THE JOURNAL OF TROPICAL LIFE SCIENCE
VOL. 3, NO. 2, pp. 127 – 131, May, 2013

Genetic Variation of *Dacrycarpus imbricatus* in Bromo Tengger Semeru National Park (BTS-NP), East Java Based on *trn*L (UAA) Intron Region

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ABSTRACT

The conservation of *Jamuju Dacrycarpus imbricatus* in Java Island has been considered important. One of the the limitation of such program is related to the viability data on the genetic diversity of species target. The aim of study was to determine genetic variation of *D. imbricatus* in Bromo Tengger Semeru National Park (BTS-NP), East Java based on *trn*L (UAA) intron region. DNA sample was collected from several *D. imbricatus* seedling population in BTS-NP in East Java. DNA was isolated and amplified using PCR. Genetic variation was estimated using *trn*L (UAA) intron sequences. This study confirm that *D. imbricatus* in BTN-SP has low genetic diversity. Based on the phylogenetic tree, *D. imbricatus* population from BTN-SP is closely related to *D. imbricatus* from Sabah-Malaysia and Hainan-China with 100 % similarity value. These data implies that population and habitat management of *D. imbricatus* in BTN-SP should be designed to enhance the population survival in the future.

Keywords:*Jamuju conservation, Genetic variation, mountain tropical forest*

INTRODUCTION

Jamuju *Dacrycarpus imbricatus* is one of the native and endemic plant trees species which grow in Indonesia including Java, Southwest and Central Celebes, and all Lesser Sunda Island (Bali-Timor). *D. imbricatus* is individual tree in the wild can grow up to 50 m in talland belongsto Podocarpaceae family (Gymnosperm). The species has ecological function in carbon storage, support soil microbial community, increase soil nutrition, and in soil and water conservation. The habitat of *D. imbricatus* confirm to the mountain tropical forest [1, 2, 3]. In Indonesia, however, the status of *D. imbricatus* could be vurnerable and endangered due to rapid deforestation and massive illegal logging. As far, there are no population assess-ments and evaluation has been done during the past decades.

Bromo Tengger Semeru National Park (BTS-NP) is one of the biodiversity hot spot areas in Java Island. In such park, *D. imbricatus* grows wild with manynative plant trees in primary and secondary forest. The existence of *D. imbricatus* and many plant trees species which has economical value, however, becomes target of illegal logging. The wood of *D. imbricatus* was reported has good quality for material contruction. Our previous survey confirms that recent distribution of *D. imbricatus* was limited to forest area surrounding Ngadas and Ranupani. These implies that conservation of *D. imbricatus* was important.

Recently, genetic aspect has been considered as one of the important aspect in plant conservation strategy. Fundamentally, plant genetic variation and population size contribute to the plant's fitness and life [4]. There are many techniques available to identify genetic diversity through sequences analysis. Currently, the uses of *trn*L intron has been introduced. The *trn*L intron is non-coding region of chloroplast DNA which can detect genetic variation in plant. The chloroplast *trn*L (UAA) intron has advantage that is easy to amplification in a large number of plants (highly success PCR) to amplification in a large number of plants. Size of trnL intron is small enough allows the production of a complete DNA sequences [5, 6]. Sequence variations of the chloroplast *trn*L (UAA) intron were detected in *Taxus wallichiana* [7], *Panicum virgatum* [8], and *Raphanus* [9].

The information of genetic diversity of jamuju is one of the important aspects to define the conservation strategy. As far, there is no in-

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formation available. The aim of study was to determine genetic variation of *D. imbricatus* in BTS-NP, East Java based on *trn*L (UAA) intron sequences.

MATERIALS AND METHODS

Sites of study

DNA sample was collected from several *D.imbricatus* seedling population in BTS-NP, East Java. The seedlings of *D. Imbricatus* appear after rainy season in June-July. In these periode, seedling grows and young leaf available to collect as a sources of DNA materials. Ecologically, BTS-NP area is humid mountain tropical forest. The national park cover an area about 50,276 ha and spread from 1,200 to 2,450 m asl. The biodiversity of mountain flora and fauna was considered high. Many plant species were endemic to the park. Our previous survey found *D. imbricatus* distribute at several point in secondary forest at Ngadas and Ranupani.

