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Determination of genetic diversity and genetic relatedness using ISSR markers in Duabanga moluccana

Diyanah Musfirah Jamal[a], Wei-Seng Ho[a]*, Kit-Siong Liew[a], Shek-Ling Pang[b]

[a]Forest Genomics and Informatics Laboratory (fGiL), Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak

[b]Applied Forest Science and Industry Development (AFSID), Sarawak Forestry Corporation, 93250 Kuching, Sarawak

*Corresponding Authors: e-mail: wsho@unimas.my

Abstract

Duabanga moluccana or locally known as Sawih is a widely known forest tree species for its multi-purpose timber and other natural products such as fibers. Genetic diversity is important for the maintenance of the viability and the adaptive potential of populations and species. This information will be a basis for establishing tree improvement programme and for management or conservation of natural communities. The present study was aimed to assess the genetic diversity and genetic relatedness of D. moluccana trees collected from three natural forests in Sarawak, namely, Mukah, Tatau and Niah using inter simple sequence repeat (ISSR) markers. A total of 73 loci from 90 individuals were successfully amplified using three selected ISSR primers, namely, $(AC)_{10}$, $(ACC)_6G$ and $ACG(GT)_7$. The Shannon's diversity index showed that D. moluccana trees in Mukah (0.499) was the most diverse population compared to Tatau (0.380) and Niah (0.330). Neighbor joining tree was also constructed to determine the genetic relationship among the three D. moluccana populations. The populations were completely clustered into three main groups, in accordance to their corresponding population and origin. Based on the results, it implies that D. moluccana trees are genetically diverse and related both within and among populations.

Keywords: Inter Simple Sequence Repeat (ISSR), Duabanga moluccana, genetic diversity, genetic relatedness

1. Introduction

Establishment of forest tree plantations is becoming more crucial as forest resource reduction increases exponentially due to human activities. Most losses are measured in square kilometers, but a more precise loss of forest tree resources cannot be measured. As forests disappear, so do their genetic resources (Arnold, 1991; Sedjo and Lyon, 1990). For this reason, forest tree plantation development is a necessity rather than a choice to alleviate the problem arisen from forest degradation, to reinstate forest system function and productivity (Kidd and Pimentel, 1992). Forest tree such as timber has a lot of benefits and has been long used by human for many purposes. It has been used as sawn timber, construction materials, fodder, fuel wood, shelter and medicine. Traditionally, forest tree plantations use planting material from wild-type tree. Today, biotechnology allows selection of genetically good traits tree that gives better quality and higher yields. Obviously, forest tree plantation has a key role to play in the long-term timber production.

Duabanga moluccana or locally known as Sawih is one of the selected timber tree species to be planted in Sarawak due to its fast-growing properties and high commercial value in wood industry (Figure 1). Apart from making boxes and firewood, they are also numerously used for house and boat building. Additionally, D. moluccana is suitable for interior paneling, matches, moulding and pulping (CIRAD, 2010). Plantation of D. moluccana has become an important management strategy in rehabilitation activities such as the planting of the trees in the attempt of re-establishing and enhancing forest structure and diversity.

Inter simple sequence repeats (ISSR) are DNA fragments between two adjacent, oppositely oriented microsatellite regions (Moreno et al., 1998). ISSR marker is chosen as molecular marker for this study as it permits detection of polymorphism in microsatellites and inter microsatellites loci without previous knowledge of the DNA sequence (Gupta et al., 1994). Furthermore, ISSRs are randomly distributed throughout the genome. ISSR produces informative loci which is suitable to discriminate closely related genotype variants (Roose et al., 1997).

A study carried out by Moreno et al. (1998) demonstrated that the reproducibility of ISSR (91.8%) was higher than RAPD (85.8%) due to the use of longer ISSR primers (16 - 25 mers) which permits much more stringent annealing conditions. ISSR also generates a large number of markers by amplifying multiple microsatellite loci, thus allowing screening of a large number of samples in a single gel (Nagaraju et al. 2001). Therefore, these markers are useful in studies on genetic diversity (Okun et al. 2007, Chezhian et al. 2010), gene tagging (Ammiraju et al. 2001), cultivar identification (Wong et al. 2009), genome mapping (Zietkiewicz et al. 1994, Godwin et al. 1997) and phylogenetic analysis (Dogan et al. 2007). In the present study, we aimed to determine the genetic diversity and genetic relatedness of D. moluccana from Niah, Tatau and Mukah populations using ISSR markers. We hope this baseline information could pave the way to formulate a better conservation and tree improvement programmes of D. moluccana in future.

