A CONSTITUTIVE PROMOTER FOR EXPRESSING FOREIGN GENES IN PLANTS: TRANSLATIONALLY CONTROLLED TUMOUR PROTEIN (TCTP)

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MPOB has identified genetic engineering as a promising technology which can be utilized to face challenges such as labour shortage, limited land resources and fluctuations in commodity prices. The production of transgenic oil palm with useful genes has a clear advantage as it could increase return per unit area. Expression of foreign genes in a particular plant species requires a constitutive or tissue-specific promoter to ensure that the gene (s) will be functional and will enhance the production of the targeted trait. Constitutive promoters are important for ensuring that a specific gene transferred into a plant will be functional in all the 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. Constitutive promoters can be used to express any gene in any plant species, other than in the species from which they were isolated.

CONSTITUTIVE PROMOTERS

In order to functionally express a gene in a plant, a transgene must have a promoter that is recognized by the RNA polymerase in the plant cells. A promoter initiates and regulates transcription, the first and the most important step in gene expression (Xiao *et al.*, 2005). Promoters can be divided into two major classes: i) constitutive, which are expressed in all tissues/cells, and ii) tissue-specific, which can be expressed only in particular tissues. As promoters affect transcription both quantitatively and qualitatively, the success of gene transfer technologies, varying from basic research to crop improvement and to biopharming, depends on their efficacious selection and use (Potenza *et al.*, 2004). Plant promoters that are capable of driving high and constitutive expression of transgenes have become a valuable tool in plant genetic engineering. These promoters are required for high level production of protein. High expression of a selectable marker is important to inhibit the growth of untransformed cells and to allow only resistant transformants to survive and finally regenerate into transgenic plants (Parveez, 1998). Otherwise, a majority of untransformed cells will dominate the culture and this will result in chimeric plants (Christou, 1992; Ritala *et al.*, 1994). High expression of reporter proteins such as GUS, GFP and CAT in plant cells can also be achieved by using constitutive promoters. Moreover, the use of constitutive promoters is essential in producing compounds that are required ubiquitously during all stages of plant development.

A CONSTITUTIVE PROMOTER FROM OIL PALM

Constitutively expressing genes from oil palm were identified through DNA microarray analysis. Northern and reverse Northern analyses were used to confirm the constitutive nature of the genes (*Figures 1* and *2*). An identified constitutive gene was later confirmed to be translationally controlled tumour protein (TCTP) after comparing the gene sequences against the GenBank database using BLAST 2.0 (Altchul *et al.*, 1997). The promoter sequences of the gene were later isolated using the genome walker approach. Prediction of the location of the transcription start site (TSS) was carried out using the Softberry database. Identification for cis-acting regulatory elements was performed using the MOTIF search at the publicly accessible databases. The databases are Softberry (http://www.softberry.com/berry.phtml), PLACE (http://www.dna.affr.go.jp/PLACE) and PLANTCARE (http://bioinformatics.psb. ugent.be/webtools/plantcare/html/). The promoter was attached to a reporter gene to produce a transformation vector for further analysis (*Figure 3*). The vector was bombarded into a number of oil palm tissues to confirm its constitutive nature via transient gene expression studies (*Figure 4*). A number of cells expressing the reporter gene were evaluated, calculated and compared against other established constitutive promoters from other plants or organisms. The oil palm tctp promoter is now ready to

