. This promoter is TATA-less but it has an initiator element (Inr motif) that could be used to direct basal transcription. A similar structure was also reported for the majority of nuclear encoded photosynthetic genes. It has been proposed that TATA-independent transcription mechanisms are crucial for regulated expression of photosynthesis nuclear genes (Nakamura *et al*., 2002). Furthermore, a few putative *cis*-acting elements responsive towards light, wounding, abscisic acid and heat-shock were also found in the distal and proximal regions of the promoter.

Transient expression analysis of oil palm tissues bombarded with constructs containing the *LSO1* promoter was conducted to assess the specificity of the promoter using GFP and GUS as reporter genes. Further characterisation was carried out using transgenic *Arabidopsis* system containing GUS reporter driven by oil palm *LSO1* promoter. Based on GFP assay analysis, the expression was only detected in the leaf tissues bombarded with pLS01: GFP but not in mesocarp slices (*Figure 4B*). Further confirmation was carried out using GUS as a reporter gene and as expected, the GUS expression was only observed in leaf tissues and no GUS expression was detected in the control tissue(mesocarp)(data not shown). In agreement with this finding, GUS expression analysis on transgenic *Arabidopsis* seedlings transformed with *LS01* promoter showed specific expression in the leaf tissues but not in other tissues such as stem and root (*Figure 4C*). However for plants transformed with *CaMV 35S*::GUS construct, all the tissues stated above expressed GUS. Based on this study, *Arabidopsis thaliana* may be used as a model system for analysing oil palm leaf-specific promoter but there are limitations depending on the type of promoters and tissue dependent expression. This promoter is currently being used in the oil palm genetic engineering programme for the production of PHB and polyhydroxybutyrate-co-valerate (PHBV) in oil palm leaf tissues (Abdul Masani *et al*., 2009; Masani and Parveez, 2008; Hanin *et al*., 2016).

CONSTITUTIVE PROMOTER

Constitutive promoters are important for ensuring that a specific gene transferred into a plant will be functional in all plant tissues. They are also important for expressing reporter and selectable marker genes required for establishing a reliable transformation system for a particular plant species. A promoter that is derived from an oil palm native gene is likely to be more advantageous for oil palm as the efficiencies of promoters are sometimes species dependent. Moreover, the availability of a wide range of effective promoters would also make possible the introduction of multiple transgenes into plant cells with reduced risks of homology-dependent gene silencing (Xiao *et al*., 2005).

Ubiquitin Extension Protein Gene (*uep1*) and Translationally Control Tumour Protein (*TCTP*) Gene Promoter

The isolation of oil palm constitutive promoters was carried out through identification of constitutively expressed genes in oil palm. A number of EST clones were found to be constitutively expressed in all oil palm tissues by reverse +36 analysis. These putative clones were subjected to Northern analysis to further confirm their constitutive nature. Two cDNA clones that code for the ubiquitin extension protein gene (*uep1*) and *TCTP* genes were found to be constitutively expressed in oil palm (Masura *et al*., 2010; 2011). The promoter regions of these genes were isolated using genome walking and PCR approaches (Masura *et al*., 2010; 2011). Detailed sequence analysis have been performed using available databases and software to identify features in the gene architecture that could contribute to constitutive *uep1* and *TCTP* expression. These promoters contain several motifs