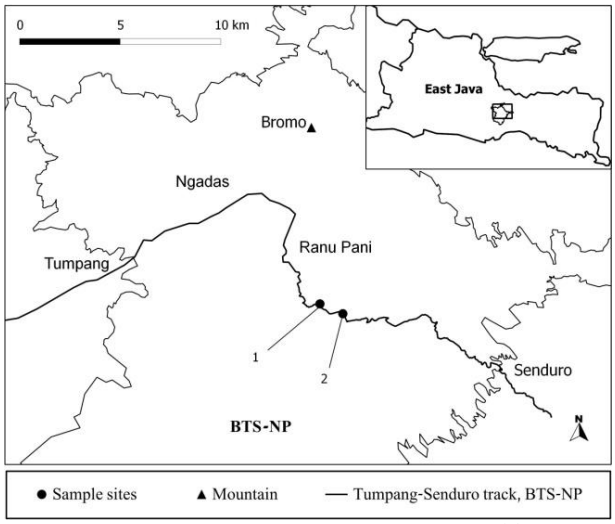

Figure 1. Map of *Dacrycarpus imbricatus* sample sites. BTS-NP: Bromo Tengger Semeru National Park

DNA extraction

Total genomic DNA was extracted from young leaf tissue of *D. imbricatus* seedling using the Doyle and Doyle CTAB method [10]. For the DNA purification DNA precipitation, the methods was modified by adding Phenol Chloroform-isoamyl alcohol and cold absolute ethanol. Quality of the extracted DNA was determined using gel electrophoresis and DNA concentrations were determined by measuring with a UV spectrophotometer. Isolated plant genomic DNA was preserved at -20 °C.

PCR amplification

Primer combinations trnL were:
c:(5'CGAAATCGGTAGACGCTACG-3')
d: (5'GGGGATAGAGGGACTTGAAC-3')
that were used for amplification of the trnL (UAA) region [5]. PCR was carried out in 30 µl volume reaction mixture. The reaction mixture contained 6 µL ddH_2O, 15 µL PCR mix 2× solution (intRON biotechnology), 3 µL DNA (100-350 ng/µL), 3 µL primer c and 3 µL primer d (30 pmol/µL). The trnL (UAA) intron thermocycling profile was: 95°C for 5 minutes, 35 cycles of 95 °C for 45 second, 61.7 °C for 45 second, and 72 °C for 45 second, with a final extension of 72 °C for 10 minutes. The PCR products were visualized on 1.5 % agarose gel stained with ethidium bromide, sequenced using 3730×l automated sequencer (Applied Biosystems, Macrogen Inc., Seoul, South Korea) and evaluated using ABI sequence Scanner v.10 (Applied Biosystems).

trnL intron analysis

Genetic variation was estimated using trnL (UAA) intron sequences from GenBank (Table 1). Sequences were aligned using Bioedit 7.0.9.0 and genetic distances were computed using MEGA 5.03. with Kimura-2-Parameter (K2P) evolution model [11]. The phylogenetic tree was inferred using Neighbor Joining (NJ), Maximum Likelihood (ML), and Maximum Parsimony (MP) method. Boot-strapping was performed with 1000 replicates.

RESULTS AND DISCUSSION

Evaluating genetic diversity is crucial in plant conservation strategy. This research confirms that seedling population of *D. imbricatus* in BTS-NP has no genetic variation. It is shown by low level of polymorphism which was detected in the cpDNA *trn*L (UAA) region (Figure 2). The alignment of *trn*L (UAA) intron shows the absence of deletions or insertions of nucleotides among the 10 samples. The *trn*L (UAA) intron sequences from Gen Bank detect only two variable sites were found, one insertion and one transversion. The bases in position 350 show insertion of adenine (A) and the bases in position 452 show transversion of timine (T) to guanine (G). Transversion are changes from purine bases (A or G) to pyrimidine bases (C or T) or pyrimidine bases to purine bases [12]. The result showed that the *trn*L intron region was highly conserved.

Table 1. Accessions of different taxa for *trn*L intron sequence variation

	Taxon	Distribution	Collector	GenBank accession number
Outgroup taxa:	*Podocarpus neriifolius*	Nepal-New Zealand	Zhou, *et al.*	AY013736.1
Ingroup taxa:	*Dacrycarpus kinabaluensis*	Sabah, Malaysia	Biffin, *et al.*	JN001415.1
	Dacrycarpus cinctus	Sulawesi-New Guinea	Biffin, *et al.*	JN001413.1
	Dacrycarpus imbricatus 1	Sabah, Malaysia	Sinclair, *et al.*	AY083140.1
	Dacrycarpus imbricatus 2	Hainan, Cina	Zhou, *et al.*	AY013727.1