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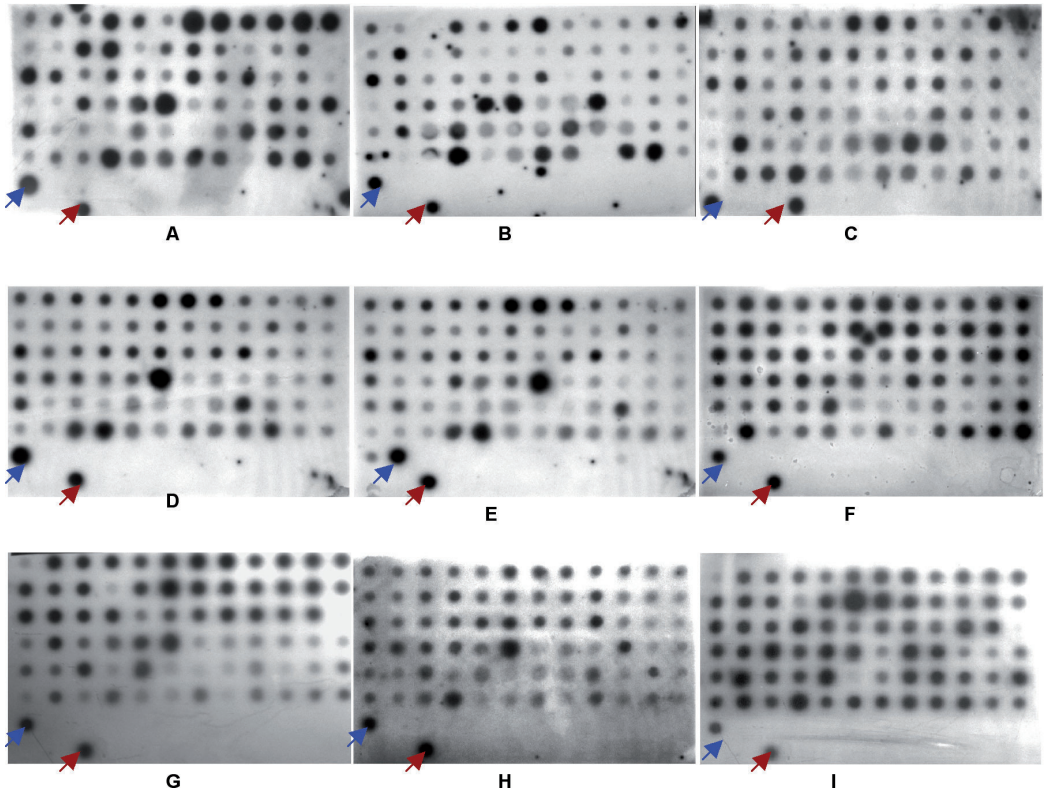

Figure 1. Reverse Northern analysis to screen expression pattern of 73 cDNA clones generated through microarrray approach. The membranes were hybridized with first strand cDNA probes from various tissues such as mesocarp 14 WAA, mesocarp 17WAA, kernel 14 WAA, frond and flower. Blue and red arrows indicate the locations of tctp cDNA clone and ribosomal DNA, respectively.

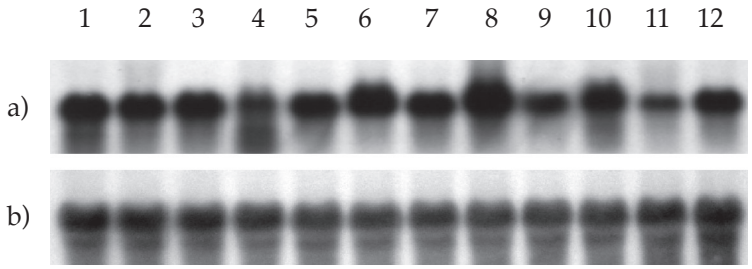

Figure 2. Northern analysis for pOP-SFB1500 cDNA (a). Each lane contains 15 ug of total RNA prepared from different tissues of oil palm. Lane 1-4: mesocarp at 5, 14, 17, 20 WAA, lane 5-6: kernel at 14, 17 WAA, lane 7: flower, lane 8: frond, lane 9: embryoid, lane 10: root, lane 11: plantlet, lane 12: young leaf. Equal loading of RNA was verified with 28S ribosomal DNA (b).

be used to drive any gene, and to transform it into oil palm or other plant species.

BENEFITS OF THE CONSTITUTIVE PROMOTER

Various constitutive promoters have been isolated and used to drive various genes in transgenic plant species such as rice, soyabean, maize and wheat. Similarly, constitutive promoters, such as the maize ubiquitin promoter (*Ubi1*), rice actin promoter (*Act1*) and cauliflower mosaic virus 35S promoter (CaMV 35S), have been used to drive the expression of various reporter, selectable and useful genes in transgenic oil palm (Chowdhury *et al*., 1997). The tctp promoter from oil palm can also be used to drive various selectable and useful genes.

