

# INVESTIGATION OF CRY (INSECT RESISTANT) PROTEIN EXPRESSION IN TRANSFORMED TEAK (*Tectona grandis*) CLONE

A. Norwati, B. Norlia, H. Mohd Rosli, M. Norwati & S. Anee Suryani

Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor Darul Ehsan

([norwatia@frim.gov.my](mailto:norwatia@frim.gov.my))

## Introduction

Teak has been planted in Malaysia for considerable number of years and there are many systematic trials have been carried out in the north of West Malaysia and East Malaysia. Its high quality and aesthetically appreciated wood have made teak the primer timber for furniture making, carving and as an excellent building materials. However, teak plantations are confronted with problems include susceptibility of the species to various pests and diseases. Skeletonisation of leaves is a serious problem in teak plantations in Malaysia, recurring yearly during the flushing of new leaves.

Genetic engineering is one of the many approaches to solve the problems arises in teak. This approach is a very attractive technique for basic and applied forestry science, as it allows the investigator to add to or modify a specific trait of the target organism, without altering the genetic background of the selected clone. This is one reason for the effort in recent years directed to establishment of transformation protocols for forest trees of economic importance, such as *Eucalyptus grandis* (Maunder, 1997) or *Pinus radiata* (Walter et al., 1998).

Research on insect resistance included insertion of the toxin gene from *Bacillus thuringiensis* such as *cry1A(b)* gene or transfer of proteinase inhibitor genes such as cowpea trypsin inhibitor (CpTI) gene from other plant species has been carried out. Advances in tissue culture and transformation techniques have enabled gene transfer in woody plant species. Transgenic plants of *Larix decidua*, *Pinus radiata* and *Havea brasiliensis* have been produced.

At FRIM, teak tissue culture technique and genetic transformation system on the basis of a biolistic approach have been developed to produce transformed teak. This protocol was used in transferring *cry1Ab* gene into teak nodule segments. The transformed plants regenerated subsequently from these explants. These transformed plants were planted in the green house at FRIM's nursery. The present and the stability of *cry1Ab* gene in transformed teak were confirmed by molecular analysis, i.e. Polymerase Chain Reaction (PCR), Reverse Transcriptase PCR (RT-PCR) and Southern Blotting.

The *cry1Ab* gene which was inserted in teak genomic DNA, was resistant to certain insect especially *Paliga damastesalis* larvae that caused skeletonisation phenomena at teak trees. The expression of cry protein has been carried out through the insect bioassay study. The results from this study are important because the stability of *cry1Ab* gene in teak genomic DNA and its expression can make that transformed teak as an elite teak clone for the forest plantation programme.

## **Objectives**

The objectives for this study are:

- 1) To confirm the stability of inserted cry1Ab gene in genomic DNA of transformed teak
- 2) To study the expression of cry protein in transformed teak

Several activities have been carried out to achieving those objectives and the activities as listed below:

- 1) Sampling samples, DNA and RNA extraction and purification
- 2) PCR screening to check the stability of the cry1Ab gene in DNA genomic of transformed teak.
- 3) RT-PCR screening to check the stability of cry1Ab gene in RNA of transformed teak.
- 4) Bioassay – challenging the transformed leaves to the *Paliga damastesalis* larvae

## **Materials and methods**

### **DNA and RNA extraction and purification**

Transformed teak leaves have been collected from the sheltered house at FRIM's nursery and used for DNA and RNA extraction. The DNA extraction has been carried out using modified CTAB method (Doyle and Doyle 1987), and purified using High Pure PCR Template Preparation Kit (Roche Diagnostics, GmbH, Mannheim, German). RNA extraction was carried out by using Aurum Total RNA Mini Kit (Bio-Rad) and the extracted RNA was freeze/stored at  $-80^{\circ}\text{C}$ .

### **PCR and RT-PCR screening**

The pure DNA samples were used for PCR analysis of *cry1Ab* gene according to Norwati, 2005. Meanwhile for pure RNA, it was used for cDNA synthesis by using Plant RNA Extraction Maxi s-Prep Kit (GeneTACG BioScience) before proceed to RT-PCR analysis. The PCR product of DNA and RNA were used for PCR-Southern Blotting or RT-PCR Blotting by Southern hybridization technique (Southern 1975).

### **Insect Bioassay with *Paliga damastesalis* larvae**

A detached leaf bioassay was used to test for feeding efficacy of the transgenic plants. Teak leaves were collected in the morning from control containment at FRIM's nursery. Then the leaves were washed under tap water and the petiole was covered with the wet tissue paper and aluminium foil. The leaves were then placed in the PVC chamber and fifteen *P. damastesalis* larvae were released on it. The observation was carried out for 5 days.