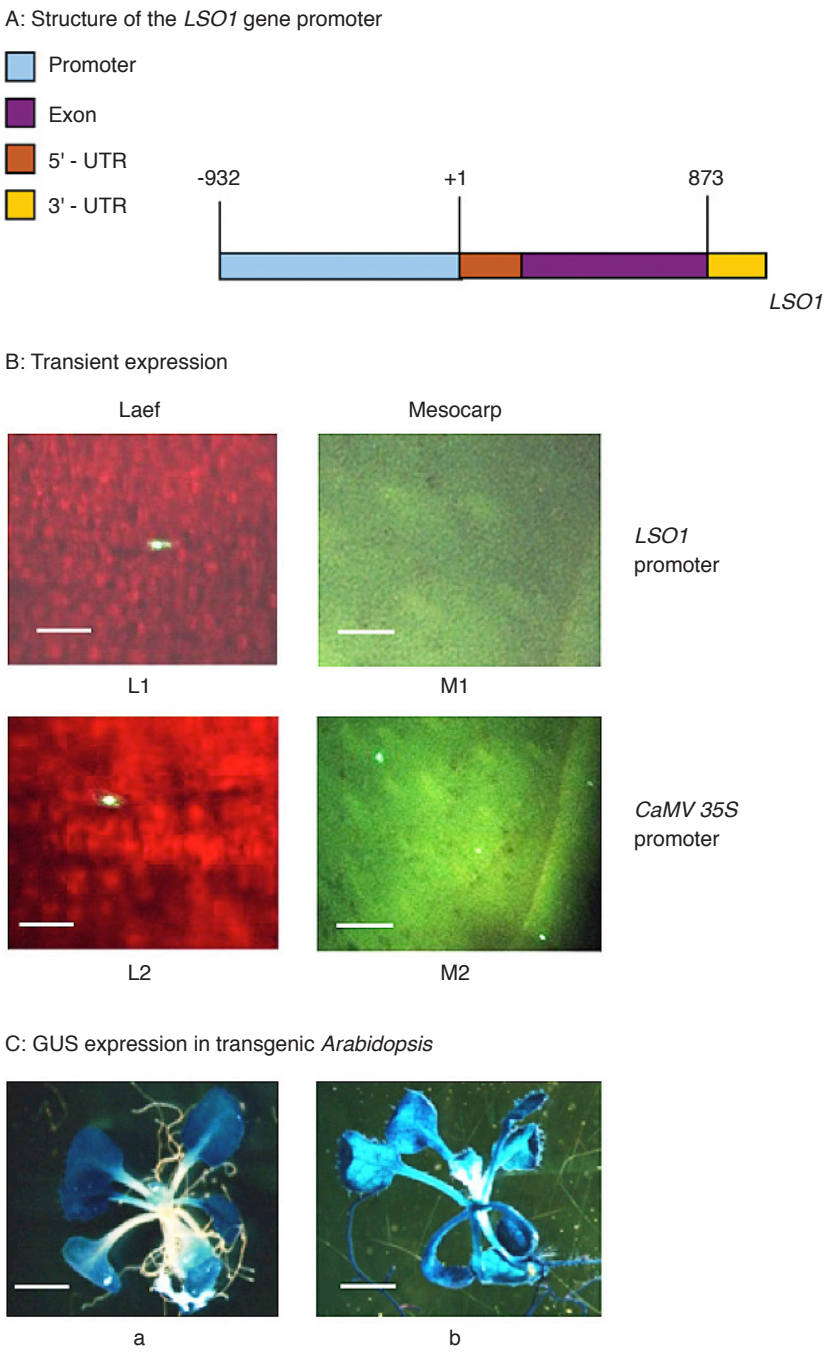

Figure 4. Characterisation and functional analysis of LS01 *gene and promoter. (A) Genomic structure of the* LS01 *promoter. (B) Transient expression using histochemical GUS assay and green fluorescence protein (GFP) detection to confirm the leaf specificity of* LS01 *promoter. L1 and M1 represent GUS expression in leaf and mesocarp tissues derived using* LSO1 *promoter and L2 and M2,* GUS *expression derived from* CAMV 35S *promoter (C) β-gluchronidase (GUS) expression analysis in transgenic* Arabidopsis thaliana *to confirm the leaf specificity of* LS01 *promoter, a = constitutive promoter and* LSO1 *promoter. B, bar = 500 µm; C, bar = 5 mm.*

suggesting its potential multiple roles in hormonal signaling pathways, light regulation, biotic and abiotic stresses.