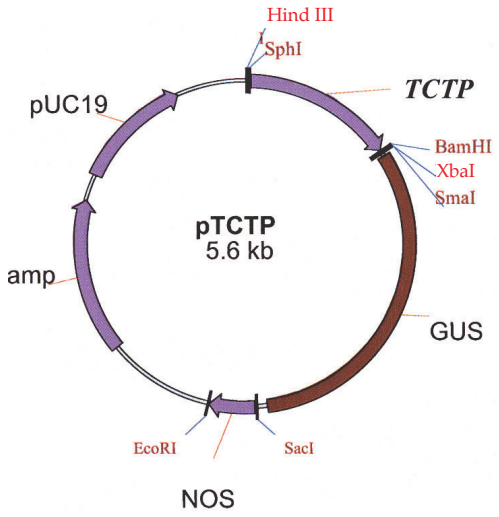

Figure 3. Transformation vector, pTCTP, carrying oil palm tctp promoter driving gusA reporter gene.

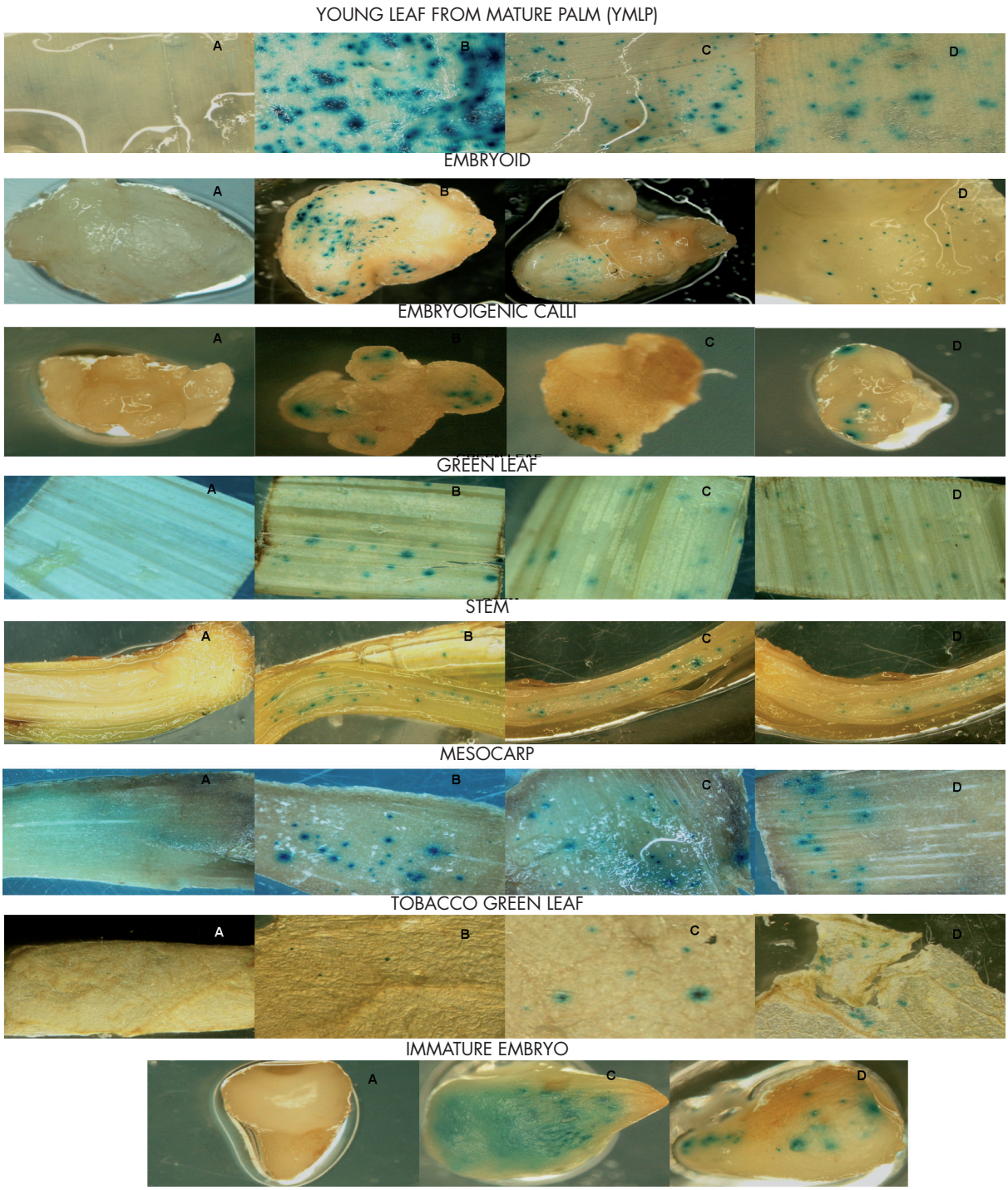

*Figure 4. Comparison of transient histochemical gusA gene expression in various oil palm tissues and in tobacco after bombardment with plasmids carrying different promoters. (A) none (bombarded without plasmid DNA), (B) pAHC25 (Ubi1), (C) pBI221 (CaMV 35S), and (D) pTCTP (*TCTP*).*

Besides driving transgenes in oil palm, this promoter can also be used to express genes in many other plants that are important to Malaysia, such as rice, banana, rubber, cocoa, tobacco, durian and forest trees.

WHO SHOULD BENEFIT

Molecular biologists or biotechnologists from the oil palm industry can benefit from using the promoter to drive any of their genes of interest. Similarly, molecular biologists and biotechnologists from local universities, research institutions and research-based companies can benefit from this promoter for their research, either to study gene expression or to regenerate transgenic plants. As offered earlier, MPOB has services to make transformation vectors and RNAi constructs as well as to regenerate transgenic plants using both microprojectile bombardment and *Agrobacterium*-mediated transformation approaches (Parveez, 2003; Masani and Parveez, 2005; Dayang *et al*., 2008; Masani and Parveez, 2008).

INTELLECTUAL PROPERTY

A patent application has been filed for the TCTP promoter isolated from oil palm (PI 20091934).

AWARD

Bronze Medal Award, Malaysia Technology Expo 2010. 4 - 6 February 2010. Putra World Trade Centre, Kuala Lumpur.

REFERENCES

ALTCHUL, S F; MADDEN, T L; SHAFFER, A A; ZHANG, J; ZHANG, G; MILLER, W and LIPMAN, D J (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res., 25:* 3389-3402.

