ABSTRACT

Neolamarckia cadamba is a lightweight hardwood timber tree species that is known to be fast-growing. Increasing demand for high quality planting material promotes the need for producing new variations. *In vitro* mutagenesis of *N. cadamba* by using ethyl methanesufonate (EMS) could accelerate the natural mutation and breeding programme for superior planting material. Thus, the current study aimed to determine the lethal concentration 50 (LC_{50}) of EMS, evaluate responses of EMS-treated *N. cadamba* explants, and screen putative mutants using gene-specific markers. Nodal explants of *N. cadamba* were subjected to 0 (control), 0.1, 0.3, 0.6, and 1% EMS for 1-, 2-, 3-, and 4-hour treatments. The LC_{50} of EMS treatments 1.2589%, 0.3715%, and 0.3311% for 2-, 3-, and 4-hour treatment, respectively. Variability of responses among the treated explants were observed by the increasing concentrations of EMS and duration of treatments. Percentages of survival, shoot formation, and callus formation were reduced, while no clear trend was observed from plant heights, rooting percentages, and survival after the acclimatization stage. Morphological abnormality among the survived explants was also identified in the current study. The SNPs analysis in *cellulose synthase* gene from three mutation lines revealed that 166 SNPs were found in C1 (Tajima's D = -0.5612, π = 0.04235), 138 SNPs in C3 (D = -2.34147, π = 0.00567), and 82 SNPs in C5 (D = -1.83398, π = 0.00480). Meanwhile for *sucrose synthase* gene, 10 SNPs were screened in C3 (D = -1.50776, π = 0.00093), and 5 SNPs in C5 (D = -0.02839, π = 0.00083). From this molecular DNA marker-assisted screening, 46 putative mutant seedlings were identified (C1 = 12, C3 = 17, and C5 = 17). The current *in vitro* mutagenesis approach has shown the effectiveness of using EMS to increase genetic variability of *N. cadamba* trees, mimicking natural mutation process in the

wild population. Current molecular DNA marker-assisted screening approach is also considered to be practical in mutant screening.

Keywords: *Neolamarckia cadamba*, ethyl methanesulfonate, single nucleotide polymorphism, *cellulose synthase*, *sucrose synthase*

Mutasi in vitro Neolamarckia cadamba (Kelampayan) menggunakan Etil metanasulfonat (EMS)

ABSTRAK

Neolamarckia cadamba adalah spesies pokok kayu balak keras ringan yang dikenali dapat tumbuh dengan cepat. Peningkatan permintaan untuk bahan penanaman berkualiti tinggi menggalakkan keperluan untuk menghasilkan variasi baharu. Mutagenasi in vitro N. cadamba dengan menggunakan etil metanasulfonat (EMS) boleh mempercepatkan mutasi semula jadi dan program pembiakan bagi penghasilan bahan penanaman yang unggul. Kajian ini bertujuan untuk menentukan kepekatan mematikan 50 (LC_{50}) EMS, menilai tindak balas eksplan N. cadamba yang dirawat oleh EMS, dan menyaring mutan putatif menggunakan penanda khusus gen. Eksplan nod N. cadamba dikenakan 0 (kawalan), 0.1, 0.3, 0.6, dan 1% EMS untuk 1-, 2-, 3- dan 4-jam rawatan. Nilai LC_{50} rawatan EMS adalah 1.2589%, 0.3715%, dan 0.3311% bagi 2, 3, dan 4 jam rawatan masing-masing. Berbagai-bagai tindak balas dalam kalangan eksplan yang dirawat telah diperhatikan melalui peningkatan kepekatan EMS dan tempoh rawatan. Peratusan kelangsungan hidup, pembentukan tunas, dan pembentukan kalus adalah dikurangkan, manakala tiada corak perubahan yang jelas telah diperhatikan dari nilai ketinggian tanaman, peratusan pengakaran, dan peratusan kelangsungan hidup selepas tahap aklimatisasi. Keabnormalan morfologi di antara eksplan yang hidup juga telah dikenal pasti dalam kajian ini. Analisis SNPs dalam gen cellulose synthase daripada tiga kelompok mutasi mendedahkan 166 SNPs dijumpai di C1 (Tajima's D = -0.5612, π = 0.04235), 138 SNPs di C3 (D = -2.34147, π = 0.00567), dan 82 SNPs di C5 (D = -1.83398, π = 0.00480). Manakala, bagi gen sucrose synthase, 10 SNPs telah disaring di C3 (D = -1.50776, π = 0.00093), dan 5 SNPs di C5 (D = -0.02839, π = 0.00083). Melalui kaedah penyaringan dibantu penanda DNA molekul ini,

46 anak pokok mutan putatif telah dikenal pasti (C1 = 12, C3 = 17, dan C5 = 17). Kaedah mutagenasi in vitro ini telah menunjukkan kebersanan untuk menggunakan EMS dalam meningkatkan variasi genetik pokok N. cadamba, mengajuk proses mutasi semula jadi di populasi liar. Pendekatan saringan dibantu penanda DNA molekul ini juga dianggap praktikal dalam penyaringan mutan.

Kata kunci: *Neolamarckia cadamba, etil metanasulfonat, polimorfisme nukleotida tunggal, selulose sintase, sukrose sintase*