## Results and discussion

The *cry1Ab* gene in the transformed teak need to be stable in order for cry protein could be used as an *ex situ* bioinsecticide. Two methods have been used to analyse the gene stability in transformed teak, first, by using the extracted and purified DNA (Figure 1). The crude extracted DNA (Fig. 1A) were contaminated with the RNA and need to purified (Fig. 1B) before used for PCR analyses.

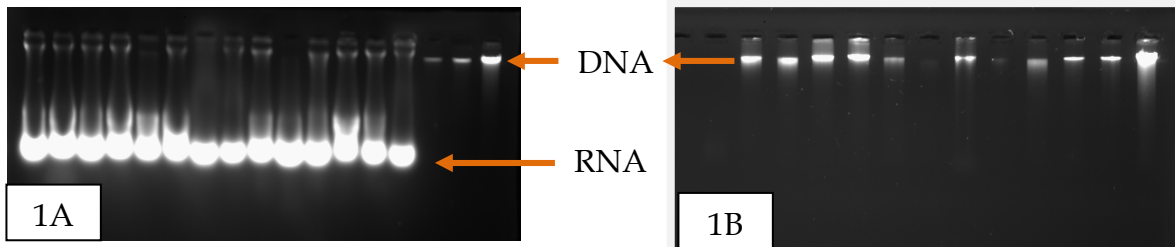


Figure 1: 1A - Crude DNA extracted from teak leaves  
1B – Purified DNA

The purified DNA used to check the presence of transferred *cry1Ab* gene in transformed teak using the PCR technique. Primers for *cry1Ab* gene were designed to amplify a 746 bp internal fragment of the *cry1Ab* gene. Following PCR amplification the expected 746 bp band was visible on the agarose gel (Figure 2A).

PCR-Southern blot was carried out on PCR product from each/number of plants. As shown in Figure 2B, bands were detected in transformed plants representing the fragment of *cry1Ab* gene in size 746bp.

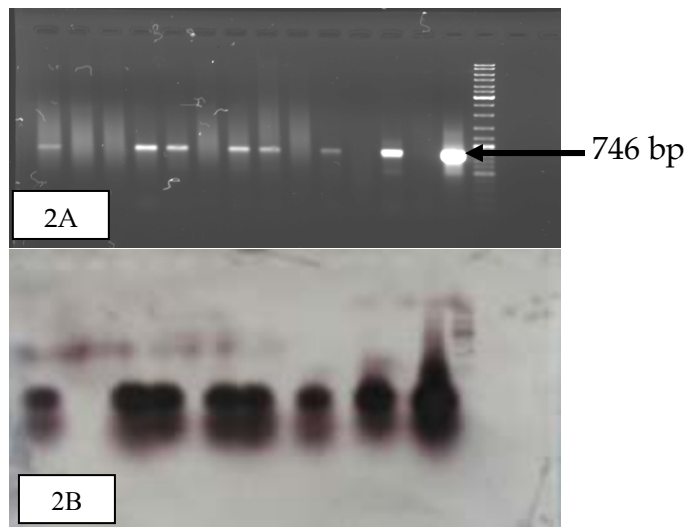


Figure 2: 2A - PCR product for *cry1Ab* gene  
2B – PCR-Southern for *cry1Ab* gene

Second method to check the stability of the inserted gene was by using extracted mRNA. Transcription of mRNA is involved in the gene expression study and several techniques are currently available to detect gene expression. These include the Northern Blot, *in situ* hybridization, and the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) (William and 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). Because of this high sensitivity, RT-PCR is being used increasingly to quantitate small but physiologically relevant changes in genes expression that would otherwise be undetectable.

Extracted RNA was also used to study the gene stability. The *cry1Ab* mRNA synthesis from the transformed plants was analyzed by PCR following reverse transcription of total RNA as a template. Total RNA extracted (Figure 3) was used for reverse transcription to synthesis cDNA and were later used in subsequent PCR amplifications.

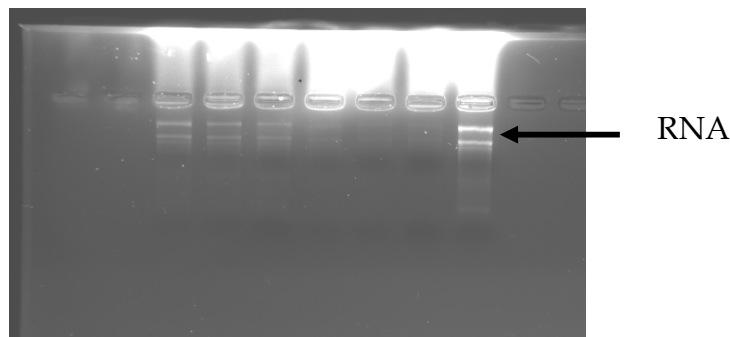


Figure 3: Extracted RNA from transformed teak leaves

The amplified DNA product of RT-PCR was detected in the agarose gel at the site corresponding to 746 bp in length (Figure 4A), representing the *cry1Ab* gene. This RT-PCR product was further confirmed by RTPCR-Southern as shown in Figure 4B.

