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Genetic diversity of *Rhizophora mucronata* on the western coast of Timor Island

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Abstract

The mangrove (*Rhizophora mucronata*) grows on the western coast of Timor Island and is threatened by anthropogenic activities. Because *R. mucronata* is an important species, a strategy for sustainable development is required. Information on the genetic diversity of the species is essential in order to plan for coastal management and conservation. This study investigates mangrove genetic diversity on the western coast of Timor Island in the Indonesian Archipelago. Mangrove leaf samples were collected from 10 locations. Samples underwent DNA isolation and random amplified polymorphic DNA testing using six primers. The *R. mucronata* population formed three main groups and 12 subgroups with a similarity coefficient value of 0.42. The genetic proximity among populations is not related to geographical distance. Further analysis suggests that the genetic diversity of *R. mucronata* on Timor Island can be classified as moderate. The highest degree of genetic diversity was found in Sumlili (Ne = 2.999, Na = 11.167, He = 0.667), whereas the lowest genetic diversity was found in Sulamu (Ne = 2.967, Na = 11.833, He = 0.663). Environmental stress and dry climate conditions might be a factor related to the genetic diversity of the mangrove at a moderate level.

Keywords: East Nusa Tenggara; Mangrove; Polymorphism; *Rhizophora mucronata*; Timor Island.

1. Introduction

Mangrove vegetation is essential to produce organic material. It acts as a buffer between the land and the sea by dampening wave action and preventing erosion (Noor *et al.*, 2006). The mangrove species *Rhizophora mucronata* grows on the western coast of Timor Island in East Nusa Tenggara Province (Koda 2013; Koda, 2014; Ernawati & Majid, 2013; Rusydi, 2010; Rusydi. *et al.*, 2015 and Yahyah, 1997). Mangrove must be protected against various anthropogenic activities such as logging for firewood and other household needs (Koda, 2013; Koda, 2014).

The first step towards conservation is careful study. Information on genetic diversity is essential in developing a strategy to conserve species and ensure sustainable development. Genetic diversity plays a significant role in the adaptation of a species. Gene variations are required for the species to survive and adapt to environmental change. The genetic diversity of a population is a buffer and determines the success and endurance of the population in extreme environmental conditions (Haig, 1998). Heterozygosity, population size, and quantitative genetic variation are all significantly and positively correlated with population adaptation (Reed & Frankham, 2003). Low genetic variability in a population is influenced by environmental factors such as soil fertility (Karron, 1987). In addition, rare plants always have a lower genetic diversity than abundant plants (Karron, 1987).

2. Methodology

The genetic variability in a population can be assessed through the number and percentage of polymorphic genes in a population (Primack, 1993; Haymer,1994) which can be determined by random amplified polymorphic DNA (RAPD).

2.1 Sample collection and DNA extraction

Samples of young *R. mucronata* mangrove leaves were collected at 10 locations in the western region of Timor Island from March to August 2017 (Figure 1). Samples were taken, cleaned with running water, put into individual plastic bags containing silica gel, and labeled. DNA was successfully extracted from the leaves by the CTAB method (modified by Pharmawati (2009)). The leaves (0.1 g) were crushed until smooth using a mortar and pestle, and 1 mL of extraction buffer [2% CTAB. 1.4 M NaCl. 0.2% β-mercaptoethanol. 50 mM Na_2EDTA (pH 8.0) and 100 mM Tris-HCl (pH 8.0)] was added.

The samples were then incubated at 65ºC in a water bath for 60 minutes with tubes agitated every 10 minutes. Thereafter, 700 µL of chloroform-isoamyl alcohol (24:1) were added, and the samples were homogenized. Samples were then centrifuged at 12,000 rpm for 5 minutes, and the supernatant was moved to a new tube. Cold ethanol was added according to the supernatant volume, and the tube was agitated again and incubated for 1 hour at -20ºC. The tubes were centrifuged at 12,000 rpm for 3 minutes, and the resulting pellets were washed with 400 µL of 70% alcohol. The pellets were dried and then resuspended in 100 µL of sterile H_2O (Uslan and Pharmawati, 2015). The DNA was check by electrophoresed on a 0.8% agarose gel.

