Genetic Structure of *Calophyllum inophyllum* L. in Dompu Natural Forest

I L G Nurtjahjaningsih, I Prihatini, M Qiptiyah and A Rimbawanto

Center for Forest Biotechnology and Tree Improvement, Forest Research, Development and Innovation, Ministry of Environment and Forestry Republic Indonesia, Jalan Palagan Tentara Pelajar Km.15 Purwobinangun, Pakem, Sleman Yogyakarta, 55582

E mail : iluh_nc@biotifor.or.id

**Abstract.** *Calophyllum inophyllum* is an indigenous species and distributed mostly at shoreline areas in Indonesia. Genetic conservation of this species inits natural habitats is very important. A study aimed to assess genetic diversity and structure of *C. inophyllum* at Dompu natural forest was carried out. Leaf samples were collected from three natural populations at Dompu regency (Kilo, Kempo and Pekat). Genetic analysis was carried out using six simple sequence repeats (SSR) markers. Private alleles were only found at Kilo population. Number of detected alleles (NA), values of alleles diversity (AR) and gene diversity (HE) were also higher at Kilo (NA: 17; AR: 2.219; HE: 0.339) than at Kempo (NA: 10; AR: 1.661; HE: 0.219) and Pekat (NA: 12; AR: 1.941; HE: 0.276). The values of the inbreeding coefficients (FIS) were insignificant among the populations. The medium-scale genetic structure showed that genetic relations. Fine-scale genetic structure analysis showed insignificant genetic structure among trees in distance of 2 m at Kilo and 400 m at Pekat, respectively, suggesting that *C. inophyllum* was dispersed as far as 2 m to 400 m.

1. **Introduction**

The Genus of *Calophyllum* consists of 190 species; it is extensively distributed and mostly in shoreline areas in Indo-Malesian region, Micronesia, Melanesia and northern Australia, Central and South America. In Indonesia 35 species are found in Sumatra and 65 species in Borneo. *Nyamplung (Calophyllum inophyllum* (L.)) is one of the *Calophyllum* that is distributed naturally at the Equator. In Indonesia, this species is widely distributed on sites ranging from Sumatra to Papua. *Nyamplung* is valued for timber, biofuel, and medicine [1].

Patterns of genetic structure of tropical plant species are influenced by their biological characteristics, landscape features, and human perturbations through gene flow process by seed and pollen dispersal. The most favored biological characteristics of species in tropical forest is that the production of flowers and fertilization occurs year around, thus various overlapping flowering and fertilization are quite common. Physically pollen and seed movement commonly relate to animal behavior, climate change, and gravitation. Landscape features such as sea or ocean barrier, mangrove forest, river floodplain contribute to shaping isolated populations that have strong effect on genetic
differentiation [2]. Human activities such as land-use changes and timber exploitation have been reported to significantly affect habitat fragmentation. Using RAPD markers, the previous studies on genetic diversity in wide-scale and indirect reproductive system of nyamplung have been initiated to construct breeding and conservation strategies efficiently. The genetic diversity (HE values) of nyamplung in natural distributions were found to be at a moderate level[3]. Value of coefficient inbreeding was insignificant[4]. Flowering might be in synchrony and insect such as wasps, bees was an effective pollinator agent that causes a random mating system [5]. All these studies suggested that genetic structure at large scales were independent from geographical locations. Furthermore, indirect mating system analysis showed that reproductive system was in the panmixia. However, to obtain more comprehensive information, studies on the genetic population dynamic of nyamplung in small islands are still limited. Thus, the aim in this study was to assess genetic diversity and structure at the medium and fine scales of nyamplung at Dompu forests.

2. Materials and Methods

2.1. Study site and samples collection
Three populations located at Dompu regency, Sumbawa Island were studied. The geographic position of each adult tree was measured by GPSMAP 78s (Garmin). Leaf samples were collected in the natural forests of nyamplung at Kilo (8°23’41.0” S 118°21’17.4” E), Kempo (8°30’40.7” S 118°13’03.1” E) and Pekat (8°21’31.1” S 117°55’14.5” E) (Figure 1).

![Figure 1](image_url)

Figure 1. The locations of collected nyamplung leaf samples at Dompu

2.2. DNA Extraction and SSR Analysis
DNAs of leaf samples were isolated using CTAB method [6] described in the previous study [3]. Six SSR markers had been developed in C. inophyllum (Nurtjahjaningsih et al., unpublished data). PCR solution used AmpliTaq Gold Master-Mix (Applied Biosystem). PCR conditions was as described by Nurtjahjaningsih et al. (unpublished data). PCR process used PCR machine Thermal cycler GeneAmp9700 (Perkin Elmer). PCR product was separated using electrophoresis capillary based. Genotyping was visualized using GenMapper (Applied Biosystem).
2.3. Data Analysis

Parameters to assess the genetic diversity within populations were the number of detected alleles (NA), alleles diversity (AR), private allele (PA), gene diversity (HE) and coefficient of inbreeding (FIS). A dendrogram was constructed to describe genetic structure among populations. A fine scale genetic structure was analyzed to assess significances of differentiation among trees within a population, thus seeds dispersal could be estimated. The genetic diversity parameters were calculated by FSTAT software [7]; genetic structure among populations was constructed by POPTREE [8] and fine scale genetic structure was assessed using GenAlex software [9].

