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

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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*

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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.

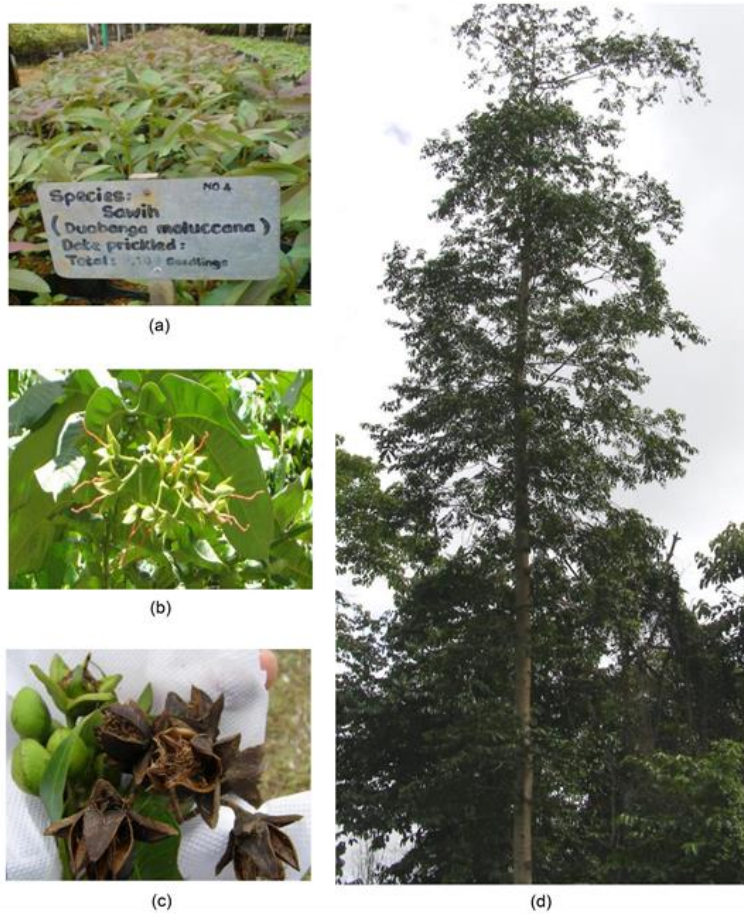


Figure 1. *Duabanga moluccana* (a) Seedlings, (b) Flowers, (c) Matured fruits and (d) Mature tree.

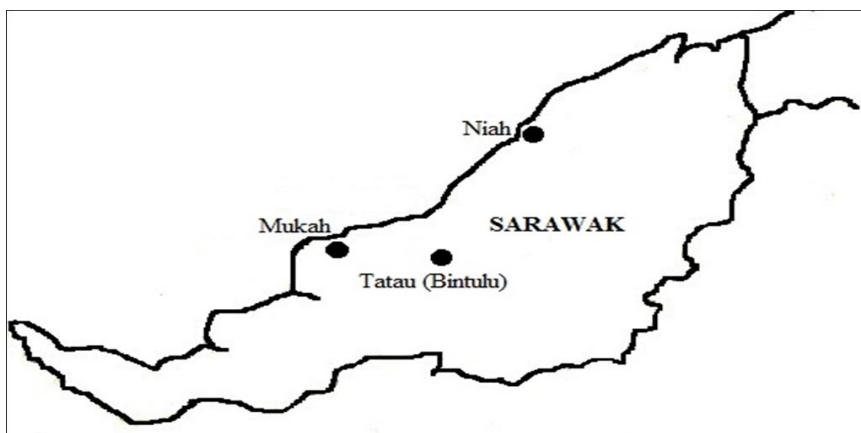


Figure 2. Locations of the *Duabanga moluccana* populations studied.

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 MgCl₂, 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 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 Stain (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).

