Natural Rubber Research, **25**(1): 21-30, 2012

EFFICIENT SCREENING OF AFLP PRIMER COMBINATIONS FOR EVALUATING GENETIC DIVERSITY AMONG CULTIVATED RUBBER (*HEVEA BRASILIENSIS*) CLONES

C. Bindu Roy, Minimol Ravindran and Thakurdas Saha

Rubber Research Institute of India, Kottayam-686 009, Kerala, India

Received : 20 October 2011 Accepted : 26 December 2011

Roy, C.B., Ravindran, M. and Saha, T. (2012). Efficient screening of AFLP primer combinations for evaluating genetic diversity among cultivated rubber (*Hevea brasiliensis*) clones. *Natural Rubber Research*, **25(**1): 21-30.

Multilocus amplified fragment length polymorphism (AFLP) markers are used in genetic mapping, diversity analysis and genotyping. Success of the technique mainly depends on the identification of the primer combinations for selective amplification. In this study a simple method was demonstrated to identify the potential primer combinations using a mini polyacrylamide gel electrophoresis (PAGE) followed by silver staining. A modified silver staining protocol was developed to save time without compromising on the sensitivity. Further, the utility of the protocol in developing AFLP markers in rubber (*Hevea brasiliensis*) for assessment of genetic diversity among cultivated clones from different South East Asian rubber growing countries was demonstrated.

Keywords: AFLP, Denaturing mini PAGE, Genetic diversity, *Hevea brasiliensis*, Silver staining

INTRODUCTION

Molecular markers are a pre-requisite for genome analysis of any organism. During the last two decades, several techniques *viz*. RFLP, RAPD, AFLP, microsatellite and SNP have been developed for polymorphism studies. AFLP marker system is widely used for genomic characterization, genome mapping and also to develop diagnostic markers that are linked to various agronomic traits. This technique is PCR-based and produces dominant markers having a high multiplex ratio because of the random placement of restriction sites between different genomes combined with nucleotide sequence variability within a short stretch of DNA directly flanking these restriction sites (Vos *et al*., 1995).

Although the AFLP technique is tedious and time consuming, this appeared to be the marker of choice for its multi-allelic properties. Success in developing AFLP markers depends on several important steps: (1) complete digestion of DNA samples with two restriction enzymes; (2) adaptor ligation of the restricted DNA; (3) pre-amplification of the adaptor ligated DNA sample using complementary oligonucleotides as primers; (4) selective amplification of the pre-amplified product using different primer combinations with a change at their 3´ ends

Correspondence: Thakurdas Saha (Email: saha@rubberboard.org.in)