Fig. 1. Sampling location at ten places of *R. mucronata* at western coast of Timor island, East Nusatenggara province, Indonesia. sample were taken in ten locations (circle mark), there were Pulau pasir, Sumlili, Salupu, Tesabela, Oematnunu, Paradiso, Nunkurus, Bipolo, Pariti, and Sulamu.

2.2 RAPD analysis

RAPD analysis was done with six primers: OPA05 5'AGGGGTCTTG; OPA07 5'GAAACGGGTG; OPA08 5'GTGACGTAGG; OPA10 5'GTGATCGCAG; OPA11 5'CAATCGCCGT; and OPA14 5'TCTGTGCTGG (Riyantini *et al.*, 2014). The PCR mixture contained 0.2 mM dNTPs, 3 mM $MgCl_2$, 1 U Taq of DNA polymerase, 1 x polymerase buffer, 1.9 µM primers, 50 ng DNA, and steriled water up to 25 µL volume. Amplification was carried out in a thermocycler instrument using a specified protocol. This involved initial denaturation at 94ºC for 5 minutes, followed by denaturation at 94ºC for 1 minute, annealing at 37ºC for 1 minute, and elongation at 72ºC for 2 minutes, for up to 44 cycles. The last stage was a final elongation at 72ºC for 8 minutes (Sahao *et al*., 2007). The PCR product was assessed by electrophoresis on a 1.8% agarose gel at 100 V for 40 minutes. Ladder DNA VC 100bp Plus (Vivantis. NL1407) was used to determine the size of the DNA bands. PCR product observations were performed using a GelDoc UV Transilluminator (Sambrook and Russell 2001).

2.3 Scoring and data analysis

Data scoring was performed using PyElph software version 1.4. Cluster analysis was performed using the PhylTool software. To determine the informativity level of the primers, a polymorphic information content (PIC) calculation was performed. Dendrogram analysis was performed using Mega6 software and included an observed number of alleles (Na), an effective number of alleles (Ne), and gene diversity (H) as described by (Finkeldey and Hattemer, 2007; Yunanto, 2006).

3. Results and discussion

The six RAPD primers (i.e., OPA-05, OPA-07, OPA-08, OPA-10, OPA-11, and OPA-14) successfully amplified the DNA in sizes ranging from 200 to 1500 bp, with a PIC ranging from 0.965 to 0.966 (Table 1). The genetic similarity among the populations from the 10 locations was assessed by comparing the DNA band pattern from PCR amplification. The analysis showed that *R. mucronata* had a similarity coefficient of 0.42, or 42%, and that the genetic proximity among populations is not related to geographical distance. *R. mucronata* from Salupu 2 and Bipolo 3 had the highest genetic distance, whereas the lowest genetic distance of similarity was found in Sulamu 2 and Sulamu 3.

Table 1. Polymorphic information content (PIC) of mangrove generated by RAPD.

No	The Name of Primer	Size Range bp	The Number of Band	The Number of Polymorphic DNA Bands	Percentage of Polymorphism	PIC
1	OPA-05	320-1350	101	101	100%	0.965
2	OPA-07	300-1480	93	93	100%	0.966
3	OPA-08	210-1500	128	128	100%	0.966
4	OPA-10	200-1190	116	116	100%	0.966
5	OPA-11	200-1200	133	133	100%	0.966
6	OPA-14	200-1210	109	109	100%	0.965
	Total		680	680		

Further analysis based on the genetic relationship by the phylogenetic tree revealed that the *R. mucronata* population has three main groups and 12 subgroups with a similarity coefficient value of 0.42 or 42%. The first group formed two subgroups. Subgroup-1 consisted of Pariti 2, Pariti 3, Sulamu 1, Sulamu 2, and Sulamu 3, while subgroup-2 included Bipolo 1, Bipolo 2, Bipolo 3, and Pariti 1. The second group consisted of six subgroups: subgroup-1, consisting of Paradiso 1 and Oematnunu 3; subgroup-2, consisting of Oematnunu 2 and Tesabela 3; subgroup-3, consisting of Tesabela 1, Tesabela 2, and Oematnunu 1; subgroup-4, consisting of Salupu 2 and Salupu 3; subgroup-5, consisting of Salupu 1 and Sumlili 3; and subgroup-6, consisting only of Sumlili 2. The third group consisted of four subgroups: subgroup-1, consisting of Nunkurus 1, Nunkurus 2, Paradiso 2, and Paradiso 3; subgroup-2, consisting only of Sumlili 1; subgroup-3, consisting of Nunkurus 3 and Pulau Pasir 2; and subgroup-4, consisting of Pulau Pasir 1 and Pulau Pasir 3 (Figure 2).