Functional characterisation of the isolated promoters were subsequently performed. Transformation vectors containing GUS as a reporter gene and nos terminator were constructed independently and designated as pUEP1 and pTCTP (*Figures 5A* and *5B*). The vectors were bombarded into oil palm tissues and tobacco leaves. The functionality of the promoters was evaluated by comparing the GUS activity driven by native promoters with GUS activity driven by maize polyubiquitin (*ubi1*) and *CaMV 35S* promoters. Results indicated that even though *uep1* and *TCTP* demonstrated a high GUS activity, the strength of these promoters was slightly lower than the controls in some tissues, particularly to pAHC25 [*Figures 5A* (*a-g*) and *5B* (*a-g*)]. The pAHC25, which is based on maize ubiquitin promoter has been shown to be the

most effective promoter for oil palm (Chowdhury *et al*., 1997; Parveez *et al*., 2015b). In general, slight variation in the promoter strength was determined in the different tissues tested. Even though a strong constitutive promoter is certainly desired, sometimes the use of a weak promoter resulted in a higher transformation efficiency (Mengiste *et al*., 1997). It is hope that the isolation of *uep1* and *TCTP* provides more alternative promoters for introducing multiple transgenes into plants. These promoters, by itself or after enhancement could be effective promoters for oil palm transformation, as well as for other dicotyledonous plants.

POTENTIAL APPLICATION OF THE ISOLATED PROMOTERS AND THE WAY FORWARD

Isolation of the three tissue-specific promoters, *i.e*., the *MT3-A* and lipase class 3 promoters from mesocarp, and glutelin promoter from kernel, have the capacity to be used as genetic engineering tools to improve oil quality and yield. The availability of the oil palm genome database with abundant transcriptome information allows us to identify more potential oil palm candidate genes and promoters. We managed to discover 41 putative candidate mesocarp-specific genes from the oil palm genome information. Identification of these mesocarp-specific gene candidates provides a valuable basis for further research especially for oil palm genetic engineering work. Candidates of root-specific genes and genes that are predominantly expressed in root have been identified. These genes are heavy metal associated domain containing protein, peroxidase, glycine rich protein and cyp86. Root-specific promoters are potentially useful to develop plants with better tolerance to various types of abiotic stress such as soil types and soil pH and also microbial stresses. The study on leaf-specific promoter has contributed to the advancement of knowledge on promoter sequence and regulatory elements for controlling and driving leaf-specific gene expression in plants, particularly in the oil palm. The leaf-specific promoter is valuable for crop improvement and production of novel products through genetic engineering such as the production of insect resistant palms. Isolation of the two constitutive promoters derived from *uep1* and *TCTP* genes has contributed to the scientific community by providing new promoters for evaluation and testing in oil palm as well as other plants.

A total of seven oil palm derived promoters have been isolated and characterised (*Table 1*). With the availability of the oil palm genome data (Singh *et al*., 2013), more tissue-specific promoters can be isolated such as stem-specific promoter to control oil palm