3. Results and Discussion

3.1. Genetic diversity and coefficient inbreeding

Mean value of HE of nyamplung was categorized as moderate level (HE: 0.2777; Table 1). However, coefficient of inbreeding at overall populations was insignificantly deviated from Hardy-Weinberg equilibrium. Similar results were also reported in previous studies, where-based on RAPD markers-the genetic diversity of nyamplung at natural forests and a conservation plot ranged from 0.186 to 0.375 [3][5]. Nyamplung tended to have abundant flowers and high synchronized flowering [5]. Moreover, nyamplung produces flowers year around [1]. Bisexual flowering of nyamplung is pollinated by insects such as bee which is an effective pollinator [5][10]. Considering these biological characteristics, nyamplung tended to have a high outcrossing rate and able to maintain its genetic diversity [11].

Table 1. Parameters of genetic diversity of nyamplung from three natural forests at Dompu using six SSR markers

| Population | N  | NA | AR | PA | HE    | FIS  |
|------------|----|----|----|----|-------|------|
| Kilo       | 50 | 17 | 13 | 5  | 0.3388| -0.004ns|
| Kempo      | 24 | 10 | 10 | 0  | 0.2187| -0.228ns|
| Pekat      | 14 | 12 | 12 | 0  | 0.2757| -0.049ns|
| Total/Mean | 88 | 13 | 12 | 1.67| 0.2777| ns    |

N: Number of samples, NA: Number of detected alleles, AR: Allelic diversity, PA: Private alleles, HE: Gene diversity, FIS: coefficient inbreeding

In consensus with genetic population studies on species that have widespread, continuous distribution and high population densities can maintain genetic diversity. In large and continuous population, gene flows by pollen are unlimited, thus an ideal reproductive system might be occurred [12][13]. Those phenomena might also happen in nyamplung at Kilo that represents a large and continuous population. Kilo had the highest level of genetic diversity values (NA: 17, AR: 13, HE: 0.3388) and private alleles (PA: 5) that was the unique alleles found in this population.

3.2. Genetic structure among populations

A dendrogram analysis shows that Kempo and Pekat populations were grouped in one cluster, while Kilo formed another cluster (Fig. 2); this clustering was in concordance with their geographical position. This result also revealed a consistent pattern that geographic distance and sea barrier strongly affect genetic structure of the populations.
3.3. Fine-scale genetic structure

Fine-scale analysis was used to measure genetic structure among individual trees within population. This scale estimated gene flow by seeds. The structure among neighbor trees in Kilo was insignificantly different within 2 m of distance (Fig. 3). The structure was not detected in Kempo (Fig. 4), while it was insignificant within 400 m in Pekat population (Fig. 5). It suggested that seeds are dispersed in short distances (2 m) at Kilo (a continuous forest) and far away (400 m) at Pekat (savannah).

Figure 2. A dendrogram among three natural forests of nyamplung

Figure 3. Spatial auto-correlation of 50 individual trees of nyamplung in Kilo
Figure 4. Spatial auto-correlation of 24 individual trees of nyamplung in Kempo

Figure 5. Spatial auto-correlation of 24 individual trees of nyamplung at Pekat

Gene flows by seeds are affected by seed dispersal agents and tree distribution density [10]. Fruit dispersal in nyamplung is by both gravity and frugivore animal such as bat [5]. Fruits dispersed by gravitation are often scattered close to their mother trees. In contrast, fruits dispersed by frugivore animals are separated far away from their parent trees [14]. Short-distance of fruit dispersal at Kilo, ca 4 m from the mother tree indicated that fruits were dispersed by gravity. The human activities such as tourism or settlements in Kilo might cause frugivore animals not coming to the area. Thus, fruits dispersed by gravity were more dominant than by bats. In contrast, Pekat and Kempo populations were surrounded by buffalo farm, which can reduce human disturbance; making seed dispersal by bats apparently more dominant. Seeds dispersed by other frugivore species such as birds can reach 17 m [10] to 4 km even in fragmented forests or savannah [15] [16][17] and might be as far as hundreds of kilometers, even in distributed and mountainous areas [18].

4. Conclusion

Based on SSR markers, the genetic diversity of nyamplung at Dompu natural forest was relatively low. However, coefficient of inbreeding values was insignificantly deviated from Hardy-Weinberg equilibrium. The reproductive system was in panmixia. Genetic structure among populations was in concordance with their geographical distances. Seeds/plants dispersal of this species can reach from 2 to 400 m.
Implication for conservation and tree improvement

This study indicated that reproductive system of nyamplung was in random mating. The effectivity of bees as pollinator and flowering synchronization might contribute to the mating system. Therefore, to obtain high genetic diversity, seeds should be collected when flowering is in synchrony or at peak flowering season. Kilo has private alleles. For a conservation strategy, seedlings from Kilo should be planted separately from others populations to prevent gene contamination. However, for a tree improvement strategy, Kilo has a potency to broaden genetic diversity. This study also showed that nyamplung has short-distance seed dispersal, consequently to reduce siblings, seeds/seedlings should be collected outside of the dispersal range.