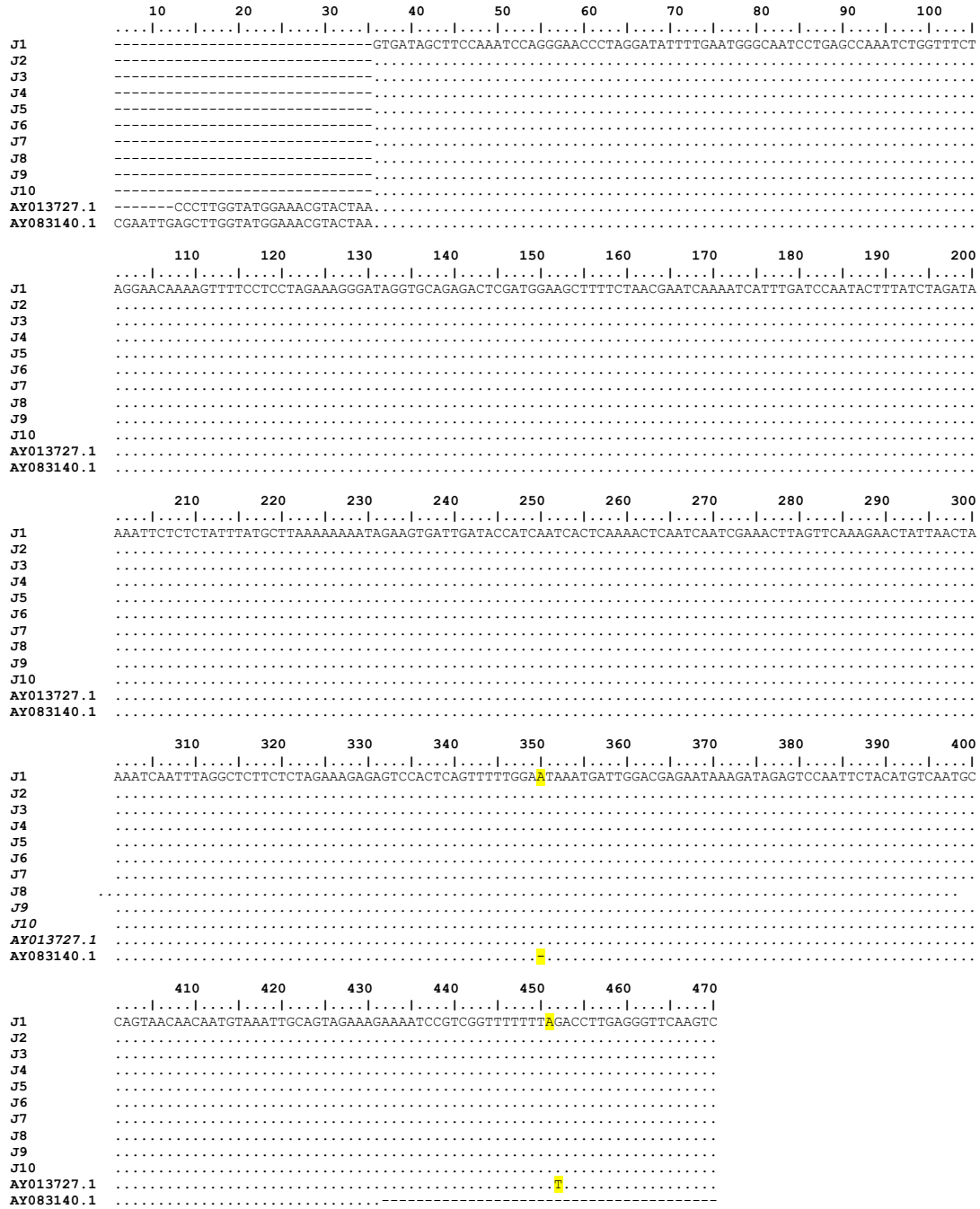

Figure 2. Alignment of the *trn*L intron of *D. imbricatus*. J1-10: Sampled population from BTS-NP. AY013727.1: The GenBank sequence accession number from Hainan-China. AY083140.1: The GenBank sequence accession number from Sabah-Malaysia. Dot (.) indicates that the character states are the same. Dash (-) indicates alignment gap