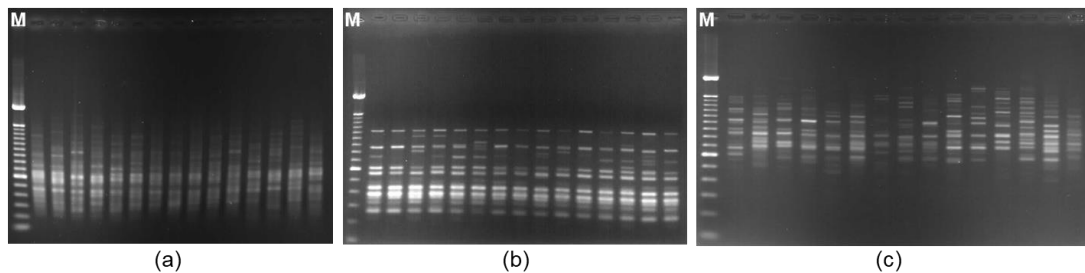


Figure 3. DNA profile using (a) (AC)₁₀, (b) (ACC)₆G and (c) ACG(GT)₇ primers. M is 100 bp DNA ladder.

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>I</i>	<i>h</i>	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. moluccana* 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).

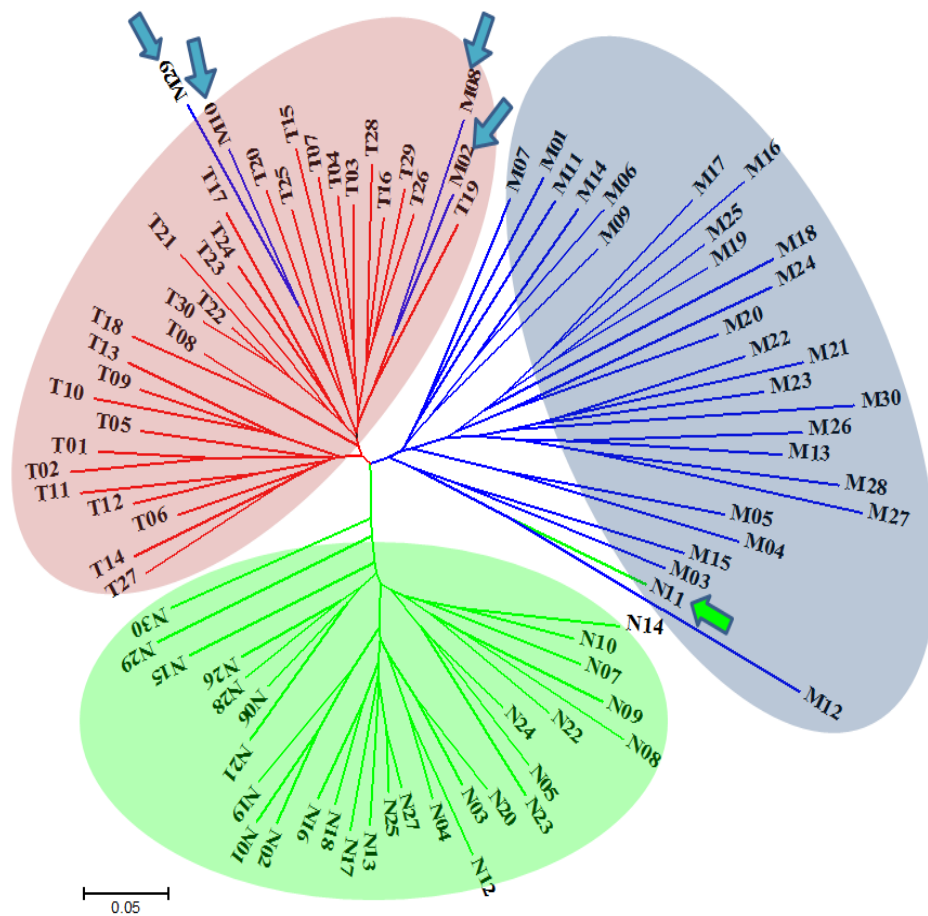


Figure 4. Neighbour-joining tree of *D.moluccana* populations in Sarawak based on shared alleles matrix.