2.0 Materials and Methods

2.1 Sample collection and DNA isolation

The leaf samples of D. moluccana were collected from three natural forests in Sarawak, namely Mukah, Tatau and Niah (Figure 2). The leaf samples were preserved using CTAB NaCl method. Total genomic DNA was extracted from leaf samples using a modified CTAB method (Doyle and Doyle, 1990). The quality and quantity of the extracted DNA were estimated spectrophotometrically and verified on 0.8% agarose gel. The DNA was then subjected to ISSR-PCR amplifications.

Figure 1. Duabanga moluccana (a) Seedlings, (b) Flowers, (c) Matured fruits and (d) Mature tree.

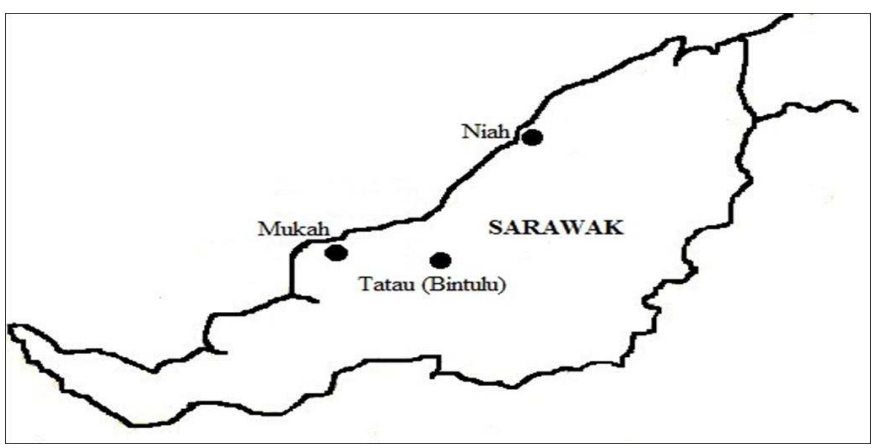

Figure 2. Locations of the Duabanga moluccana populations studied.

2.2 ISSR-PCR assay

Three microsatellite primers, namely, $(AC)_{10}$, $(ACC)_6G$ and $ACG(GT)_7$ were used in this study to amplify the ISSR regions. PCR was carried out using a Mastercycler Gradient PCR (eppendorf, Germany). PCR amplification was carried out in 25 µl reaction volume containing 1 x PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.5 unit of Taq DNA polymerase (Invitrogen, USA), 10 pmol of primer and 5 ng/µl of DNA. The thermal cycling profile was programmed at 94°C for 2 minutes as initial denaturation step, 40 cycles of 30 seconds at 94°C, 30 seconds at at 59°C for $(AC)_{10}$, 60.0°C for $(ACC)_6G$ and 48.0°C for $ACG(GT)_7$, 1 minute at 72°C and final extension step at 72°C for 10 minutes. The amplification products were subjected to 1.5% agarose gel electrophoresis at 80V for 2 hours and stained with 1x Gelstar Nucleic Acid Gel Satin (Cambrex, USA) for 30 minutes. The gel with amplification products was visualized using the UV transilluminator and documented using Geliance 200 Imaging System (PerkinElmer, USA).

2.2 Data analysis

Amplified DNA bands at different loci were analysed based on the method described in Ho et al. (2010) and Tiong et al. (2014). The genetic diversity parameters were estimated using POPGENE version 1.32 software (Yeh et al., 1997). To determine the genetic relationship between all D. moluccana trees, a consensus neighbour-joining (NJ) tree was constructed based on shared allele distance, D_{SA} (Chakraborty and Jin, 1993). The PowerMarker 3.25 software was used to generate the shared alleles matrices while NJ tree was generated using MEGA 4 software (Tamura et al., 2007).

3.0 Results and Discussion

All the D. moluccana trees collected from Mukah, Niah and Tatau were successfully screened using the three selected primers. The range for scoring the amplified bands was determined at 200 bp to 1,500 bp as all the primers employed in the ISSR-PCR generated a consistent banding profile in this range (Figure 3).