CHOWDHURY, M K U; PARVEEZ, G K A and SALEH, N M (1997). Evaluation of five promoters for use in transformation of oil palm (*Elaeis guineensis* Jacq). *Plant Cell Rep., 16:* 277-281.

CHRISTOU, P (1992). Genetic transformation of crop plant using microprojectile bombardment. *The Plant J., 2*: 275-281.

DAYANG, I A M; PARVEEZ, G K A and MASANI, A M Y (2008). Transgenic oil palm mediated by *Agrobacterium tumefaciens*. *MPOB Information Series No. 401.*

MASANI, A M Y and PARVEEZ, G K A (2005). Construction of transformation vectors for genetically modifying plants. *MPOB Information Series No. 10.*

MASANI, A M Y and PARVEEZ, G K A (2008). Construction of transformation vectors for efficient down-regulation of genes in transgenic plants. *MPOB Information Series No. 49.*

PARVEEZ, G K A (1998). Optimization of parameters involved in transformation of oil palm using the biolistic method. Ph. D. thesis. Universiti Putra Malaysia, Serdang. 243 pp.

PARVEEZ, G K A (2003). Microprojectile bombardment for generating transgenic oil palm. *MPOB Information Series No. 189.*

POTENZA, C; ALEMAN, L and SENGUPTA-GOPALAN, C (2004). Targeting transgene expression in research, agricultural and environmental applications: promoters used in plant transformation. *In Vitro Cell Dev. Biol. Plant*, *40*: 1-22.

RITALA, A; ASPEGREN, K; KURTEN, U; SALMENKALLIO-MARTTILLA, M; MANNONEN, L; HANNUS, R; KAUPINEN, V; TEERI, T H and ENARI, T (1994). Fertile transgenic barley by particle bombardment of immature embryos. *Plant Mol. Biol*., *24*: 317-325.

XIAO, K; ZHANG, C; HARRISON, M and ZENGYU, W (2005). Isolation and characterization of novel plant promoter that directs strong constitutive expression of transgene in plants. *Molecular Breeding*, *15*: 221-231.

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