Phylogeny of Kemenyan Toba (*Styrax sumatrana*) Inferred from *trnl-trnf* Chloroplast DNA Sequence

Arida Susilowati[1], Henti Hendalastuti Rachmat [2], Wiza Noni Fadilah[1] and Yosie Syadza Kusuma[1]
[1]*Faculty of Forestry, Universitas Sumatera Utara. Jl. Tri Dharma Ujung No. 1, Kampus USU, Medan 20155, North Sumatra, Indonesia*
[2] *Forest Research, Development and Innovation Agency Ministry of Environment and Forestry. Jl. Gunung Batu No. 5. PO Box 165, Bogor 16001, West Java, Indonesia. Tel.: +62-251-8633234; 7520067. Fax. +62-251-8638111*

Keywords: DNA, *Styrax sumatrana*, trnL-trnF, Phylogeny.

Abstract: *Styrax sumatrana* is a member of genus styrax that cultivated by local comunities in North Sumatra due to its higher rosin and cinnamic acid content compared to others. This species is widely distributed in Tapanuli Utara, Pakpak Bharat, and Humbang Hasundutan District. The information on *Styrax sumatrana* molecular phylogeny in North Sumatra has not determinet yet, whereas it is important for future breeding and conservation efforts. Therefore, this research was conducted to determined the phylogenetic relationship *Styrax sumatrana* in North Sumatra and other member of genus Styrax in the world. The material for genetic analysis were leaves sample from 10 individuals and collected from Humbang Hasundutan, Tapanuli Utara, and Pakpak Bharat. Samples then extracted by using CTAB (*Cetyl Trimethyl Ammonium Bromide*) method. DNA amplification was perform using PCR with annealing temperature 50°C. Sequence data analysis was conducted by using BioEdit software and phylogenetic tree construction was using Mega 5.05. The results showed that 3 sampled populations of *S. sumatrana* were grouped into four haplotypes. Phylogenetic tree analysis result showed that *Styrax sumatrana* has the closest relationship with *Styrax suberifolius* and *Styrax chinensis, both are Chinese kemenyan species,* with 63% bootstrap value.

1 INTRODUCTION

Indonesia known as megabiodiversity country with huge number of endemic flora and fauna. Information on plant species diversity in Indonesia is needed for future conservation strategy and loosen the rate of diversity loss. Among those of important native and multipurpose tree in North Sumatra comes from Styrax Genus, and locally are known as kemenyan species. Steenis (1953) mentions four species of kemenyan which were found and cultivated by local farmer in North Sumatra, those were: kemenyan toba (*Styrax sumatrana* J.J.SM), kemeyan durame (*Styrax benzoin*), kemenyan siam (*Styrax tonkinennsis*) and kemenyan bulu (*Styrax paralleloneurum*). There are several local names standing both for similar and different species of the Styrax.

Styrax sumatrana known by local people as the best rosin producer in North Sumatera compared to other because of it whitish color (preffered by market) and stronger odor in which they were assumed have higher rosin and cinnamic acid content (Hidayat et al. 2018). Rosin from this family has long been known for local medicinal, traditional event and pharmaceutical purpose and globally named as benzoin resin (Susilowati et al. 2017).

The general purpose of phylogenetic reconstruction using molecular evidence is done on the basis of a homology sequence by aligning DNA sequences (Thomy et al. 2018). Variations in the sequence of nucleotide caused by substitution of base or the indel. Compared to another marker, the secondary structure of the intron trnL is often constructed to infer homology position, for example in Annonaceae (Pirie et al. 2007). Among the plant DNA regions, non-coding areas such as *trnL-trnF* and *trnH-psbA* chloroplast markers usually exhibit high-level variations, including indel polymorphism, and for some cases can provide good capacity for species identification (Rachmat et al. 2017)

The use of non-coding region sequences of chloroplast genome also has potential in phylogenetic research (Soltis and Soltis 1998). Non-coding region *trnL-trnF* is region with the highest mutation frequency so that in most varied *trnL-trnF* sequence plants (Taberlet et al. 1991). Therefore, our research

