The genetic potential of *Taxus sumatrana* medicinal plants in Kerinci Regency

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**Abstract.** Taxus - a taxol-producing medicinal plant that mostly found in highland area- is a species in genus Taxus and family Taxaceae. This study was aimed to determine the genetic diversity within and between population of *T. sumatrana* in Kerinci Regency, i.e. Mount Kerinci and Mount Tujuh, based on altitude. The genetic diversity was analyzed with RAPD analysis. The altitude was categorized as low (<2000 m asl) and high (>2000 m asl). The cambium extraction was carried out based on CTAB method. DNA amplification was conducted in RAPD method on machine of PCR System 9700 Applied Biosystems. Nine RAPD primers were used in this study. The results revealed that the average of polymorphic locus was 53.89%. Genetic diversity within population was fairly high with value of 0.1799 and Shannon index of 0.2746. Among the four populations, the population of High Tujuh showed the highest level of variability (He=0.2044). The Nei genetic distance between populations was ranging from 0.0567 to 0.1302. The potential of High Tujuh population is still large enough so that it can still be explored for genetic conservation and cultivated as a taxol-producing material which is useful for medicine.

1. **Introduction**

The Indonesian Forest is rich with the potential for biopharmaceuticals. Taxus or Sumatran pine (*Taxus sumatrana*) is one of those biopharmaceutical potentials. This plant contains taxane diterpenoid namely paclitaxel (commercially called taxol) that has an anticancer capacity [1]. A cancer patient would require an administration of 2-2.5 g of taxol which equivalent to 6-8 of mature taxus trees [2]. The price of taxol is USD 70 for 10 mg [3], therefore it would cost a cancer patient USD 17,500 of treatment.

Cancer is the third highest cause of death in Indonesia after heart disease and stroke [4], with 400 thousand new cases and 230 thousand death cases [5]. In order to meet the market demand of taxol, most species in genus *Taxus* were extensively exploited that led to the decline in the populations [6-8]. The high-rate taxus population decline has caused all species in this genus listed in the Appendix II Cites and IUCN Red List. Indonesia has ratified the protection of *Taxus sumatrana* as the only *Taxus* species found in a fully tropical environment with the Regulation of Minister of Environment and Forestry No. 106/MENLHK/SETJEN/KUM.1/12/2018. *Taxus sumatrana* was found distributed in a group pattern with a density of 19.1 trees/ha in Mount Kerinci [9] and 10.19 trees/ha in Mount Tujuh [10]. The population of this species in those two
locations were dominated by the tree stage but lack of rejuvenation stages of seedlings, saplings, and poles [9]. The species can be found starting at altitudes of 1800 m asl.

Assessing plant genetic diversity without the influences of environment and age can be done with molecular markers [11]. Random Amplified Polymorphism DNA (RAPD) is a molecular marker that is commonly used in genetic diversity analysis. RAPD gives faster results, is easier to carry out, relatively low cost, produces a large number of DNA band polymorphisms [12], and random primers required is relatively easy to find to analyze the genomes of almost all organisms [13]. Moreover, RAPD is a molecular marker that can be used to analyze genetic diversity within population, population structure, differentiation, and genetic dynamic [14,15].

This study was aimed to analyze genetic diversity within and between the populations of *Taxus sumatrana* along the gradients of altitudes based on RAPD marker in Kerinci Regency, those were populations in Mount Kerinci and Mount Tujuh.

2. Materials and Methods

2.1. Location and time

The samples for genetic materials were collected from Taxus trees in Kerinci Regency, Jambi Province (Figure 1). The genetic assay was carried out in the Laboratory of Molecular Genetics, Biotechnology and Forest Tree Improvement Research and Development Center, Yogyakarta Province. The research was done from March to August 2016.

