COMPARATIVE GENETIC DIVERSITY STUDIES OF *SHOREA CURTISII* (DIPTEROCARPACEAE): AN ASSESSMENT USING SSR AND DAMD MARKERS

W. S. Ho[1,*], R. Wickneswari[2], M. C. Mahani[2] & M. N. Shukor[2]

[1]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[2]School of Environmental and Natural Resource, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Darul Ehsan, Malaysia

Received April 2004

HO, W. S., WICKNESWARI, R., MAHANI, M. C. & SHUKOR, M. N. 2006. Comparative genetic diversity studies of *Shorea curtisii* (Dipterocarpaceae): an assessment using SSR and DAMD markers. Genetic diversity of *Shorea curtisii* from different age cohorts, namely, seedlings, saplings and adult trees were determined using six SSR loci and 33 DAMD loci. To quantify genetic diversity in *S. curtisii* we used standard genetic diversity measures for SSR data, and both phenotypic and genotypic methods with null-allele frequency corrected for deviation from Hardy-Weinberg equilibrium (HWE) with SSR markers for DAMD data. Results showed that the genetic diversity measured using DAMD genotypic method was lower than those derived from SSR data based on the same set of samples. This suggests that DAMD allele frequencies corrected from HWE deviation using fixation index derived from SSR data may be underestimated. The genetic distance matrix generated from SSR data was significantly correlated with DAMD genotype data ($r = 0.990$, $p < 0.05$), indicating a similar genetic structure of *S. curtisii* being depicted by both marker types among the age cohorts tested. The relationship between sample size and genetic diversity measures demonstrated a threshold level, i.e. $n = 20$ and $n = 30$ for seedlings and saplings respectively, and $n = 15$ and $n = 20$ for adult trees revealed by SSR and DAMD markers respectively. Genetic diversity measures dropped drastically below these levels. These results further imply that a highly heterogeneous population was observed in *S. curtisii* from each age cohort. Collectively, both SSR and DAMD markers have good genome coverage in the *S. curtisii* genome.

Keywords: DNA analyses, polymorphisms, genetic distance, tropical timber species, hill dipterocarp forest

HO, W. S., WICKNESWARI, R., MAHANI, M. C. & SHUKOR, M. N. 2006. Kajian perbandingan kepelbagaian genetik *Shorea curtisii* (Dipterocarpaceae): satu penilaian menggunakan penanda SSR dan DAMD. Kepelbagaian genetik bagi *Shorea curtisii* daripada kohort umur berbeza, iaitu anak benih, anak pokok dan pokok matang telah ditentukan daripada enam lokus SSR dan 33 lokus DAMD. Bagi menganggar kepelbagaian genetik dalam *S. curtisii* kami menggunakan parameter kepelbagaian genetik piawai untuk data SSR. Bagi data DAMD pula kami menggunakan kedua-dua kaedah fenotip dan genotip dengan frekuensi alel nol dibetulkan penyisihannya daripada keseimbangan Hardy-Weinberg (HWE) dengan penanda SSR. Hasil kajian menunjukkan bahawa anggaran kepelbagaian genetik dengan kaedah genotip DAMD adalah lebih rendah berbanding dengan data SSR menggunakan set sampel yang sama. Ini mencadangkan frekuensi alel nol DAMD yang dibetulkan daripada sisihan HWE menggunakan indeks penetapan daripada data SSR mungkin di bawah jangkaan. Matriks jarak genetik yang dijana daripada data SSR berkorelasi secara signifikan dengan data genotip DAMD ($r = 0.990$, $p < 0.05$). Ini menunjukkan bahawa struktur genetik yang serupa bagi *S. curtisii* telah digambarkan oleh kedua-dua jenis penanda antara kohort umur yang diuji. Hubungan antara saiz sampel dengan kepelbagaian genetik memaparkan satu aras ambang, iaitu $n = 20$ dan $n = 30$ masing-masing bagi anak benih dan anak pokok, serta $n = 15$ dan $n = 20$ bagi pokok matang yang masing-masing dicerap oleh penanda SSR dan DAMD. Nilai kepelbagaian genetik menurun dengan mendadak di bawah nilai ambang. Hasil ini turut menunjukkan bahawa *S. curtisii* daripada setiap kohort umur mempunyai tahap keheterogenan yang tinggi. Secara keseluruhan, kedua-dua penanda SSR dan DAMD meliputi taburan yang luas dalam genom *S. curtisii*.