Susilowati, A., Rachmat, H., Fadilah, W. and Kusuma, Y.
Phylogeny of Kemenyan Toba (Styrax sumatrana) Inferred from trnL-trnF Chloroplast DNA Sequence.
DOI: 10.5220/0008387300260029
In *Proceedings of the International Conference on Natural Resources and Technology (ICONART 2019)*, pages 26-29
ISBN: 978-989-758-404-6

was conducted to determined phylogenetic relationship of kemenyan toba (*Styrax sumatrana*) in North Sumatra. An understanding of genetic identity of the species and/or population is great practical importance both to conservation biologists and silviculturist.

2 MATERIAL AND METHODS

2.1 Plant Material

Kemenyan leaf sample collected from tree different populations those were Humbang Hasundutan, Pakpak Bharat and North Tapanuli (Figure 1). This population were choosen based on their different of altitude and also the presence of physical barrier of Bukit Barisan mountain range. Ten individuals *S. Sumatrana* was taken from healthy and mature trees of each population.

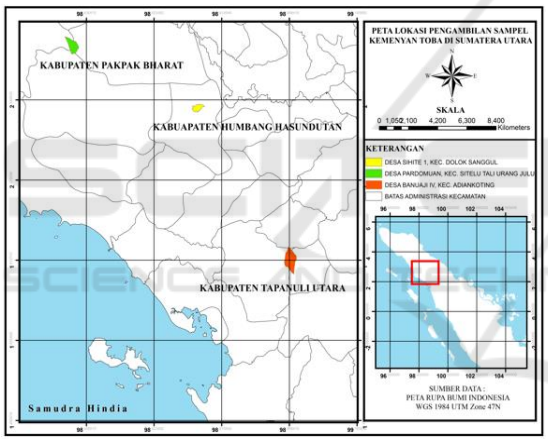

Figure 1. The origin of kemenyan sample from tree population.

The total of 30 samples were sampled originated from 15 years or more mature trees with the average of dbh was 21 cm. Leaf samples were cut to ± 2 cm x 2 cm size and then stored into an plastic clip filled with silica gel at a ratio of 1:5, and kept until all leaf samples ready for extracted. The total genomic DNA was extracted by using a modified CTAB (*Cetyl Trimethyl Ammonium Bromide*) method according Murray and Thompson (1980).

2.2 DNA Sequencing of *trnL-trnF* Chloroplast DNA (cpDNA)

The *trnL-trnF* cloroplast region was amplified by PCR using the universal c and f primers described in Taberlet et al. (1991). PCR process was performed using 20 uL of a solution containing 10 ng of genomic DNA, 5 pmol of each forward and backward primer, and 10 uL of Go Taq® Hot Start Colourless Master Mix (Promega, Wisconsin, 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 at 50°C and polymerization at 72°C for 45 min, and final extension at 72°C for 7 min. Prior to sequencing, the PCR products were purified and automatic sequenced by Genetic Science (Singapore). DNA sequencing was performed for both strands with the primers were used for the PCR amplifications.

2.3 Data Analysis

The successful rate of amplification then assembled using a nucleotide assembly software. In this study the assembly of nucleotides more clearly using BioEdit software (Hall, 1999). Sequences are aligned using MEGA 5.05 software (Tamura et al. 2011) in the ClustalW menu (Larkin et al. 2007) and then manually adjusted.

Phylogenetic studies were analyzed using MEGA 5.05 software on the phylogeny menu using the Neighbor-Joining (NJ) method. The consistency of NJ phylogenetic trees was tested by the bootstrap method (Felsenstein, 1985) of 1,000 repetitions. Genetic distance between samples was analyzed using Kimura 2-parameter method (K2P) (Kimura, 1980). Phylogenetic studies of another Styrax species using *trnL-trnF* primers can be obtained from the sequence databases of various deposited Styrax types in NCBI (https://www.ncbi.nlm.nih.gov), the DNA sequence is then aligned with Mega 5.05 using Align by Muscle and then select the Phylogeny menu to obtain the phylogenetic tree.

