A CONSTITUTIVE PROMOTER FOR EXPRESSING FOREIGN GENES IN PLANTS – UBIQUITIN EXTENSION PROTEIN

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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 the 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 tissue of any plant species, in addition to the species from which it was 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: constitutive, which are expressed in all cells, and 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 generate 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). The high level 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 to produce 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 after DNA microarray analysis. Northern and reverse Northern analyses were used to confirm the constitutive nature of the genes (*Figure 1*). A putatively identified constitutive gene was later confirmed to be *ubiquitin extension protein* (*uep1*) after comparing the gene sequences against the GenBank database BLAST 2.0 (Altchul *et al.*, 1997). The constitutive gene was then used to isolate its promoter sequences *via* methods such as genome walking. Prediction of the putative location of the transcription start site (TSS) was carried out using the Softberry database. Identification of *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 2*). The vector was bombarded into a number of oil palm tissues to confirm its constitutive nature *via* transient gene expression studies (*Figure 3*). A number of cells expressing the reporter gene were evaluated, calculated and

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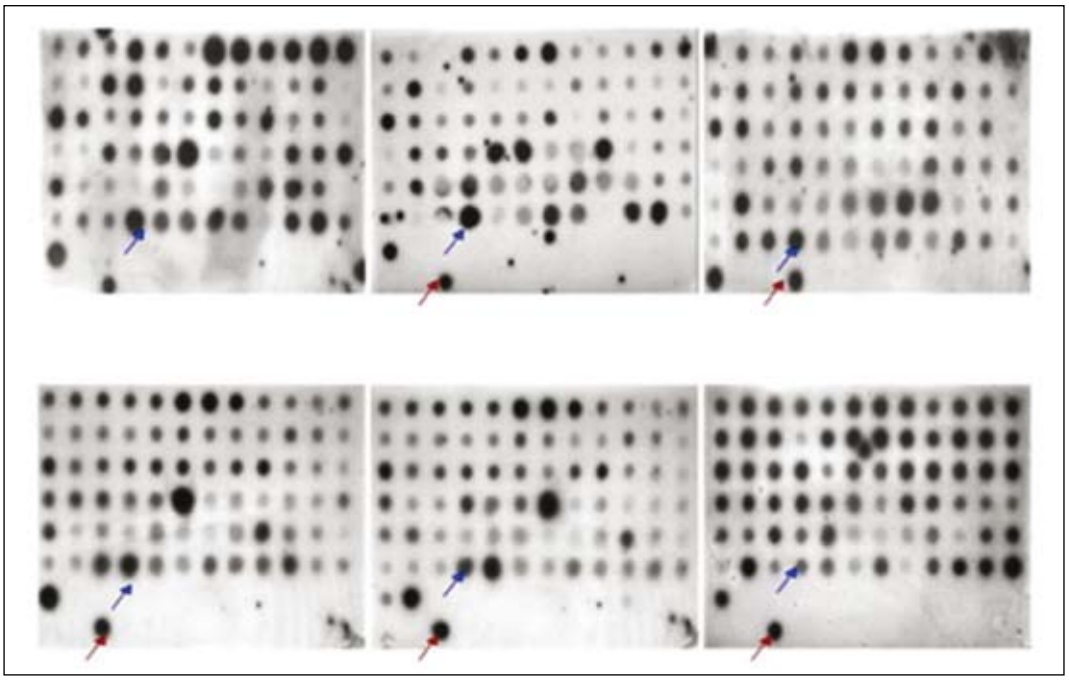

Figure 1. Reverse Northern analysis to screen expression pattern of 73 cDNA clones generated through the microarrray approach. The membranes were hybridized with first strand cDNA probes from various tissues such as mesocarp (14 WAA and 17 WAA), kernel 14 WAA, frond and flower. Blue and red arrows indicate the locations of uep1 cDNA clone and ribosomal DNA, respectively.

