Short Communication:
Genetic diversity and conservation strategy considerations for highly valuable medicinal tree of *Taxus sumatrana* in Indonesia

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Manuscript received: 17 February 2016. Revision accepted: 10 June 2016.

**Abstract**. *Rachmat HH, Subiakto A, Kamiya K. 2016. Genetic diversity and conservation strategy considerations for highly valuable medicinal tree of Taxus sumatrana in Indonesia. Biodiversitas 17: 487-491*. Genetic variation is considered to be the key factor for long-term survival of the species. The recognition of the existing genetic diversity is the preliminary phase in development of an effective strategy for conservation of forest tree species. *Taxus sumatrana* or is confined to grow naturally only in Asia, it is a rare and endangered species that in several Asian countries needs both ex situ and in situ protection program. In its natural distribution, *T. sumatrana* is the only *Taxus* species that reached its southernmost distribution to Sumatran forest-Indonesia and locally named as Sumatran Yew. The objective of this research was to determine the genetic variation of *T. sumatrana* as baseline information for designing conservation strategy of the species. Leaves samples were collected from two natural population of *T. sumatrana* in Mt. Kerinci (Sungai Penuh, Jambi) and Mt. Dempo (Pagaralam, South Sumatra), both sites are located along Bukit Barisan Mountain Ranges of Sumatra. We sequenced two non-coding chloroplast DNA (cpDNA) regions of *trnL-trnF* and *psbC-trnS* that each yielded 808 bp and 1092 bp, and *rbcL* gene of 523 bp, in which the total length covered 2423 bp. Surprisingly, we found no variation for all individuals and population, which means that the species is similar and both populations are not genetically structured. This study also revealed on how a proper conservation strategy should be practiced for the species as we know that without a sufficient amount of genetic variation, a population cannot evolve in response to changing environmental conditions. In situ conservation program is a must that can maintain the existence of the species while at the same time keeping the sustainability of the entire systems; in other side ex situ conservation strategy can take place as an additional effort to secure the genetic resources in case of the catastrophic events that might diminish their limited natural habitat.

**Key words**: Genetic variation, *cpDNA*, *rbcL* gene, Sumatran Yew

**INTRODUCTION**

Plants of the genus *Taxus* are sources of a number of physiologically and pharmacologically active compounds of different classes, especially the anti-cancer paclitaxel and many other taxane derivatives. The species of *Taxus* are more geographically than morphologically separable. The genus *Taxus* has included eight geographically defined species; including *T. sumatrana* (Miq.) de Laub (Spjut 2007). *T. sumatrana* (Miq.) de Laub, locally names as Sumatran Yew, is naturally distributed in Taiwan, Sulawesi, and reached its southernmost distribution to Sumatra mainland (de Laubenfels 1988). In Sumatra, *T. sumatrana* is an endangered conifer with a scattered distribution. The highly valuable timber is usually distributed in shady valleys and slopes at high altitudes, e.g at 1700- 2200 m asl. in Mt. Kerinci-Jambi.

Plants within the Genus of *Taxus* are highly known for their taxol production. Taxol is a blockbuster anticancer drug which is widely used for clinical application against different types of cancer (Zhou et al. 2010). The drug is known to bind to microtubules and essentially freeze them in place, prevent them from separating the chromosomes when cell divides. This mechanism will kill dividing cells, particularly cancer cells (Weaver 2014). In other part of the world, species in the Genus have been facing serious threats because of human overexploitation and habitat destruction that lead to the decline and fragmentation of populations. Yet, there have been any reports of the exploitation for its valuable bark or other tree parts. However, increasing pressure on forest and land and also their narrow and scattered distribution at only several spots have made the species to be the priority for conservation (Hidayat et al. 2014). Field exploration on the potency and distribution of *T. sumatrana* (Rachmat 2008; Hidayat et al. 2013) in Mt. Kerinci and Mt. Dempo of Sumatra, Indonesia found that this species occurred in a narrow habitat range with low numbers of mature individuals consisting of 13-19.