3 RESULT AND DISCUSSION

Not all of 30 samples produce clearly chromatographic sequences and graph, at the end we only used 26 individuals that yield clear and unbiased sequence read. The four excluded individuals were originated two from Humbang Hasundutan (SS13HB, and SS20HB) and two more individuals from Tapanuli Utara (SS23TU and SS24TU).

After alignment we obtained 941 bp of sequence length from all individuals which were divided into 4 haplotypes (Rachmat et al. 2017). Phylogenetic analysis was performed using Mega 5 software, using

Neighbour Joining (NJ) method. The analysis of the *trnL-trnF* gene involved 53 data, containing 26 Styrax sumatrana sequences and 27 other Styrax species, including:. Others styrax sequences references were downloaded from NCBI. *Styrax suberifolius, Styrax chinensis, Styrax gentryl, Styrax pentlandianus, Styrax nunezii, Styrax latifolius, Styrax peruvianus, Styraz camporum, Styrax leprosus, Styrax pohlii, Styrax obtusifolius, Styrax ferrugineus, Styrax rotundatus, Styrax acoustic, Styrax tomentosus, Styrax lanceolatus, Styrax glaber, Styrax portoricensis, Styrax martii, Styrax laberi, Styrax ubargenteus, Styrax officinalis, Styrax benzoin, Styrax aureus, Styrax japonicus,* and *Styrax agrestis.* The reference sequences were aligned using the Align by Muscle menu. The phylogenetic tree is a graph used to describe the interconnecting kinship between species consisting of a number of nodes and branches with only one branch connecting the two closest nodes. Each node represents the taxonomic units and each branch represents the relationships between units that describe the hereditary relationship with the ancestor.

The phylogenetic tree produced by the Neighbour Joining (NJ) method produces a hypothesis of kinship relationships between samples based on the genetic distance in the *trnL-trnF* gene. In the present study, phylogenetic trees were tested statistically using the bootstrap method of 1000 replications presented in Figure 2.

Reconstruction of phylogenetic trees based on molecular markers *trnL-trnF* shows the separation of several groups. The sample group of *Styrax sumatrana* is supported with 83% bootstrap values consisting of four sub-groups with bootstrap values ranging from 56 – 94 % (See Figure 2). From the phylogenetic tree, it can be seen that *Styrax sumatrana* has the closest relationship with two China species of *Styrax suberifolius* and *Styrax chinensis* with a bootstrap value of 63%. Even though shared similar habitat, the relationship among *S. sumatrana* with that of *S. benzoin* seemed to be far enough.

Considering the phylogenetic relationship as described in Figure 2, we can determine that *S. sumatrana* had close ancestry with those of China species of *Styrax suberifolius* and *Styrax chinensis* rather than *S. benzoin* which grow and share similar habitat type. Phylogenetic trees provide information about the classification of populations based on their evolutionary relationships. The roots of the tree illustrate the first branching point or origin of each population on the assumption that the rate of evolution is running constant (Dharmayanti, 2011).