Introduction

Genetic diversity provides the template for adaptation and evolution of populations and species (Thomas *et al.* 1999). Therefore, preservation and maintenance of genetic diversity of all species are important for both short-term adaptations to environmental change and long-term impact on

**E-mail: wsho@frst.unimas.my*

species and communities (Templeton 1995). Loss of genetic diversity immediately after harvesting has been recognized as a potentially serious problem in commercially managed forest tree species (Buchert *et al.* 1997, Wickneswari *et al.* 1999, Rajora *et al.* 2000, Wickneswari & Lee 2001, Lee *et al.* 2002). Hence, assessment of genetic diversity has a high priority in developing successful management guidelines and effective conservation strategies or programmes at the species level and above. Indeed, conservation of genetic diversity may be one of the most important issues influencing future forestry practices (Namkoong 1992).

In recent years, there has been increasing interest in the use of DNA-based markers for a variety of applications in population genetics, conservation and tree improvement. Both SSRs (simple sequence repeats) and DAMD (direct amplification of minisatellite-region DNA) markers show much promise in this regard. DAMD has recently been used to identify wheat (Somers *et al.* 1996, Bebeli *et al.* 1997) and rice (Zhou *et al.* 1997), and quantify genetic variation in *Phaseolus vulgaris* (Métais *et al.* 2001), *Asimina triloba* (Rogstad *et al.* 1991) and *Plantago major* (Schaal *et al.* 1991). Meanwhile SSR markers have been used to quantify genetic diversity and examine population differentiation in agricultural crops (Morgante *et al.* 1994, Maughan *et al.* 1995) and trees, including radiata pine (Smith & Devey 1994), *Pithecellobium elegans* (Chase *et al.* 1996), *Quercus robur* (Lefort *et al.* 1998) and *Pinus strobus* (Rajora *et al.* 2000).

Since DAMD is a dominant marker type, the presence and absence of a band are defined as representing two alleles at a locus and, therefore, estimation of genetic diversity values must assume Hardy-Weinberg equilibrium(HWE). Consequently, these data do not allow for the estimation of allelic richness, effective number of alleles or fixation indices. However, with the advancement of PCR technology and statistical analysis methods, this problem can be minimized. For instance, Chong *et al.* (1994) analysed RAPD data genotypically by incorporating codominant allozyme data for the estimation of null-allele frequencies. SSRs, on the other hand, are codominant and tend to have multiple alleles per locus so that individuals can be identified as homozygotes or heterozygotes. The data can be used to compare observed and expected heterozygosities and other genetic diversity parameters. However, there are several shortcomings with SSR markers, i.e. the development costs of SSR markers are high if primers are not yet available, heterozygotes may be misclassified as homozygotes when null-alleles occur due to mutation in the primer annealing sites, underlying mutation model (IAM or SMM) is largely unknown, and homoplasy due to different forward and backward mutations may underestimate genetic divergence.

This study was undertaken to quantify genetic diversity of a tropical timber species, namely, *Shorea curtisii* (Dipterocarpaceae) using SSR and DAMD markers. This species is locally known as meranti seraya and is an emergent, abundant canopy tree species occurring in the ridges of hill dipterocarp forests (300–850 m) in Peninsular Malaysia. It is found in southern Thailand, Peninsular Malaysia, Sumatra and Borneo. *Shorea curtisii* flowers heavily at irregular intervals (3–5 years) after severe drought, in synchrony with mass flowering of other emergent tree species. The flowers are hermaphroditic, pollinated by insects such as thrips and meliponid bees, and produce single-seeded fruits; more than half of the mature seeds fall within 20 m of the parent tree. It is one of the main sources of the light hardwood of dark red meranti timber, which has already established a market both locally and overseas, and are used for furniture, high-class interior finishing, flooring, panelling, moulding and veneers. In this study we also examined the correlation coefficient of SSRs and DAMD based on genetic distance on the same set of samples of *S. curtisii* used to quantify genetic diversity. Both SSR and DAMD markers have advantages and limitations as genetic markers for assessing genetic diversity, genetic erosion resulting from logging practices and population structure. Therefore, combination of these two markers would yield complementary information to quantity genetic diversity.