

Molecular characterization *Pinus merkusii* native Kerinci revealed by rbcL

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Abstract

Pinus merkusii native Kerinci, also known as Sigi, shares the same taxonomic family as *Pinus merkusii* native Aceh but differs in morphology, anatomy, and physiology. Additionally, *P.merkusii* Kerinci has a high utilization potential but its population is decreasing and the entry of *P.merkusii* native Aceh around the Kerinci population can result in the erosion of the original genetic source (germplasm). The molecular characteristics of *P.merkusii* Kerinci and its differences with *P.merkusii* Aceh will be studied using rbcL. Molecular character of *P.merkusii* Kerinci will be compared with *P.merkusii* Aceh using rbcL marker in 4 individuals. The results obtained for the rbcL analysis are the percentage of conservative characters at 100%, and the four populations of *P.merkusii* Kerinci and Aceh are located in the same cluster and branch length. This indicates a large percentage of individual similarity between the five populations. These results tend to prove that *P.merkusii* Native kerinci and Aceh are located in the same taxa species and the rbcL marker cannot distinguish between *Pinus merkusii* native Kerinci and Aceh.

Keywords: *Pinus merkusii*; Sumatera; Kerinci; RbcL

1. Introduction

The only kind of pine that grows in Indonesia is *Pinus merkusii*, one of the pines that naturally occur in Southeast Asia. Its geographic range includes Thailand, Cambodia, Vietnam, Laos, Myanmar's eastern region, Indonesia, and the Philippines [13]. *P.merkusii* is the tallest pine species in the world and has a medium-sized tree that can reach a height of up to 70 m [21]. When the tree is young, its canopy is pyramid-conical, but as it grows, it tends to flatten out and spread out [3]. Only the regions of Aceh, Tapanuli (North Sumatra), and Kerinci on the island of Sumatra naturally accommodate *P.merkusii* in Indonesia. There are numerous differences between the three populations of *P.merkusii* in Sumatra in terms of morphology, anatomy, physiology, and genetics [5], [6], [11], [19], [16]. *P.merkusii* native Kerinci differs from other *P.merkusii* species in that it has upright stems, thinner bark, and a scaly stem surface that lacks deep grooves, making it more resemblant to the shape of the stems of broad-leaved plants [11].

P.merkusii native Kerinci also referred to as Sigi by the locals, uses *P.merkusii* as firewood. *P.merkusii* is generally a plant that is frequently suggested as a pioneer plant in rehabilitation and restoration due to this population's ability to grow well on critical land (rocky soil and steep areas) and because it has potential as an indicator of climate change because it produces a clear growth circle [14], [16]. Additionally, *Pinus merkusii* has economic significance for the neighborhood, particularly as a producer of wood and latex (gondorukem and turpentine) [3]. In Indonesia, gondorukem and turpentine have long been used as fundamental components in a variety of products, including batik, paint solvents, paper, paint oil, perfume blends, detergents, flavorings, insecticides, lubricants, medicines, and plastics [18], [15].

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The *Pinus merkusii* Kerinci has a lot of potential because it produces latex (gondorukem) more quickly and in greater quantities than the other two native pines, even before the stem in the latex has emerged on its own. Additionally, *P.merkusii* Kerinci's wood is superior because of its large, upright trunk [18]. Before breeding and other uses, *P.merkusii* Kerinci was becoming less common because of encroachment or field use disturbances by the community, extremely low *P.merkusii* Kerinci regeneration, and the emergence of *Pinus merkusii* native Aceh near the *Pinus merkusii* Kerinci population's habitat [8], which may lead to the loss of the *Pinus merkusii* native Kerinci and the erosion of the original genetic source (germplasm). This issue is made worse by the lack of a distinct taxonomic status or additional character analysis that separates the Kerinci population from the Aceh population. Genetic data in the form of the molecular character of *P.merkusii* Kerinci is required for properly directing efforts to conserve plants and germplasm sources.

