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

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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*, *tnH-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, *trn*L-*trn*F, *psb*C-*trn*S, and *rbcL* gene respectively. The sequence for each of the region are described below.

(i) sequence of *trn*L-t*rn*F region of all studied *T. sumatrana* individuals:

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CCTTGGTATGGAAACTTACTAAGTGATAGCTTCCAAATA
CAGGGGAACCCTGGAATATTTTGAATGGGCAATCCTGAT
CCAAATCCGTATTATAGGAACAATAATTTTATTTTCTAG
AAAAGGGATAGGTGCAGAGACTCAACGGAAGATATTCTA
ACGACTTAATATCATTTGAATTTGAACCAATATTCTATC
TACAGAGTGTAGTATGTTATTGAAAACTTTGAAGTGTTC
TGTATCATCGTTAAAACTTGTTTCAACGATTAGAACTTG
AGTTGTTCTAGGCTTGCCTAGCTTAATGAATACTTAATT
AAAGTAATTCAATTAAGAAATAAATAGAATTTATTCCAT
TTTTGAATTATTGGACGAGGATAAAGATAGAGTCCAATT
CTACATGTAAATGCCAACAACAACAATGCAAATTGCAGT
AGTCGGAAAATCCGTTGGTTTTATAAACCGTGAGGGTTC
AAGTCCCTCTATCCCCAGGTGTATTTCCGAATTAAAGAA
AGATCAAATATTATTACTCTTGACAATTTTATAAGCAAT
CCAGAATATAGAGCTATATTCCCATAAATTTAGAAAGGT
TGATCGTAAGATCAACTCATACATTTTGTCAGATATAAC
ATTTGTGTATGTATAATTGTATTATACATACAATTTAAA
TTTATAATAGAAATTGATAATGGTAACTTACCAATCCAA
AAGTATAATTTAAAAAGGGAAATAAAAAAAGGATTTTCT
TTTGTCTTTTTAGTTGACCTGAGCTCAGGTTCTGCGCTA
GGATGATAAACAGGGAAGAGTCGGGATAGCTC;
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(ii) Sequence of *psbC-trnS* region of all studied *T. sumatrana* individuals:

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TAAATTACTTGGGGCTCACGTGGCTCATGCCGGATTAAT
TGTATTCTGGGCTGGAGCAATGAATCTATTTGAAGTGGC
TCATTTTGTATCGGAAAAGCCTATGTATGAACAAGGATT
GATTTTACTTCCCCATCTAGCTACTTTAGGATGGGGAGT
CGGTCCTGGTGGGGAAATTGTGGACACTTTTCCCTATTT
TGTATCTGGGGTACTTCACTTAATTTCTTCTGCAGTTTT
AGGTTTTGGTGGTATTTATCACGCACTAATCGGACCCGA
AACTTTAGAAGAATCTTTTCCATTTTTTGGTTATGTCTG
GAAAGATAGAAATAAAATGACTACAATTTTAGGTATTCA
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CTTAATTTTGCTAGGTGTTGGTGCTTTTCTTCTAGTCTT
CAAGGCTTTGTATTTTGGTGGCATATATGATACCTGGGC
TCCTGGTGGTGGAGATGTAAGAAAAATTATGAACCTTAC
GCTTAACCCAGTTGCTATATTTGGTTATTTGCTCAAGTC
TCCTTTTGGAGGAGAGGGATGGATTGTTAGCGTGGACAA
TCTAGAAGATATAATCGGAGGACATGTATGGTTAGGTTC
CACCGAGCAATAAGGACTCAGTTGGAAAAAAATATCGAA
GGATCCTGCTATCCCGTCTCCCAACATGGTAAATGAAAA
GAAATTAGATGAATTATGATTTTATCTAGTTTATTTTAT
CGTTTAATTAAGAGGGGTCATGGAAAGAACAGGTTCAAA
ATCACGATCAATTCCTTTCTCAAATCCTGCTGCAGCTGC
GCGAGCTCTTCCTGCATGCCACAAATGACCCACGAAAAA
GAAAAATCCTAGAACAAAATGAGAGGTGGCTAACCAACT
TCGGGGTGATACATAATTCACCGCATTAATCTCGGTAGC
TACACCACCCACAGAATTTAAAGAACCTAAAGGAGCATG
AGTCATATATTCCGCTGAACGTCGTTCTTGCCAGGGTTG
TATGTCCTTTTTCAACTTACTCAGGTCCAAACCATTAGG
ACCCCTTAGAGGTTCCAACCAGGGAGCACGAAGATCCCA
AAAACGCATTGTTTCTCCTCCGAAGATAATTTCTCCAGT
;

