NOTE

EXPRESSION OF *BT* GENE IN TRANSGENIC TEAK

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Teak (*Tectona grandis*) is an economically important, large deciduous and semi-evergreen tree. It is predominantly distributed in tropical and subtropical regions of South-East Asia (Baghel et al. 2008). It is an important species for forest plantation in the tropics and valued worldwide for its wood. Genetic improvement of teak by conventional breeding is still an obstacle due to long reproductive cycles. It takes more than a decade from the initiation of a genetic improvement programme until improved seeds are available and it needs another four to five decades before the timber from the first rotation of improved planting stock can be harvested (Suseno & Wibisono 2000).

Teak has been planted in Malaysia for a considerable number of years and many systematic trials are being carried out in the north of West Malaysia and East Malaysia. However, teak plantations face problems including susceptibility to various pests and diseases. Among them is the teak skeletoniser *Paliga damastesalis* (Intachat 1998, 1999), the most common teak defoliator species in Malaysia. Complete defoliation has been recorded during serious outbreaks. These outbreaks, however, have been observed to be seasonal, especially in the wetter periods (Chey 2000).

Research on insect resistance including insertion of the toxin gene from *Bacillus thuringiensis*, namely, *Bt*/*cry*1A(b) gene or transfer of proteinase inhibitor genes from other plant species such as cowpea tripsin inhibitor (*CpTI*) gene has been carried out (Lingling et al. 2005, Zaidi et al. 2006). Advances in tissue culture and transformation techniques have enabled gene transfer in woody plant species; thus, efforts in recent years are directed towards the establishment of transformation protocols for forest trees of economic importance such as *Pinus* spp. (Trontin et al. 2007).

Bacillus thuringiensis (*Bt* gene donor) is the most widely used environment-friendly alternative to chemical insecticides for the biological control of forest and agricultural pests (Lambert & Pefereon 1992). During sporulation, different strains of this gram-positive bacterium produced crystalline parasporal known as δ-endotoxin. Endotoxin has high levels of specificity against different species of lepidopteran, dipteran and coleopteran insects (Höfte & Whiteley 1989). This crystalline protein is highly toxic to insect if ingested, whereby the crystals are dissolved, digested to yield a truncated and more soluble state, leading to epithelium pore formation, inflammation, starvation and death (Karim & Dean 2000). Its toxic property has been recognised for more than 40 years and is still in extensive use for control of pest infestations in crops (Soberon et al. 2007).

At the Forest Research Institute Malaysia (FRIM), teak tissue culture technique and genetic transformation system on the basis of a biolistic approach have been developed to produce transgenic teak (Norwati 2005). Biolistic particle delivery device (PDS 1000He, BioRad) was used for all transformation experiments. Gold particles were coated with DNA as described by Sanford et al. (1993) and Walter et al. (1994). The following physical bombardment conditions were used for all experiments: rupture disc-pressure 1100 psi, gap distance from rupture disc to macrocarrier 6 mm, macrocarrier travel distance 16 mm, microcarrier travel distance 6 cm and gold size 1.6 μm. This protocol was used to transfer recombinant plasmid pCAMSB1 that

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contained the *Bt* gene into teak nodule segments. Transgenic plants were regenerated subsequently from these explants and these transgenic plants were planted in the green house at FRIM.

The expression of *Bt* gene in transgenic teak was studied using transcription of mRNA and insect bioassay. The *Bt* mRNA synthesis from the transgenic teak was analysed by PCR following reverse transcription of total RNA as a template and these were carried out according to RevertAid™ First Strand cDNA Synthesis, Fermentas.

The amplified DNA product of reverse transcriptase–polymerase chain reaction (RT–PCR) was detected in the agarose gel at the site corresponding to 746 bp in length, representing the *Bt* gene. This RT–PCR product was further confirmed by RTPCR–Southern (Southern 1975) as shown in Figure 1. RTPCR–Southern is a procedure in which the cloned, labelled segment of DNA (*Bt* gene) is hybridised to the *Bt* gene fragment on a Southern blot.

RT–PCR was used to detect gene expression (William & Jeffrey 1995). In the RT–PCR analysis, fewer than 10 copies of target DNA are required and it has been successful when the RNA was isolated from a single cell (Razin et al. 1991). Due to this high sensitivity, RT–PCR is increasingly being used to quantitate small but physiologically relevant changes in gene expression that would otherwise be undetectable.