![Figure 1. The map of *Taxus sumatrana* sampling in the Kerinci Regency.](image-url)
2.2. Chemical and equipment
The genetic materials were collected from cambiums of 16 trees samples from Mount Kerinci and Mount Tujuh population based on altitude. The altitude was categorized as low (<2000 m asl) and high (>2000 m asl). Total of populations were four, namely Low Tujuh, High Tujuh, Low Kerinci and High Kerinci. The chemicals used to extract DNA were Cetyl Trimethyl Ammonium Bromide (CTAB) extraction buffer and Purified Sterile Distilled (PSD) HO; chloroform; sodium acetate (NaOAc); isopropanol; 70% and 100% ethanol. Chemicals for DNA precipitation were isopropanol, 70% and 100% ethanol, PSD H2O, and sodium acetate (NaOAc). Agarose gel was composed from PSD H2O, 20X TBE, agarose and Ethidium bromide. Chemicals for PCR were DNA, PSD H2O, ice cubes, KAPA Taq Extra HotStart DNA Polymerase (KK 3504), 5x KAPA Taq HotStart Buffer (KK 1508), dNTPs, MgCl2, and 9 RAPD primers (Operon RAPD 10 Mer Kit).

2.3. Analysis and research design
DNA extraction was done with a modified CTAB method [16] by Shiraishi and Watanabe [17] using Mini beadbeater-8. Following the extraction process, the resulted DNA was purified with re-precipitation. The purified DNA was then counted with NanoVue. The reaction process of PCR DNA was carried out by dissolving the DNA into a concentration of 2.5 ng DNA/µl. The PCR system 9700 Applied Biosystem machine was used for PCR with a total of 10 µl/sample. PCR reaction was conducted in three stages: (1) DNA template denaturation, (2) primer annealing, and (3) extension (Table 1).

| The stages of PCR reaction | Temperature | Time (s) | Remark |
|---------------------------|-------------|----------|--------|
| Preheat                   | 94°C        | 3 seconds|        |
| Incubation                | 95°C        | 60 seconds|       |
| Denaturation              | 94°C        | 30 seconds|       |
| Annealing                 | 37°C        | 30 seconds|       |
| Extension                 | 72°C        | 90 seconds|       |
| Final Extension           | 72°C        | 5 minutes|        |
| Storage                   | 4°C         | -        |        |

45 cycles

The results of PCR amplification were electrophoresis in 1.25% gel agarose, 20X TBE buffer, and 0.5% Ethidium bromide for 2.5 hours at 120 Volt. The results of electrophoresis were then subsequently UV visualized by using Gel DocTM EQ Imaging System (BIORAD) with Quantity One (BIORAD) computer program [18].

2.4. Data analysis
The pictures of RAPD bands were transformed into binary data by scoring 1 (one) in the existence of band and 0 (zero) in the non-existence of band at the same position for each compared-individual. The resulted binary data was analyzed with POPGENE 3.2. The analyzed parameters to assess genetic diversity within and between populations were: Percentage Of Polymorphic Locus (PPL), effective allele count (Ae), Shannon Index (I), and genetic diversity Nei-s/expected heterozygosity (He). The results of grouping analysis were presented in dendrogram, the clustering analysis of populations in a concept of genetic distance was done by UPGMA (Unweighted Pair-group Method with Arithmetic Averaging) method.

3. Results and Discussion
3.1. Genetic diversity
Nine RAPD primers were selected to analyze 4 (four) populations of T. sumatrana in the Kerinci Regency. The size of DNA bands were between 250-1500 bp. Total of 67 polymorphic bands/loci were produced from the nine selected primers (Table 2).
Table 2. The polymorphic loci of the RAPD primers for *T. sumatrana*.