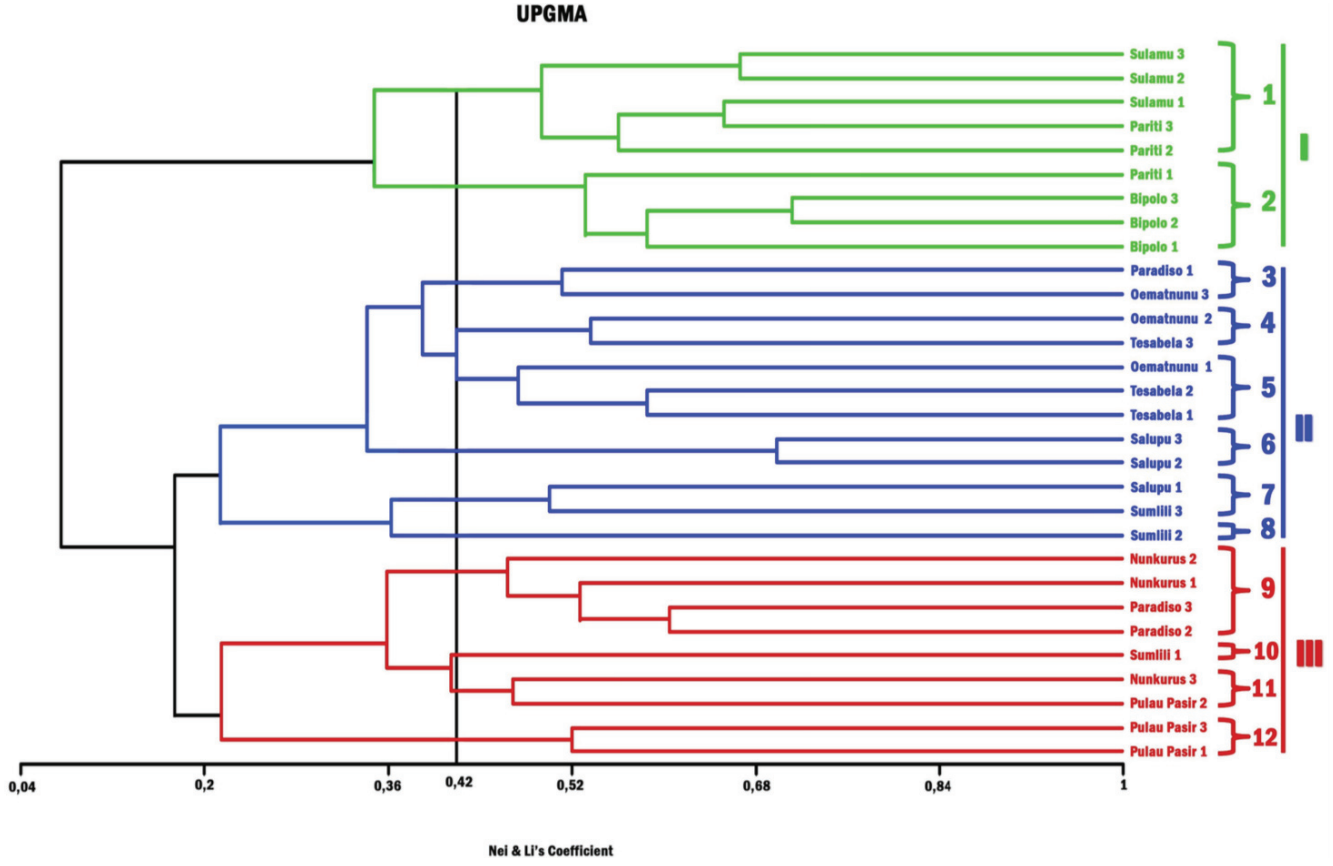

Fig. 2. Dendrogram of 30 samples of mangrove *R. Mucronata.* Numbers below dendrogram show values of Nei and Li's similarity coefficient.

