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GENETIC FIDELITY ASSESSMENT OF TISSUE CULTURE-DERIVED *Neolamarckia cadamba* PLANTLETS USING DNA-BASED MARKERS

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ABSTRACT

Neolamarckia cadamba is one of the fast-growing tree species selected for industrial tree plantations (ITPs). As demand and supply of true-to-type planting material are increasing, micropropagation of elite *N. cadamba* planting material is required for the sustainable development of ITPs. However, somaclonal variation among tissue culture-derived plantlets is a bottleneck in micropropagation. The present study described the genetic fidelity of *in vitro* regenerated *N. cadamba* plantlets from first, second, third and sixth subcultures. These plantlets were obtained from axillary shoot multiplication and repeated subcultures of microshoots with three weeks interval period, which initially developed from nodal explants. By using five random amplified length polymorphism (RAPD) and two inter-simple sequence repeats (ISSR) primers, a total of 9,334 bands and 2,760 bands were amplified respectively, from a total number of 164 tested plantlets. The banding profiles for each primer was highly uniform, and the DNA bands were monomorphic across all tissue culture-derived plantlets from every four subcultures compared to the stock plants. A target gene-specific marker was also employed to detect single nucleotide polymorphism (SNP) within the targeted genomic sequence of *Susy* gene. There was no SNP detected from all the analysed plantlets. The current findings ascertained the efficiency and reliability of the *N. cadamba* micropropagation protocol at least up to six subculture cycles for mass production of true-to-type plantlets.

Key words: *Neolamarckia cadamba*, genetic fidelity, DNA marker, SNP, micropropagation

INTRODUCTION

Micropropagation of *Neolamarckia cadamba* (Roxb.) Bosser is vital for large scale commercial production of quality planting material for industrial tree plantations (ITPs) in Malaysia in order to fulfil the increasing demand for timber in future. This tree species is belonging to Rubiaceae family and widely distributed in South Asia and Southeast Asia, such as Malaysia, Indonesia, China, India, Thailand, Vietnam and Papa New Guinea (Krisnawati *et al*., 2011; Tchin *et al*., 2018). It is a light hardwood species that frequently harvested as raw materials for plywood, hardboard, paper, and furniture. It is also used for ornamental purposes, while its leaf and fruit extracts and dried bark are used for pharmaceutical purposes (Zaky *et al*., 2014; Dwevedi *et al*., 2015; Pandey & Negi, 2016).

Micropropagation is a well-established plant tissue culture technique that adopted to propagate commercially important plants by using part of the plants as explants. This technique is widely used for rapid clonal propagation in order to supply a large scale of planting material for afforestation and elite genotypes preservation (Kataria *et al*., 2013; Alizadeh *et al*., 2015). Hence, a high degree of genetic fidelity among the tissue culture-derived plantlets is very critical. The somaclonal variations could be detected in tissue culture-derived plantlets, which is the major bottleneck in the micro-propagation of plants. Level of plant growth regulators, types of explants, time in tissue culture conditions and subculture number are frequently reported as the main factors of this variation (Bairu, 2011; Krishna *et al*., 2016). It is reported that shoot proliferation from explants with pre-existing meristems poses a lower genetic instability risk (Mohanty *et al*., 2012; Behera *et al*., 2018). Besides,

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