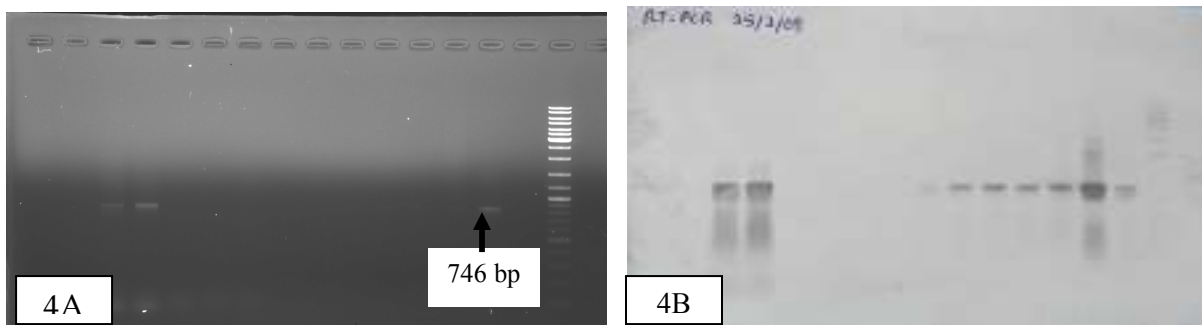


Figure 4: 4A – RT-PCR product for *cry1Ab* gene  
4B – RTPCR- Southern for *cry1Ab* gene

In the insect bioassay, fifteen *P. damastesalis* larvae were released on transgenic and non-transgenic teak leaves. The result showed that the skeletonization area on the non-transgenic teak leaves were about 42-57cm<sup>2</sup> and most of the survival larvae were in the 3<sup>rd</sup> instar. The skeletonisation area that occurred on the transgenic teak leaves was mostly small at about 8 – 36cm<sup>2</sup> (Figure 5). Small number of larval survival or growth was observed on the transgenic teak leaves. Kumar, H. & Kumar V., (2004) reported there was no larval survival or growth was observed on the BT plants.

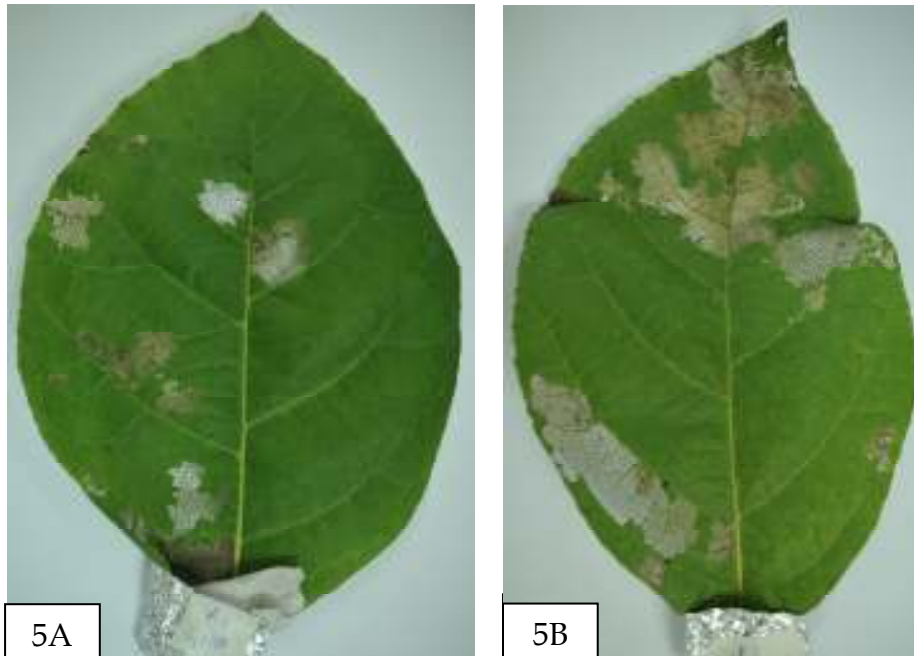


Figure 5: Skeletonisation phenomena that caused by *Paliga damastesalis* larvae;  
5A: Transgenic leaf  
5B: Non-transgenic leaf

In conclusion, PCR analyses on transformed teak confirmed that the inserted *cry1Ab* gene is stable in the teak genomic DNA as the *cry1Ab* gene was been found transcribed to mRNA in the plant. Whilst, the insect bioassay showed that the cry protein has been expressed in the transgenic teak leaves.

#### **Acknowledgement**

The authors would like to thank the staffs of Genetic Laboratory, FRIM, especially Mrs. Sharifah Talib and Miss Suryani Che Seman for their assistance in this study. The study was funded by the Ministry of Science and Innovation, Malaysia under e-Science fund (02-03-10-SF0068).

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