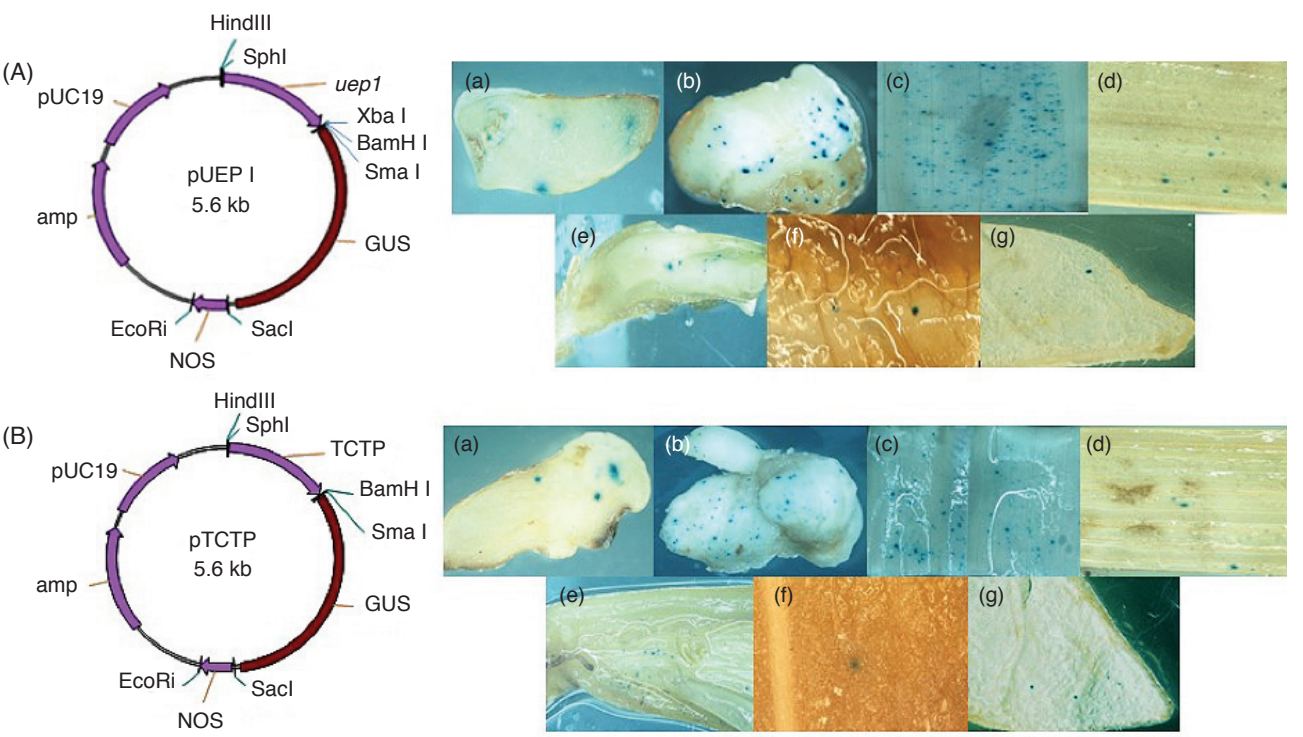

Figure 5. Isolation of constitutive promoters from oil palm. A) Transformation vectors containing uep1 *promoter (pUEP1) and transient histochemical assay in various oil palm tissues and tobacco leaves bombarded with plasmid carrying β-gluchronidase (GUS) gene driven by* uep1. *(B) Transformation vectors containing* TCTP *promoter (pTCTP) and transient histochemical assay in various oil palm tissues and tobacco leaves bombarded with plasmid carrying GUS gene driven by* TCTP. *The GUS expression were detected in oil palm tissues, a: immature embryo, b: embryoid, c: young leaflet from mature palm, d: green leaves, e: plantlet stem, f: mesocarp and g: tobacco leaves.*

TABLE 1. LIST OF ISOLATED OIL PALM PROMOTERS AND POTENTIAL USES

Type of promoter	Promoter name	Functional analysis	Potential usage
Messocarp-specific	*MT3-A*	Transient expression in oil palm tissues, Siti Nor Akmar and Zubaidah (2008)	To improve oil quality
	FLL1	Transient expression in oil palm tissues, Nurniwalis *et al*. (2015)	
Kernel-specific	*pOP-KT21*	*In situ* hybridisation, Siti Nor Akmar *et al*. (2014)	Nutraceutical and pharmaceutical products
Inducible root-specific	*MT3-B*	Transient expression in oil palm tissues, Zubaidah and Siti Nor Akmar (2003)	Disease resistant palms
		Arabidopsis thaliana, Zubaidah and Siti Nor Akmar (2010)	
Leaf-specific	*LS01*	Transient expression in oil palm tissues, Chan *et al*. (2008)	Pest resistant palm, bioplastics
		Arabidopsis thaliana, Hanin *et al*. (2016)	
Constitutive	*Uep1*	Transient expression in oil palm and tobacco tissues, Masura *et al*. (2010)	Production of transgenic oil palm
Constitutive	*TCTP*	Transient expression in oil palm tissues and tobacco, Masura *et al*. (2011)	

height, root-specific promoters that can utilised for root-associated agronomic traits and plant response to abiotic stresses and more mesocarp and kernel promoters for novel oil products. Isolation and characterisation of more than one tissue-specific promoters allow for more choices of promoters to be used to direct the accumulation of genetically engineered products to targeted tissues, as different promoters have different strengths and specificities to ensure stable expression of the transgene

CONCLUSION

Seven oil palm-derived promoters (two mesocarp-specific promoters, one kernel-specific promoter, one root-specific promoter, one leaf-specific promoter and two constitutive promoters) have been isolated and characterised. Findings from these studies, have generated knowledge through basic research that can be applied to develop the necessary innovations for the industry. The isolated promoters are potentially useful and can be used to improve traits as well as diversify the whole industry into a more sustainable and high income generating industry.

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