| Primer | Sequence     | Number of Polymorphic loci | Number of Monomorphic loci | Total Loci |
|--------|--------------|----------------------------|---------------------------|------------|
| OPA01  | CAGGCCCTTC   | 10                         | 1                         | 11         |
| OPA03  | AGTCAGCCAC   | 10                         | 4                         | 14         |
| OPA08  | GTGACGTAGG   | 8                          | 4                         | 12         |
| OPA15  | TTCCGAACCC   | 7                          | 4                         | 11         |
| OPA19  | CAAACGTCGG   | 8                          | 6                         | 14         |
| OPR01  | TGGCGGGTCCT  | 7                          | 5                         | 12         |
| OPR03  | ACACAGAGGG   | 7                          | 5                         | 12         |
| OPR04  | CCCGTAGCAC   | 6                          | 2                         | 8          |
| OPR06  | GTCTACGGCA   | 4                          | 4                         | 8          |
| Total  |              | 67                         | 35                        | 102        |
| Mean   |              | 7.44                       | 3.89                      | 102        |
| Percentage |                          | 65.69%                     | 34.31%                    |            |

Primer OPA01 and OPA03 produced the highest numbers of polymorphism loci, 10 loci for each primer. The selected primers produced a total of 67 loci with an average of 7.44 loci/primer. The number of loci produced by the primers depends on the distribution of homologous sites of the corresponding genomes [19].

The diversity between individuals can be reflected in the number of the produced polymorphic loci. [20]. The DNA locus is a result of pairing the primer nucleotides with the plant genome nucleotides, therefore more primers in larger genome sequences would be represented and more actual plant genomes would be depicted. The distribution of primer annealing sites on the DNA genomes, the number of amplified fragments, the purity and concentration of the DNA genomes in the reaction affect the intensity of amplified DNA loci [20]. The genetic diversity of *T. sumatrana* was depicted by the number of polymorphic loci in this current study. Higher result in loci polymorphism reveals higher genetic diversity between individuals [21].

3.2. Analysis of cluster between individuals and populations

Based on the Nei’s [22], the genetic diversity within the population of *T. sumatrana* was varied from 0.164 to 0.204. The highest genetic diversity within population was shown by the High Tujuh population (altitude >2,000 m asl), while the lowest diversity was found in the Low Kerinci population (< 2,000 m asl). The genetic diversity within population of the four populations is presented in Table 3.

Table 3. The genetic diversity of four populations of *T. sumatrana*.

| No | Population   | Numbers of sample | Na          | Ne          | NPL          | PPL        | He          | I            |
|----|--------------|--------------------|-------------|-------------|--------------|------------|-------------|--------------|
| 1  | Low Tujuh    | 5                  | 1.568±0.45  | 1.292±0.34  | 84           | 56.76%     | 0.178±0.18  | 0.274        |
| 2  | High Tujuh   | 3                  | 1.568±0.50  | 1.339±0.35  | 84           | 56.76%     | 0.204±0.19  | 0.308        |
| 3  | Low Kerinci  | 5                  | 1.554±0.50  | 1.257±0.31  | 82           | 55.41%     | 0.164±0.17  | 0.258        |
| 4  | High Kerinci | 3                  | 1.466±0.50  | 1.292±0.36  | 69           | 46.62%     | 0.173±0.19  | 0.258        |
|    | Average      | 1.539±0.50         | 1.2949±0.08 | 53.89%      | 0.179±0.18  | 0.275±0.27 |

Remarks:
Na : number of observed alleles
Ne : number of effective alleles
NPL : number of polymorphic loci
PPL : percentage of polymorphic loci
He : heterozygosity (genetic diversity)
I : Shannon’s Information index
The genetic diversity of High Tujuh population was higher than that of High Kerinci population, with Nei’s index of 0.204 and 0.173, respectively (Table 3). This implied that High Tujuh had a more varied population than High Kerinci, although the previous study found that the total potential density of the Mount Kerinci population was higher than that of the Mount Tujuh population with 19.1 trees/ha [10] and 10.19 trees/ha [9], respectively.

The average of genetic diversity within populations was 0.179 (Table 3) which was lower in comparison with that of tropical trees (0.910) and conifer (0.207) [23]. This low genetic diversity presumably was due to the distinctly geographically limited distribution of *T. sumatrana* in the Kerinci Regency. This plant requires a specific growing climate that would be contributed by the altitude of the growing site.