Insect bioassay was carried out to further confirm the expression of *Bt* gene in transgenic teak and this was done according to Bortan et al. (1987). Newly hatched *P. damastesalis* larvae were used in this study. The larvae were allowed to feed for three to four days prior to transfer to the test leaves. Tests were conducted using the whole leaf tissue in a PVC chamber, with 10 larvae per chamber. The larvae were placed directly on transgenic leaves and for control, on non-transgenic leaves. Feeding trials were carried out for five days, with daily monitoring of reductions in feeding and larval death.

The result showed that the skeletonisation area, i.e. leaf area eaten by larvae, on the non-transgenic teak leaves was 42–57 cm^2 (Figure 2a) whereas on the transgenic teak leaves, only 8–36 cm^2 (Figure 2b). Of the 10 larvae used in the bioassay, only six survived on the transgenic teak leaves while there was no mortality on the non-transgenic teak leaves (Figure 3). Death of larvae was due to the *Bt* toxins which directly affected the ionic permeability of the apical membrane of the midgut following ingestion of leaves. These *Bt* toxins are solubilised in the highly alkaline midgut lumen of the larvae and converted to active toxins by trypsin-like proteases. The activated toxins cross the peritrophic membrane to bind to specific receptors on the brush border apical membrane of the midgut columnar cells before being inserted into the membrane. Pore formation disrupts ionic gradients and osmotic balance across the apical membrane and eventually causes the epithelial midgut cells to lyse. This leads to a massive disruption of the epithelium and ultimately, the death of the larvae by starvation or septicaemia (Knowles 1994).

In conclusion, the insect bioassay showed that the *Bt* gene had been expressed in the transgenic teak leaves. Likewise, PCR analysis on transgenic teak confirmed that the inserted *Bt* gene was stable in the teak genomic DNA as the *Bt* gene was found transcribed to the mRNA in the transgenic teak.

Figure 1 Southern blot analysis of RTPCR-positive transgenic teak and non-transformed teak; lane 1: PCR product for negative control (RNA from non-transformed teak), lane 2: PCR product for positive control (recombinant plasmid, pCAMSB1), lane 3–15: PCR product for transgenic teak (RNA form transgenic teak); M: marker

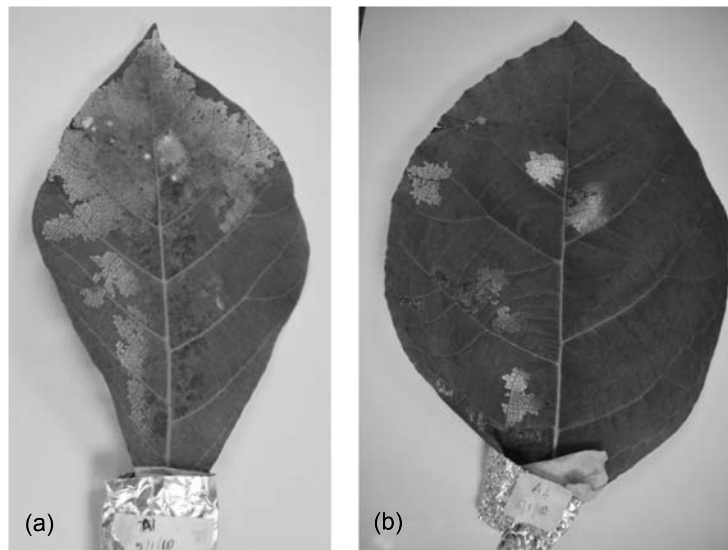

Figure 2 Skeletonisation phenomena on (a) non-transgenic and (b) transgenic teak leaves caused by *Paliga damastesalis* larvae

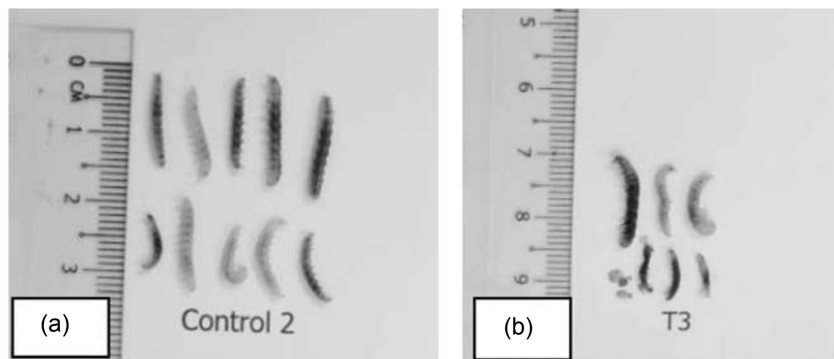

Figure 3 Larvae after being fed (a) non-transgenic and (b) transgenic teak leaves

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