Acknowledgment

This study has been supported by FORDIA Ministry of Environment and Forestry of Republic Indonesia. We appreciated Dr. H. Kurokochi, University of Tokyo, Japan for his suggestions as peer-reviewer. We thank to Mrs. Wahyunisari and Mr. Triyanta who helped in laboratory works.

References

[1] Warrier KC S2015 Calophyllum inophyllum L. In Krishnakumar N, Palanisamy K, Hegde M, Warrier K C S and Krishnamoorthy M (Eds.) Manual of economically important forestry species in South India Tamil Nadu Institute of Forest Genetics and Tree Breeding pp 171–178.

[2] Toczydlowski R H and Waller D M 2019 Drift happens: Molecular genetic diversity and differentiation among populations of jewelweed (Impatiens capensis Meerb.) reflect fragmentation of floodplain forests Mol. Ecol. 28(10).

[3] Nurtjahjaningsih I L G, Haryanti T, Widyatmoko A, Indrioko S and Rimbawanto A 2015 Keragaman genetik populasi Calophyllum inophyllum menggunakan penanda RAPD (Random Amplification Polymorphism DNA) JPTH 92 pp 91–102.

[4] Nurtjahjaningsih I L G 2019 Genetic structure of Calophyllum inophyllum mother trees at a provenances seeds stand based on simple sequences repeats markers JPTI/13 1

[5] Nurtjahjaningsih I L G, Sulistyawati P, Widyatmoko A and Rimbawanto A 2012 Karakteristik pembungaan dan sistem perkawinan nyamplung (Calophyllum inophyllum) pada hutan tanaman di Watusipat, Gunung Kidul/JPTH/62 pp 65–80.

[6] Shiraishi S and Watanabe A 1995 Identifikasiik of chloroplast genome between Pinus densiflora Sieb et Zucc and P. thumbergii Parl based on the polymorphism in rbct gene. J. of Japanese For. Soc. 77 pp 429–436.

[7] Goudet J 2001 FSTAT (version 2.9.3): A program to estimate and test gene diversities and fixation indices. https://doi.org/zzz.unil.ch/izea/softwares/fstat.html

[8] Takesaki N, Nei M and Tamura K 2014 POPTREEW: Web version of POPTREE for constructing population trees from Allele Frequency Data and Computing Some Other Quantities. Molecular Biology Evolution, 31(6), 1622–1624.

[9] Peakall R and Smouse P E 2006 GenAlex 6: Genetic analysis in excel, Population genetic software for teaching and research. Mol. Ecol. Notes 6 pp 288–295.

[10] Sork V L 2016. Gene flow and natural selection shape spatial patterns of genes in tree populations: implication for evolutionary processes and applications. Evol. Appl. 9 pp 291–310.

[11] Francisco P M, Tambarussi E V, Alves, F M, Bajay S, Ciampi-Guillard M and Souza A P 2018 Genetic diversity and mating system of Rhizophora mangle L. (Rhizophoraceae) in northern Brazil revealed by microsatellite analysis. CERNE 244 pp 295–302.

[12] Lee S L, Wichneswari 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 pp 213-224.

[13] Tsuda Y, Goto S and Ide Y 2004 RAPD analysis of genetic variation within and among four natural populations of *Betula maximowicziana* *Silvae Genet.* **53** 5/6 pp 234–239.

[14] Choo J, Juenger T E and Simpson B B 2012 Consequences of frugivore-mediated seed dispersal for the spatial and genetic structure of a neotropical palm. *Mol. Ecol.* **21** 4 pp 1019–1031.

[15] Hanson T R, Brunsfeld S J, Finegan B and Waits L P 2008 Pollen dispersal and genetic structure of the tropical tree *Dipteryx panamensis* in a fragmented Costa Rican landscape. *Mol. Ecol.* **17** 8 pp 2060–2073.

[16] Moraes M A, Kubota T Y K, Rossini, B C, Marino C L, Freitas M L M, Moraes M. LT, da Silva A M, Cambuin J and Sebbenn A M 2018 Long-distance pollen and seed dispersal and inbreeding depression in *Hymenaea stigonocarpa* (Fabaceae: Caesalpinioideae) in the Brazilian savannah *Ecol. Evol.* **8** pp 7800–7816.

[17] Solis-Hernandez W and Fuchs E 2019 Effective gene flow patterns across a fragmented landscape in southern Costa Rica for *Symphonia globulifera* (Clusiaceae); a species with mobile seed and pollen dispersers. *Rev. Biol. Trop.* **67** 2 S95–S111.

[18] Bekku Y S, Kurokochi H, Matsuku Y, Nishi N and Lian C 2019 Genetic structure of *Pinus parviflora* on Mt. Fuji in relation to the hoarding behavior of the Japanese nutcracker. *Ecophere* **10** 4 pp 1–13.