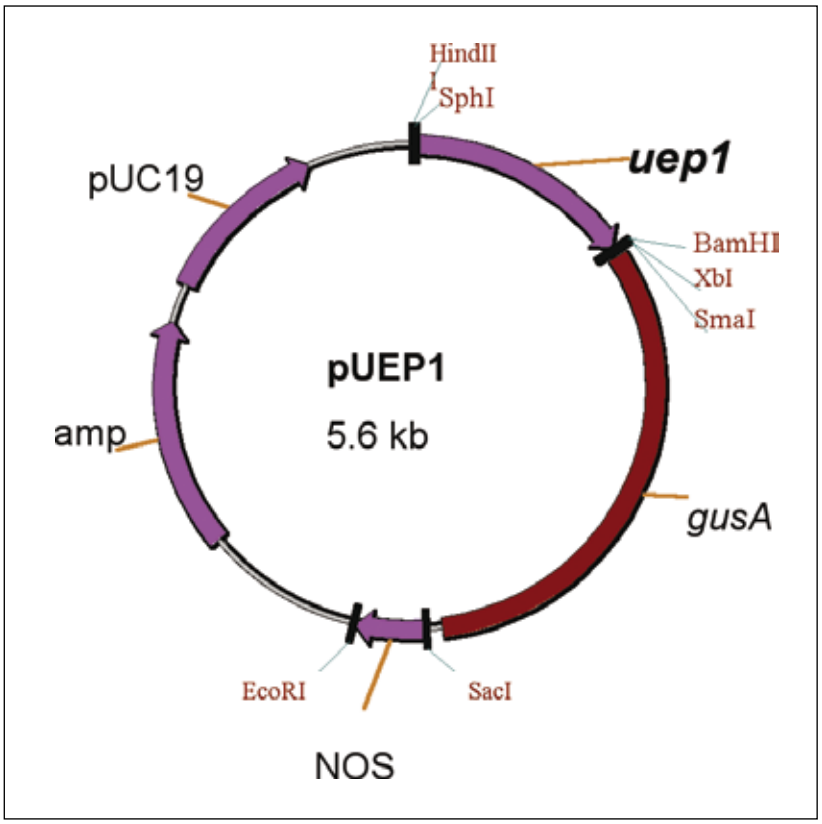

Figure 2. Transformation vector, pUEP1, carrying oil palm uep1 *promoter driving gusA reporter gene.*

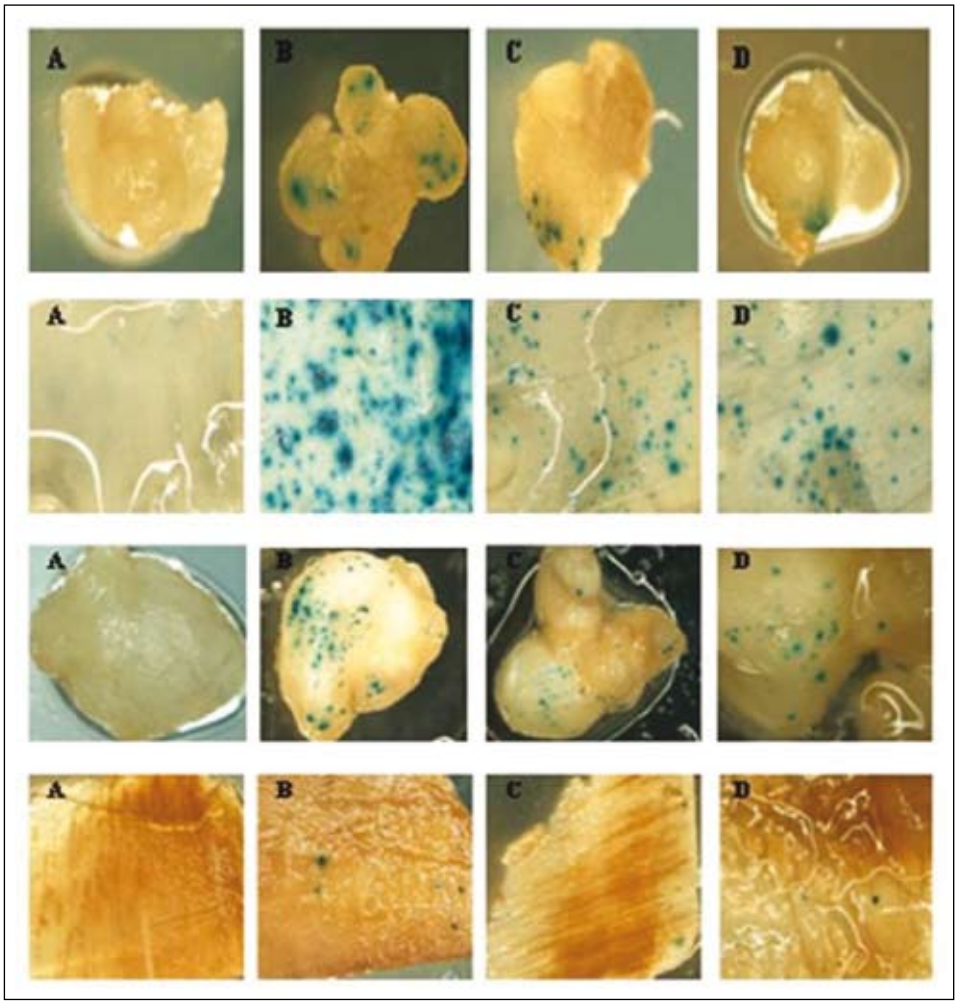

Figure 3. Comparison of transient histochemical gus A gene expression in various oil palm tissues after bombardment with plasmids carrying different promoters. (A) none (bombarded without plasmid DNA), (B) pAHC25 (Ubi1), (C) pBI221 (CaMV 35S), and (D) pUEP1.

compared against other established constitutive promoters from other plants or organisms. This promoter is now ready to be used to drive any gene, and to transform 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 reporters, selectable and useful genes in transgenic oil palm (Chowdhury *et al*., 1997; Masani *et al*., 2008). The *uep1* promoter from oil palm can also be used to drive various selectable and useful genes. Besides using it to drive genes in oil palm, this promoter can be used to express genes in many other plants of importance in 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; 2008; Dayang *et al*., 2008).

INTELLECTUAL PROPERTY

A patent application has been filed for the *uep1* promoter isolated from oil palm (PI 20090677).

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