Genetic variation refers to all the different gene versions that are present in a population. Over long time scales, decreased genetic variation can be a problem for a population because genetic variation is the raw material of
evolution (Fisher 1930). Furthermore, loss of genetic diversity in small populations of threatened species is predicted to reduce their ability to evolve, and increase their extinction risk in response to environmental change. While experimental evidence validates this prediction, there are only a few examples where extinctions of natural populations can be directly attributed to lack of genetic variation (Farkham et al. 2004).

Understanding genetic variation within and between populations is essential for the establishment of effective and efficient conservation practices for rare and or endangered species. Several aspects of conservation biology, such as loss of genetic diversity in conservation programs and restoration of threatened population, can only be addressed by detailed population genetic studies (Hamrick and Godt 1996). The objectives of this study are to examine the levels of cpDNA (chloroplast DNA) variation and genetic differentiation among T. sumatrana population growing in Sumatra. This molecular information will provide effective and efficient measures for protecting the species.

MATERIALS AND METHODS

Plant material
Leaf samples of adult trees were collected from two populations of T. sumatrana in Mt. Kerinci (Sungai Penuh, Jambi) and Mt. Dempo (Pagaralam, South Sumatra); both sites are located along the Bukit Barisan Mountain Ranges of Sumatra, Indonesia. We took leaf samples from adult trees with diameters of over 25 cm at breast height, and the minimum distance between individuals sampled was 50 m. In total, 27 individuals of T. sumatrana were analyzed in this study: 14 individuals were sampled from Mt. Kerinci and 13 individuals from Mt. Dempo.

Loci studied
At the beginning we evaluated the performance of six candidate plastid DNA regions those: trnT-trnL, trnL-trnF (Taberlet et al. 1991), psbC-trnS, trnH-trnK (Demesure et al. 1995), trnH-psbA (Kress and Erickson 2007) and rbcL gene (Hasebe et al. 1994). However, only three loci gave a good amplified product, those were trnL-trnF, psbC-trnS and rbcL and used for further analysis.

DNA isolation, amplification, and sequencing
Genomic DNA was isolated from adult leaves following the company procedure using DNeasy® Plant Mini Kit (Qiagen, Germany). PCR amplifications were performed in a volume of 20 µl containing 10 ng of genomic DNA, 5 pmol of each forward and backward primer, and 10 µl of Go Taq® Hot Start Colourless Master Mix (Promega, Madison, WI, USA) according to the manufacturer’s instructions. Initial denaturation was performed at 95°C for 2 min, followed by 30-35 cycles of denaturation at 95 °C for 1 min, annealing 52°C for trnL-trnF and 56°C for psbC-trnS and rbcL and polymerization at 72°C for 2 min, and final extension at 72°C for 7 min. Prior to sequencing, the PCR products were purified using rAPid Alkaline Phosphatase™ (Roche, Germany) and Exonuclease I (New England Biolabs, Ipswich, MA, USA). Purified products were directly sequenced on both strands using an ABI Prism 3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

Data analysis
DNA sequences were checked visually, forward and reverse traces were assembled using the ATGC program (Genetyx Corporation, Japan). Single nucleotide polymorphism would clearly be distinguishable from the electropherograms showed for each sequences in ATGC. However it was more apparent when all sequences were exported into fasta file and read in BioEdit (Hall 1999). Further analysis could not be executed from all loci studied because of the absence of nucleotide variation from all individuals in both populations.

RESULTS AND DISCUSSION

Sequences of 808, 1092 and 523 bp (2423 bp in total) were determined, for two non-coding regions of cpDNA, trnL-trnF, psbC-trnS, and rbcL gene respectively. The sequence for each of the region are described below.