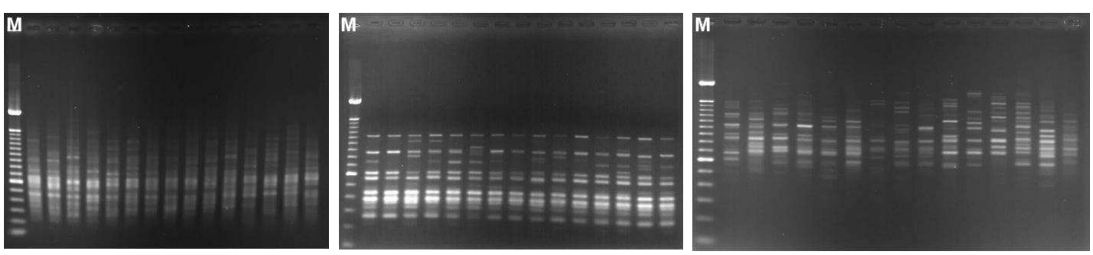

Figure 3. DNA profile using (a) $(AC)_{10}$, (b) $(ACC)_6G$ and (c) $ACG(GT)_7$ primers. M is 100 bp DNA ladder.

The genetic diversity estimation of D. moluccana is summarized in Table 1. A total of 73 loci were generated and the percentage of polymorphic loci ranged from 78.08% to 100.0%, with an average of 87.67%. The mean Shannon's diversity indices (I) ranged from 0.330 to 0.499 meanwhile the mean Nei's gene diversity was in the range of 0.214 to 0.331 with an average of 0.264. The results also showed that D. moluccana population was genetically more diverse in Mukah followed by Tatau and Niah. The genetic diversity of D. moluccana was moderately high when compared to other forest tree species such as Sonneratia

paracaseolaris with 81.37% polymorphic loci (Li and Guizhu, 2009), 85.7% in Swertia chirayita (Joshi and Dhawan, 2007), 89.11% in Dalbergia sissoo (Wang et al., 2011) and 48.41 - 58.2% in Neolamarckia cadamba (Ho et al., 2010; Tiong et al., 2014) amplified by ISSR primers.

Table 1. Genetic diversity estimation of Duabanga moluccana

Population	Genetic diversity parameter		
	I	h	P (%)
Mukah	0.499	0.331	100.0
Tatau	0.380	0.248	84.93
Niah	0.330	0.214	78.08
Mean	0.403	0.264	87.67

I = mean Shannon diversity index; h = mean Nei's genetic diversity;
P = percentage of polymorphic bands

The coefficient of population differentiation (G_{st}) of D. moluccana was at the moderate level (G_{st} = 0.133). The high G_{st} value has been reported in other species such as in Ceriops decandra (0.882) (Tan et al., 2005), Ceriops tagal (G_{st} = 0.529) (Ge and Sun, 2001), Taxus fauna (G_{st} = 0.5842) (Shah et al., 2008) and Hagenia abyssinica (G_{st} = 0.25), (Feyissa et al., 2007) and Neolamarckia cadamba (G_{st} = 0.2013) (Ho et al., 2010; Tiong et al., 2014). Meanwhile the low G_{st} value was also observed in other species such as such as Calocedrus macrolepis, G_{st} = 0.042 (Wang et al., 2003), Shorea leprosula, G_{st} = 0.085 (Lee et al., 2000), Larix potaninii, G_{st} = 0.116 (Yu et al., 2006).

According to Hamrick and Loveless (1989), tropical trees frequently express high level of genetic diversity. The genetic structure of a species is affected by many evolutionary factors. Based on its life history features, D. moluccana, is a long-lived woody plant and is pollinated by bat which is nocturnal mammal capable of sustained flight for an effective cross-pollination. Moreover, its seeds have tails that aid the seed dispersal mechanisms by wind. These characteristics confer a higher possibility of gene flow occurrences between populations, which is factor that capable of increasing genetic diversity in natural population.

Figure 4 illustrated the neighbour-joining tree that was generated by MEGA 4 software to determine the genetic relationship between all the 90 trees of D. mollucana from the three populations. Three distinct groups were identified based on the genetic relatedness of D. moluccana trees and this correlates well to the relatively moderate level of gene differentiation coefficient obtained from the present study.