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.

Acknowledgements

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References

- Arnold, M., 1991. Forestry Expansion: A study of technical, economic and ecological factors. Oxford Forestry Institute Paper No 3. Oxford.
- Sedjo, R. A. and Lyon, K.S., 1990. The long-term adequacy of world timber supply. Resources for the Future. Washington DC.
- Kidd, C. V. and Pimentel, D. 1992. Integrated resource management: Agroforestry for Development. Academic Press Inc.
- CIRAD forestry Department. *Duabanga*. Retrieved August 22, 2010 from French Agriculture Research Centre for International Development (CIRAD) website: <http://tropix.cirad.fr/asia/duabanga.pdf>.
- Moreno, S., Pedro, J. and Ortiz, J.M., 1998. Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica*. 101, 117-125.
- Gupta, M., Chyisys, R. J. and Owen, J. L., 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple sequence repeats. *Theoretical and Applied Genetics*. 89, 998-1006.
- Roose, M. L., Fang, D. Q., Federici, C.T. and Krueger, R.R., 1997. Fingerprinting trifoliolate orange germ plasm accessions with isozymes, RFLPs and inter-simple sequence repeat markers. *Theoretical and Applied Genetics*. 95, 211-219.
- Nagaraju, J., Reddy, K. D., Nagaraja, G. M. and Sethuraman, B.N., 2001. Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm, *Bombyx mori*. *Heredity*. 86, 588-597.
- Okun, D. O., Kenya, E. U., Oballa, P. O., Odee, D. W. and Muluvi, G. M., 2007. Analysis of genetic diversity in *Eucalyptus grandis* (Hill ex Maiden) seed sources using inter simple sequence repeats (ISSR) molecular markers. *Proceedings of African Crop Science Conference*. 8, 661-665.
- Chezian, P., Yasodha, R. and Ghosh, M., 2010. Genetic diversity analysis in a seed orchard of *eucalyptus tereticornis*. *New Forests*. 40, 85-99.
- Ammiraju, J. S. S., Dholakia, B. B., Santra, D. K., Singh, H., Lagu, M. D., Tamhankar, S. A., Dhaliwal, H. S., Rao, V. S., Gupta, V. S. and Ranjekar, P. K., 2001. Identification of inter simple sequence repeat (ISSR) markers associated with seed size in wheat. *Theoretical and Applied Genetics*. 102, 726-732.
- Wong, S. C., Yiu, P. H., Bong, S. T. W., Lee, H. H., Neoh, P. N. P. and Rajan, A., 2009. Analysis of Sarawak Bario Rice diversity using microsatellite markers. *American Journal of Agricultural and Biological Sciences*. 4(4), 298-304.
- Zietkiewicz, E., Rafalski, A. and Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*. 20(2), 176-183.
- Godwin, I. D., Aitken, E. A. and Smith, L. W., 1997. Application of inter simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis*. 18(9), 1524-1528.
- Dogan, B., Duran, A. and Hakki, E.E., 2007. Phylogenetic analysis of *Jurinea* (Asteraceae) species from Turkey based on ISSR amplification. *Annales Botanici Fennici*. 44, 353-358.
- Doyle, J. J. and Doyle, J. L., 1990. A rapid total DNA preparation procedure for fresh plant Tissue. *Focus*. 12, 13-15.
- Ho, W. S., Liew, K. S., Elias, A. B., Fedrick, B. J., Mohamad, K. M. A. and Phui, S.L., 2010. Genetic diversity of kelampayan using dominant DNA markers based on inter-simple sequence repeats in Sarawak. In *Proceedings of the International Symposium on Forestry and Forest Products 2010: Addressing the global concerns and changing societal needs*. The Legend Hotel, Kuala Lumpur, 5-7 October 2010. pp.332-340.
- Tiong, S. Y., Chew, S. F., Ho, W. S. and Pang, S.L., 2014. Genetic diversity of *Neolamarckia cadamba* using dominant DNA markers based on inter-simple sequence repeats (ISSRs) in Sarawak. *Advances in Applied Science Research*. 5(3), 458-463.