(i) sequence of trnL-trnF region of all studied T. sumatrana individuals:

(ii) Sequence of psbC-trnS region of all studied T. sumatrana individuals:

For the complete sequences, see Table 1.
There were no variants for all individuals and population studied. *T. sumatrana* growing in Sumatra-Indonesia occupies specific sites and very restricted with clumped or scattered distribution. Species with this kind of characteristic would show low levels of genetic variation as compared to other species in the genus with wider distributions. This is indeed the case for *T. sumatrana*. There were no variations observed both the population and distributions. This is indeed the case for *T. sumatrana* and non-

(ii) Sequence result of rbcL gene of all studied *T. sumatrana* individuals:

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. sumatrana</em></td>
<td>TTTGTTGTGAATAGATTAGTGAAGAACATTAGATGTGAGGAAATATCCGG</td>
</tr>
<tr>
<td></td>
<td>ACCCTCCTTTTTCCCAATTTCTCAAGGCAACAAACGACTTGTGTTAT</td>
</tr>
<tr>
<td></td>
<td>AGCTTCTTATTACAGACATGTGAGGAAATATCCGGTTTGGTGAATAG</td>
</tr>
<tr>
<td></td>
<td>TTTGTTGTGAATAGATTAGTGAAGAACATTAGATGTGAGGAAATATCCGG</td>
</tr>
</tbody>
</table>

When habitat is highly narrow and limited, in situ conservation effort is a compulsory to carry out with emphasizing on genetic considerations. Many plants, especially rare taxa, exhibit microhabitat preferences (Maliakal-Witt et al. 2005). When these microhabitats occur in the landscape in discrete and small-scale patches, along the time they can create opportunities for genetic divergence at a small spatial scale. To avoid this phenomenon for the narrowly scattered-clumped distribution of the *T. sumatrana*, maintaining the connectivity among clumps is a must. This will allow gene flow and might be implemented to maintain viability or even to...
increase population size. Intact and free-perturbation habitat need to be secured. In a simple word, in situ conservation is the core strategy for species conservation. How this should manage properly to assure the species conservation would require several actions in the field as described below.

Soft flesh fruit of Taxus species (aril) are highly preferred by certain birds and rodents. As seeds are the main key for natural regeneration, predatory mechanism should be checked scientifically. This will give insight to how and what actions need to overcome the predatory problems. Rarity to find natural seedling during field sampling indicated the need of special concern on this aspect. In this case, it is clearly seen the need of additional treatments to support natural regeneration. Related to their natural regeneration capability, soil condition is one of the important factors that need to be taken into account as soil moisture can be extremely limiting factor for seedling survival. Genetic variation is one of the most important factors for the survival of the population. In case of less or even no variation, artificial regeneration is essential. In addition that the population size of T. sumatrana known to be small, limited, and showed narrow habitat range, artificial regeneration could be an important way to increase the population size and yet increase the genetic variation. The success of regeneration both natural regeneration and artificial regeneration should be evaluated by regeneration survey in at least 5-10 year cycles.

Dharr et al. (2006) stated that appropriate light and microclimatic condition are needed to maintain yew population. To maintain the light availability, a continuous selective thinning reducing competition with other tree species is advocated to improve the population status. This also can be applicable for T. sumatrana growing in Indonesia when they exhibit similar ecological niche, the trees usually fill the spots on shady ridges of the hills. Artificial management on promoting light availability might support species growth and lessen the competition between others trees.

If in certain condition the trees need to be cut, it should be done at least 25 cm above from the ground as in general Taxus species can produce more sprouting buds from that origin. During field surveys it is commonly found that many sprouting comes from the fallen branches and stems. Actually this condition is beneficial, especially when alternative propagation by cutting is considered to carry out for producing new plants.

ACKNOWLEDGEMENTS

Authors are grateful to Japan Student Service Organization (JASSO) for the scholarship of the Follow-up Research Fellowship program and made the research possible to carry out. Authors are also grateful to Forest Genetic Lab, Faculty of Agriculture, Ehime University, Japan for accepting and providing all laboratory materials and equipment. Authors are also grateful to Ministry of Environment and Forestry, Indonesia for leave permission during laboratory work abroad.

REFERENCES