We analyzed the degree of genetic diversity based on population genetic parameters such as Na, Ne, and He (Finkeldey and Hattemer, 2007). The results of the analysis show that the genetic diversity of *R. mucronata* on Timor Island could be classified as moderate (Na = 2.998, Ne = 11.333, He = 0.665). The highest degree of genetic diversity was found in Sumlili (Ne = 2.999, Na = 11.167, He = 0.667), whereas the lowest genetic diversity was found in Sulamu (Ne = 2.967, Na = 11.833, He = 0.663) (Table 2). The dry climatic conditions at Timor Island are generally a form of environmental stress that has selected the mangrove *R. mucronata* over a long period of time, generating genetic endurance.

Table 2. Results of genetic diversity of *R. Mucronata* on western coast of Timor Island.

No	Population/Location	Number of Samples	Na	Ne	He
1	Pulau Pasir	3	2.996	12.667	0.666
2	Sumlili	3	2.999	11.167	0.667
3	Salupu	3	2.990	10.667	0.666
4	Tesabela	3	2.998	10.333	0.666
5	Oematnunu	3	2.982	10.833	0.665
6	Paradiso	3	2.990	10.833	0.666
7	Nunkurus	3	2.995	10.500	0.666
8	Bipolo	3	2.976	12.000	0.664
9	Pariti	3	2.987	12.500	0.665
10	Sulamu	3	2.967	11.833	0.663
	Total	**30**	**29.880**	**113.333**	**6.653**
		Average	**2.988**	**11.333**	**0.665**

Description: Na = observed number of alleles; Ne = effective number of alleles; He = genetic diversity

4. Conclusion

The genetic proximity among populations of *R. mucronata* on the western coast of Timor Island was not related to geographical distance. The genetic diversity of the mangrove was classified as moderate, and the highest diversity was found in Sumlili, whereas the lowest was found in Sulamu.

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التنوع الجيني لأشجار المانجروف من النوع (*Rhizophora mucronata*) في الساحل الغربي لجزيرة تيمور

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الملخص

تتعرض أشجار المانجروف من النوع *Rhizophora mucronata* التي تنمو على الساحل الغربي لجزيرة تيمور لأنشطة بشرية متنوعة، مثل الإحتطاب وغيرها من الاحتياجات المنزلية، وهذا يتطلب استحداث استراتيجية خاصة للتنمية المستدامة لتلك الأشجار. إن توفر المعلومات المتعلقة بالتنوع الوراثي لأنواع الأشجار أمر هام للغاية وذلك لتخطيط الإدارة الساحلية والحفاظ على أشجار المانجروف. لذلك، أجرينا دراسة حول التنوع الوراثي لأشجار المانجروف على الساحل الغربي لجزيرة تيمور حيث تم جمع عينات من أشجار المانجروف من 10 مواقع مختلفة. خضعت العينات لعملية عزل الحمض النووي (DNA) والاختبار العشوائي المتضخم متعدد الأشكال للحمض النووي باستخدام ستة فتائل. شَكَل مجتمع الشجيرات التي تنتمي للنوع *R. mucronata* ثلاث مجموعات رئيسية و12 مجموعة فرعية بقيمة 0.42 لمعامل التشابه، ولا يرتبط التقارب الوراثي بين المجتمعات بالمسافة الجغرافية. واقترحت التحاليل أن التنوع الوراثي لشجيرات *R. mucronata* في جزيرة تيمور يمكن تصنيفه على أنه متوسط. وتم العثور على أعلى درجة من التنوع الوراثي في Sumlili (Ne = 2.999, Na = 11.167, He = 0.667)، في حين تم العثور على أقل درجة من التنوع الوراثي في Sulamu (Ne = 2.967, Na = 11.833, He = 0.663)، قد يكون الضغط البيئي وحالة المناخ الجاف عاملان مهمان في التنوع الوراثي للمانجروف على الصعيد المعتدل.