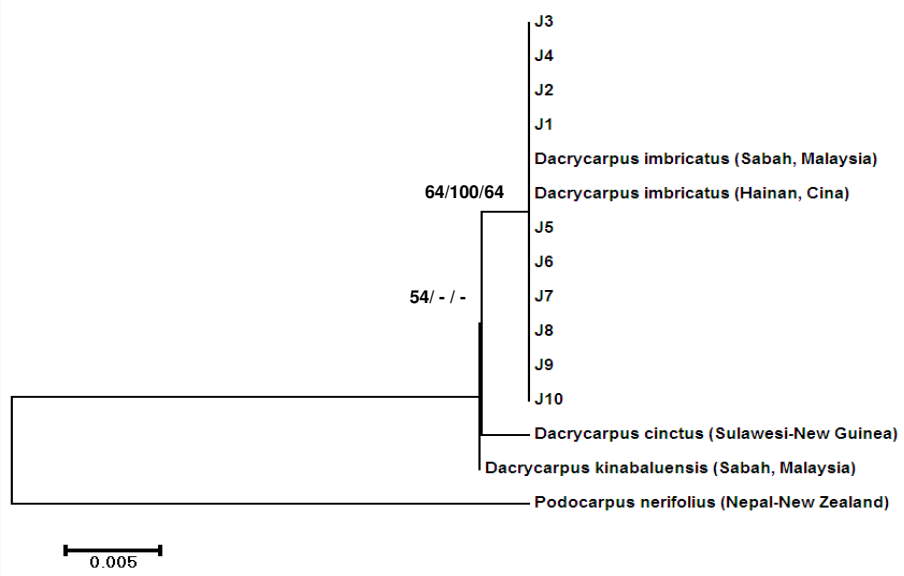

Figure 3. Phylogenetic tree of the jamuju based on *trn*L (UAA) intron sequences. J1-10: Sampled population from BTS-NP. Bootstrap percentages (NJ/MP/ML) with a frequency of more 50% are shown in the nodes of the dendrogram

The aligned sequence lengths of the *trn*L (UAA) intron regions were 470 bp. Compared to the sequences available in GenBank and also sequence produced in this research, the *trn*L (UAA)intron among ten samples of *D. imbricatu*s were identical in length. Moreover they have a very high sequence identity (identities value 99 % for *D. imbricatus*). The phylogenetic tree based on *trn*L intron show that jamuju from BTS-NP is closely related to *D. imbricatus* from Sabah-Malaysia and Hainan-China with 100 % similarity value (Figure 3). In the context of phytogeography, these species seems similar and these species has low genetic diversity.Recent plant conservation strategy has been considerd phylogenetic as a key for conservation success [13].

In this study, the phylogenetic tree showed that the *D. imbricatus* clade is closely related to *D. cinctus* (origin: Sulawesi-New Guinea) with 99.5 % similarity value and 54 % Neighbor-NJ bootstrap value (Figure 3). The phylogenetic tree showed *D. imbricatus* clade, well supported with bootstrap value (100 % in MP), 64 % in NJ and ML. The high genetic similarities among the spesies show the presence of some associations in the evolutionary processes in *Dacrycarpus* and *Podocarpus* (Figure 3). The relationship between *Dacrycarpus* and *Podocarpus* has evaluated by the low rates of nucleotide substitution within sequences, suggesting that *trn*L (UAA) intron suitable for phylogenetic studies [14].

Overall, the phylogenetic tree of the *jamuju* based on *trn*L intron (Figure 3) support a statement that ancestor of *Dacrycarpus* is from south region (Antartica) [2]. According to fossil evidence and plant distribution, it is possible because Cretaceous period (last dinosaur era), New Guinea still join with Antarctic plate, then in the mid-Tertiary period (early ice age), continent of New Guinea move away from Antartica plate and began to move closer to continent of Asia (East Java, Malaysia and Cina).

CONCLUSION

Sequence analysis revealed that the genetic variation within the seedling of *D.imbricatu*s from BTS-NP was low. Considering the low individual number and recent population were distributed pactcy, the conservation of *D. imbricatu*s becomes important. The habitat protection and plants regeneration are important approach, while in the same time establishing restoring wild seedling population is important. The *trn*L (UAA) intron was unable to detect genetic variation of *jamuju* in population level, it is useful for phylogenetic studies of Podocarpaceae.

ACKNOWLEDGEMENTS

This research was support and facilited by Japan International Cooperation Agency (JICA) and BTS-NP throught the project entitled Project on Capacity Building for Restoration of Ecosystems in Conservation Areas which is granted to Dr. Luchman Hakim.

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