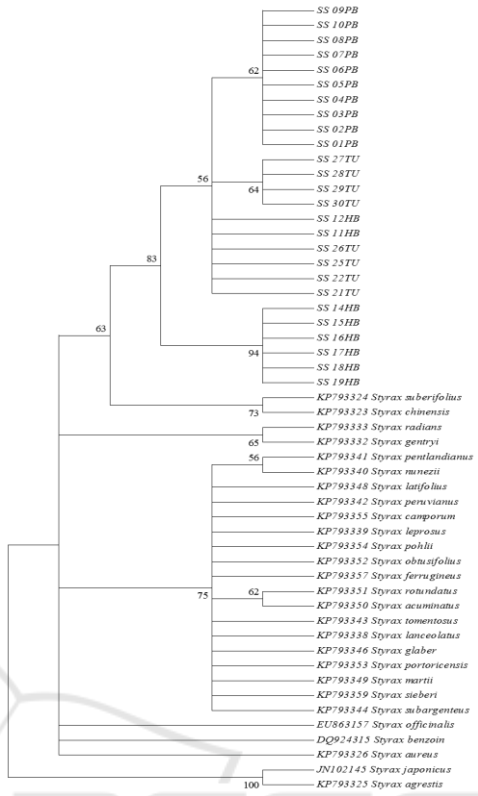

Figure 2: Phylogenetic tree of *Styrax sumatrana* and the other Styrax from all over the world with the same marker. Note : SS (*Styrax sumatrana*), PB (Pakpak Bharat), TU (Tapanuli Utara), HB (Humbang Hasundutan).

Among their inter population differences, we found that the genetic distance between *Styrax sumatrana* from Tapanuli Utara and Pakpak Bharat is 0.003 or 99.7% similarity, Tapanuli Utara with Humbang Hasundutan similarity is 99%, while Humbang Hasundutan value with Pakpak Bharat is 99.7%.

The genetic distance of *Styrax sumatrana* with *Styrax chinensis* and *Styrax suberifolius* is 0.005 or with similarity of 99.5%. While *Styrax sumatrana* with *Styrax benzoin* have similarity of 99,3%.

4 CONCLUSIONS

Our result on the phylogenetic tree of *Styrax sumatrana* showed that this species has the same monophyletic with *Styrax suberifolius* and *Styrax chinensis* with a bootstrap value of 63%. Although *S. sumatrana* and *S. benzoin* were planted together in North Sumatera, both of species separated into different group.

REFERENCES

Dharmayanti, I. 2011. Filogenetika molekuler: metode taksonomi organisme berdasarkan sejarah evolusi. *Wartazoa*:21:1

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser* 41: 95-98.

Hidayat, A., Iswanto, A. H., Susilowati, A., Rachmat, H. H. 2018. Radical scavenging activity of Kemenyan Rosin Produced by an Indonesian native plant, *Styrax sumatrana*. *Journal of the Korean Wood Sciences Technology*: 46 No.4:346-354

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* 39:783-791.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., Higgins, D. G. 2007. ClustalW and ClustalX version 2. *Bioinformatics* 23 (21):2947-2948.

Kimura, M. 1980. A simple methode for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111-120.

Murray, H. G., Thompson, W. F. 1980. Rapid isolation of high molecular weight DNA. *Nucleilc Acids Res* 8: 4321-4325

Pirie, M. D., Vargas, M. P. B., Bottermans, M., Bakker, F. T., Chatrou, L. W. 2007. Ancient paralogy in the cpDNA trnL-F region in Annonaceae. *Am J Bot* 94:1003-1016.

Rachmat, H. H., Susilowati, A., Elfiati, D., Hartini, K. S, Faradillah, W. N. 2017. Strong genetic differentiation of the endemic rosin-producing tree *Styrax sumatrana* (Styracaceae) in North Sumatra, Indonesia. *Biodiversitas* 18:(4): 1331-1335

Soltis, D. E., Soltis, P. S. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. In: Soltis, D. E., Soltis, P. S., Doyle, J. J., editors. Molecular Systematics of Plants II: DNA Sequencing. Netherlands: Kluwer Academic Publishers. ISBN : 0-412-11131-4. p. 1-42.

Steenis, V. 1953. Styracaceae. *Flora Malesiana Ser*. I, Vol.42.

Susilowati, A., Rachmat, H. H., Kholibrina, C. R., Ramadhani, R. 2017. Weak delineation of *Styrax* species growing in North Sumatra, Indonesia by *matK + rbcL* gene. *Biodiversitas* 18(3): 1270-1274

Taberlet, P., Gielly, L., Pautou, G., Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17:1105-1109.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28 (10):2731-2739.

Thomy, Z., Yulisma, A., Harnelly, E., Susilowati, A. 2018. Molecular phylogeny of peat swamp trees in Tripa Peat Swamp Forest Aceh inferred by 58S Nuclear gene. *Biodiversitas* : 19(4):1186-1193