The first group mostly coded in blue consisted of 30% individuals which were from Mukah population, except for one individual coded in green which was from Niah (i.e, N11). This sample is genetically more closely related with the individuals from Mukah. The second group which was coded in red consisted of 33% of individuals which was from Tatau population. However, there were also 4.4% individuals from Mukah population (i.e., M02, M08, M10 and M29) that clustered in this group and formed loose clusters despite being collected from different location. The third group consisted of 32.2% of individuals only from Niah population. Mukah and Tatau populations were genetically more similar compared to Mukah and Niah populations. This result can be supported by the corresponding geographical distances between the three natural forests, of which Mukah is geographically closer to Tatau (100 km) compared to Niah (145 km).

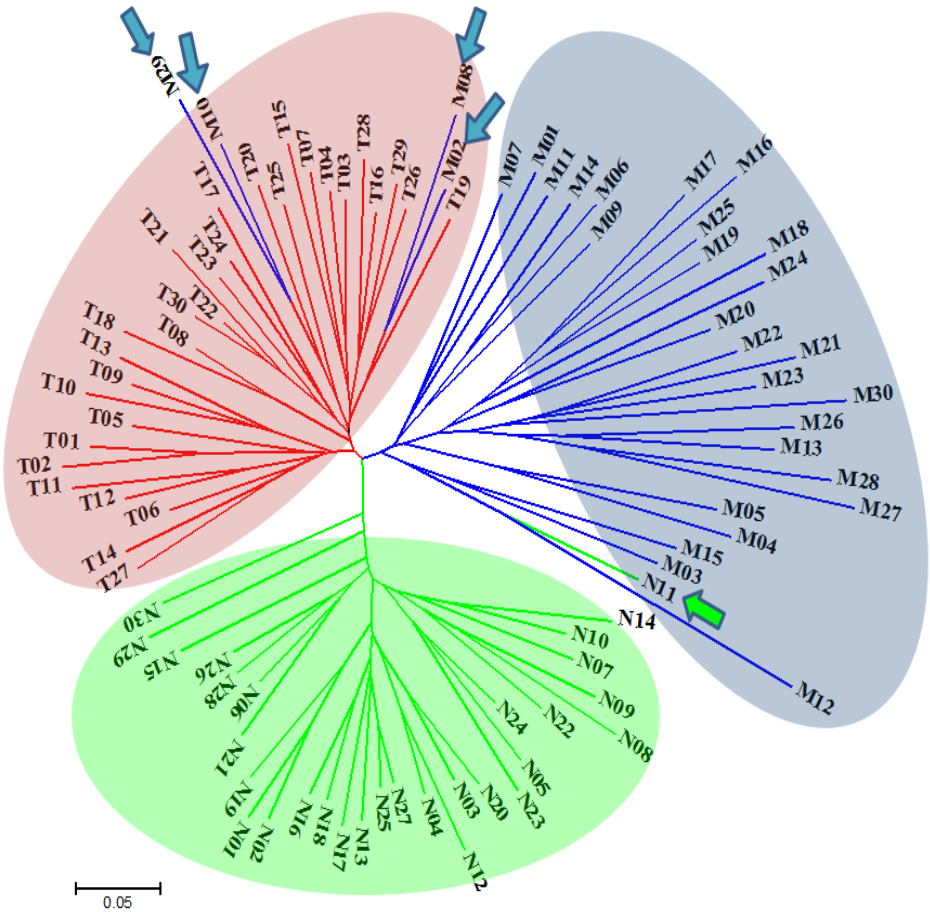

Figure 4. Neighbour-joining tree of D.moluccana populations in Sarawak based on shared alleles matric.

If migrants move from their population of origin to another population area of slightly different characteristics, they may not be well established to the new location and thus may be less likely than the local individuals to pass their genes on to the next generation (Cooper, 2000). The well-structured neighbour-joining tree which clearly established the clusters according to their population as observed in the present study supported this theory.

4.0 Conclusion

To the best of our knowledge, this is the first report on genetic diversity and genetic relatedness estimation of D. moluccana using ISSR markers. It clearly demonstrated that ISSR marker is a powerful tool for assessing genetic diversity of D. moluccana trees collected from three natural forests in Sarawak. This preliminary information will form the base for D. moluccana tree improvement and conservation programmes in future.

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