- Yeh, F. C., Yang, R. C., Boyle, T. B. J., Ye, Z. H. and Mao, J. X., 1997. POPGENE, the User-Friendly Shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.
- Chakraborty, R. and Jin, L., 1993. Determination of relatedness between individuals by DNA fingerprinting. Human Biology. 65, 875-895.
- Tamura, K., Dudley, J., Nei, M. and Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 24, 1596-1599.
- Li, H. and Guizhu, C., 2009. Genetic variation within the endangered mangrove species *Sonneratia paracaseolaris* (Sonneratiaceae) in China detected by inter-simple sequence repeats analysis. Biochemical Systematics and Ecology. 37, 260-265.
- Joshi, P. and Dhawan, V., 2007. Assessment of genetic fidelity of micropropagated *Swertia chirayita* plantlets by ISSR marker assay. Biologia Plantarum. 51(1), 22-26.
- Wang, B. Y., Shi, L., Ruan, Z. Y. and Deng, J., 2011. Genetic diversity and differentiation in *Dalbergia sissoo* (Fabaceae) as revealed by RAPD. Genetics and Molecular Research. 10(1), 114-120.
- Tan, F. X., Huang, Y. L., Ge, X. J., Su, G. H., Ni, X. W. and Shi, S. H., 2005. Population genetic structure and conservation implications of *Ceriops decandra* in Malay Peninsula and North Australia. Aquatic Botany. 81, 175-188.
- Ge, J. X. and Sun, M., 2001. Population genetic structure of *Ceriops tagal* (Rhizophoraceae) in Thailand and China. Wetlands Ecology and Management. 9, 203-209.
- Shah, A., Li, D. Z., Gao, L. M., Li, H. T. and Moller, M., 2008. Genetic diversity within and among populations of the endangered species *Taxus fauna* (Taxaceae) from Pakistan and implication for its conservation. Biochemical Systematics and Ecology. 36, 183-193.
- Feyissa, T., Nybom, H., Bartish, I. V. and Welander, M., 2007. Analysis of genetic diversity in the endangered tropical tree species *Hagenia abyssinica* using ISSR markers. Genetic Resource Crop Evolution. 54, 947-958.
- Wang, D. L., Li, Z. C., Hao, G., Chiang, T. Y. and Ge, X. J., 2003. Genetic diversity of *Calocedrus macrolepis* (Cupressaceae) in southwestern China. Biochemical Systematics and Ecology. 32, 797-807.
- Lee, S. L., Wickneswari, R., Mahani, M. C. and Zakri, A. H., 2000. Genetic diversity of a tropical tree species, *Shorea leprosula* Miq. (Dipterocarpaceae), in Malaysia: implications for conservation of genetic resources and tree improvement. Biotropica. 32(2), 213-224.
- Yu, X. M., Zhou, Q., Qian, Z. Q., Li, S. and Zhao, G. F., 2006. Analysis of genetic diversity and population differentiation of *Larix potaninii* var. *chinensis* using microsatellite DNA. Biochemical Genetics. 44(11-12), 491-501.
- Hamrick, J. L. and Loveless, M. D., 1989. The genetic structure of tropical tree populations: associations with reproductive biology. In: Bock, J. H., Linhart, Y. B. (Eds.). The evolutionary ecology of plants. Westview Press, Boulder. pp. 129-146.
- Cooper, M.L., 2000. Random amplified polymorphic DNA analysis of Southern Brown Bandicoot (*Isodon obesulus*) populations in Western Australia reveals genetic differentiation related to environmental variables. Molecular Ecology. 9, 469-479.