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

CGGCTCTACCATAATTTTTGGCAGATAGGCCCAATTTTG
GTTTTATAGTACATCCCAGCAAAGGACGACCATATTTGT
TTAATTTATCTCTTTCCACTTGGATACCATGTGGTGGGC
CTTGGAAAGTTTTTGAATAAGCAGGAGGAATTCGTAGAT
CTTCCAGACGTAGAGCTCGTAGGGCTTTGAATCCAAAGA
CATTACCTACAATGGAAGTGAACAGGTTAGTCACAGAAC
CTTCTTCGAAAAGATCTAAGGGGTAAGCTACATAGGCAA
TAAATTGATTTTCCTCTCCAGGAACGGGTTCGATATCAT
AGCATCGTCCCTTGTAACGATCAAGACTGGTAAGTCCAT
CGGTCCAAACAGTGGTCCATGTACCAGTGGAAGATTCGG
CAGCTACTGCTGCTCCCGCTTCCTCGGGGGGCACTCCCG
GTTGAGGAGTGACTCGGAATGCTGCCAAGATATCAGTAT
CTTTGGTCTGATATTGTGGAGTATAATAAGTTAGTCTGT
AATCTTTAACACCAGC.

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 species level. Our result conformed to Hamrick and Godt (1996) who stated small population size tends to have low level of genetic diversity. Our study also supports the general expectation of reduced genetic diversity in the species with a narrow geographic distribution (Hamrick et al. 1992).

Genetic structure within and between populations is important for developing a conservation strategy for endangered species, especially if not all populations can be protected. Species with low levels of population structure could be simplified, as the loss of single population may have little impact on the species-wide genetic diversity. Molecular and morphological studies of *Taxus* have distinguished genotypes that differentiate (i) individuals within populations (Collins et al. 2003; Lewandowski et al. 1995; Spjut 2007), (ii) distinct populations within geographic regions (El-Kassaby and Yanchuk 1994; Li et al. 2006, Zu et al. 2006; Spjut 2007; Zarek 2009), and (iii) alleged geographically distinct species (Collins et al. 2003; Spjut 2007). However, little attempt has been made to determine genetic variation of the *Taxus* growing in their southernmost distribution, *T. sumatrana* in Indonesia.

Genetic diversity is attributed to the capacity of long term survival of the species. Low genetic diversity in the species will increase inbreeding rate, while the reduction of genetic diversity will affect the adaptability level to the environment change (Furlan et al. 2012). Our study revealed that no variation and no population differentiation could be detected in *T. sumatrana* based on cpDNA variation, suggesting that both population from Mt. Kerinci and Mt. Dempo harbored similar genetic characteristics. It could be that the low level of genetic variation is specific in the *cpDNA* regions. However, among plant DNA regions, non-coding regions, such as the chloroplast markers *trnH-psbA* and *trnL-trnF* usually exhibit high levels of variation, including indel polymorphism (Graham et al. 2000), and for several cases can provide good capacity even for species identification (Hollingsworth et al. 2011; Taberlet et al. 2007). Moreover, in a previous study of DNA barcoding for Eurasian *Taxus* species based on five DNA regions (*rbcL, matK, trnL-trnF, trnH-psbA* and *ITS*), eleven species were clearly identified (Liu et al. 2011). Population genetic study of several *Taxus* species were also recorded using *trnL-trnF* and *petA-psbE* showed significant genetic variation (Gao et al. 2007; Liu et al. 2013; Poudel et al. 2012; Cheng et al. 2015). This observation suggests that the extremely no genetic variation in the *cpDNA* regions examined here is specific for *T. sumatrana* since those loci yielded some extent of genetic variation when assessed to other *Taxus* and non *Taxus* species.

In Sumatra, Indonesia, the species grows naturally inside the protected area or nature reserved. We can simply determine that this condition was strong enough to conserve the species. However, this fact does not reflect factual condition because protected areas and or nature reserves in actual condition are not fully free from disturbances. There are high pressures on habitat destruction of both protected and nature reserves. In this case, effort to conserve outside its natural habitat is worth to be considered and those implemented within the concepts of ex situ conservation strategy. In Indonesia we can see ex situ conservation effort for *T. sumatrana* has been conducted in Cibodas Botanical Garden, West Java.

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 impacted 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 me managed 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 with 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.

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