The total heterozygosity (HT) was 0.2298 (Table 4) which was higher than the heterozygosity within population (HS = 0.1798). This implied that genetic diversity in *T. sumatrana* was fairly distributed among the observed populations (High Tujuh, Low Tujuh, High Kerinci, and Low Kerinci). The heterozygosity within population (HS=0.2298) was pointedly higher than that of between populations (DST=0.0499). This indicated that genetic variation within population was higher than that of between populations. The total heterozygosity of the populations (HT) on the basis of area distribution for the tropical area was 0.275 for Angiospermae [23]. Those were higher than the HT of *T. sumatrana* (0.2298). The limited geographical distribution was assumed to contribute to this lower HT, considering *T. sumatrana* is the only species in the genus *Taxus* that can be found in the fully tropical area.

![Table 4. The genetic diversity gen flow of the T. sumatrana.](image)

| Number of Sample | HT    | HS    | DST   | Gst   | Nm    |
|------------------|-------|-------|-------|-------|-------|
| 16               | 0.2298| 0.1799| 0.0499| 0.217 | 1.8045|

Remarks:
HT : total heterozygosity of the populations (total gene diversity) (HS+DST);
HS : heterozygosity within population (diversity within population);
DST : heterozygosity between populations (diversity between population);
GST : genetic differentiation between population (the coefficient of gene differentiation);
NM  : gene flow

The gene flow of the *T. sumatrana* populations was considerably low (1.8045) (NM>1). Gene flow is correlated with genetic distance. Regardless of the dendrogram that produced three separate clusters, the genetic distances within population were in a range of <0.1 and the genetic variation between populations was <1%.

The genetic divergence between populations was analyzed with the Nei genetic distance [22] in the UPGMA method. In general, the populations of *T. sumatrana* were divided into 3 (three) clusters: the first cluster consisted of Low Tujuh population, the second cluster consisted of High Tujuh and Low Kerinci populations, while the third cluster consisted of High Kerinci population (Figure 2).

The population of High Tujuh and Low Kerinci had a close genetic distance (0.0561) albeit separated by a relatively long geographic distance (Figure 2). The genetic relationship is usually associated with geographic distance, as in the case of the populations of *Intsia bijuga* [24]. However, this current study showed different findings in which the close genetic relationship between the High Tujuh and the Low Kerinci was not associated with geographic distance. This was probably due to the landscape of nature, or even the role of faunas and anthropoids in the gene mixing as occurred in the case of white jabon [25]. Many previous studies also reported the human roles in shaping the genetic structure of a population, such as through intentional domestication or unintentional origin mixing [26, 27].
Figure 2. The dendrogram of the four altitude-based populations of *T. sumatrana* based on the Nei genetic distance (1978).

Information on the genetic diversity of *T. sumatrana* is essential for the conservation and tree improvement programs for this species, considering *T. sumatrana* is a potential plant for biomedicine. Information on genetic diversity would define the necessary strategies and actions to be taken, such as crossing the high genetic diversity populations with the low genetic diversity ones. The genetic information will also be important to prevent the occurrence of inbreeding that will decrease genetic quality of individuals in the population. In a tree improvement program, individuals from a high genetic diversity population are usually recommended to be selected for mother trees. For conservation program, remaining trees of *T. sumatrana* should be prevented from cultivation to maintain the genetic diversity of the species. Establishment of *in-situ* and *ex-situ* plots is very important to be carried out in order to conserve the remaining genetic diversity of the species. For *ex-situ* conservation, as many as possible seeds should be collected from remaining trees from all population, and planted in safe area.

4. Conclusion
Information on the genetic diversity of *T. sumatrana* is essential for conservation and tree improvement programs for this species, considering *T. sumatrana* is a potential plant for biomedicine. Based on nine selected RAPD primers, 4 populations of *T. sumatrana* distributed in the Kerinci Regency have low genetic diversity. Mean genetic distance among the populations was also very low. According to the results, the remaining individual trees and populations of *T. sumatrana* should be prevented from cultivation. *In-situ* and *ex-situ* conservation plots should be established as soon as possible to keep the remaining genetic diversity.

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