The CBOL (Consortium for the Barcode of Life) recommends the *rbcl* primer as one of the chloroplast DNA barcodes for plants, especially gymnosperms, because it satisfies the requirements for good primer standards, specifically in terms of universal primer properties, sequence quality, and species discrimination [4]. It has been demonstrated that the *rbcl* primer reveals genetic characteristics at the species and genus levels [22], [1]. Additionally, *rbcl* primers may reveal character variations at the intraspecies level [2].

The purpose of the study was to use *rbcl* to analyze the molecular characteristics of *P.merkusii* Kerinci and to determine whether they could be used to distinguish between *P.merkusii* native Kerinci and the *P.merkusii* native Aceh. This study aims to enhance the efficacy of genetic conservation and the preservation of *P.merkusii* native Kerinci germplasm.

2. Material and methods

Sampling was done at four different sites in West Sumatra, three of which were populations of *P.merkusii* grown from seeds imported from Aceh (South Rao District, Alahan Panjang, and TAHURA) and one of which was *P.merkusii* native Kerinci population from Bukit Tapan Kerinci. Four samples from each location were used for the *rbcl* sequencing DNA analysis. The CTAB method was modified for DNA isolation [7].

2.1. Amplification of *rbcl* Primer

The obtained DNA isolation results are then used for the amplification procedure using the Cocktail PCR composition, which consists of mastermix taq green 11 μ l, 9 μ l nuclease-free water, 3 μ l isolated DNA, and 1 μ l of forward and reverse primer. Then, 40 cycles of PCR were carried out, each cycles consisting of the following steps: initial denaturation at 95 °C for 2 minutes, denaturation at 95 °C for 20 seconds, annealing at 55 °C for 60 seconds, extension at 72 °C for 1 minute, and final extension at 72 °C for 10 minutes [17].

2.2. Data Analysis

Using the MEGA X program [20], the results of the *P.merkusii* *rbcl* sequences are then analyzed for sequence characters and phylogenetic analysis using the Neighbor-Joining (NJ) method.

3. Results and discussion

To determine the DNA base arrangement in each *P.merkusii*, four samples of *P.merkusii* from the populations South Rao, TAHURA, Alahan Panjang, and Kerinci were sequenced using DNA analysis. According to table 1, the *rbcl* region sequence length varied between 1291 and 1315 bp in individuals from four populations, with a total length of 1226 bp being used for data analysis. This value is not significantly different from the *Pinus* genus's 1262 base length of the *rbcl* region [9].

It can be said that the five sequences of the *rbcl* region do not exhibit any changes or mutations in nucleotide base substitution because the conservative character obtained in the five populations of *P.merkusii* is very high, measuring 1226 bp with a percentage of 100 percent while the variable character is 0 (zero). These findings suggest that *P.merkusii* is the same species in all five populations studied: South Rao, TAHURA, Alahan Panjang, Kerinci, and Thailand. In the genus *Pinus*, the *rbcl* marker is known to discriminate against taxa at the species level [9], [1]. However, the findings of this study indicated that the *rbcl* marker did not reveal any distinctive molecular characteristics that would have distinguished *P.merkusii* native Kerinci from *P.merkusii* native Aceh.

Table 1 Sequence characteristics of *Pinus merkusii* with rbcL marker

No	Sequence Characteristics	Value
1	Sequence Length (pb)	1291 -1315
2	Sequence Length in Data Analysis (pb)	1226
3	Conservative Character	1226
4	Percentage amount of G+C (%)	44.60%
5	Percentage amount of A+T (%)	55.39%

According to the percentage of bases, G+C's percentage (44.60 %) was lower than A+T's percentage (55.39 %). Gernandt et al. [9] found that the G+C base percentage in the rbcL area of the *Pinus* genus was 45.1 percent lower than the A+T base percentage. DNA rbcL, which exhibits high variability of the translocation structure in certain plants, is one sequence region in the chloroplast genome that has more bases A (adenine) and T (thymine) than bases G (guanine) and (cytosine).

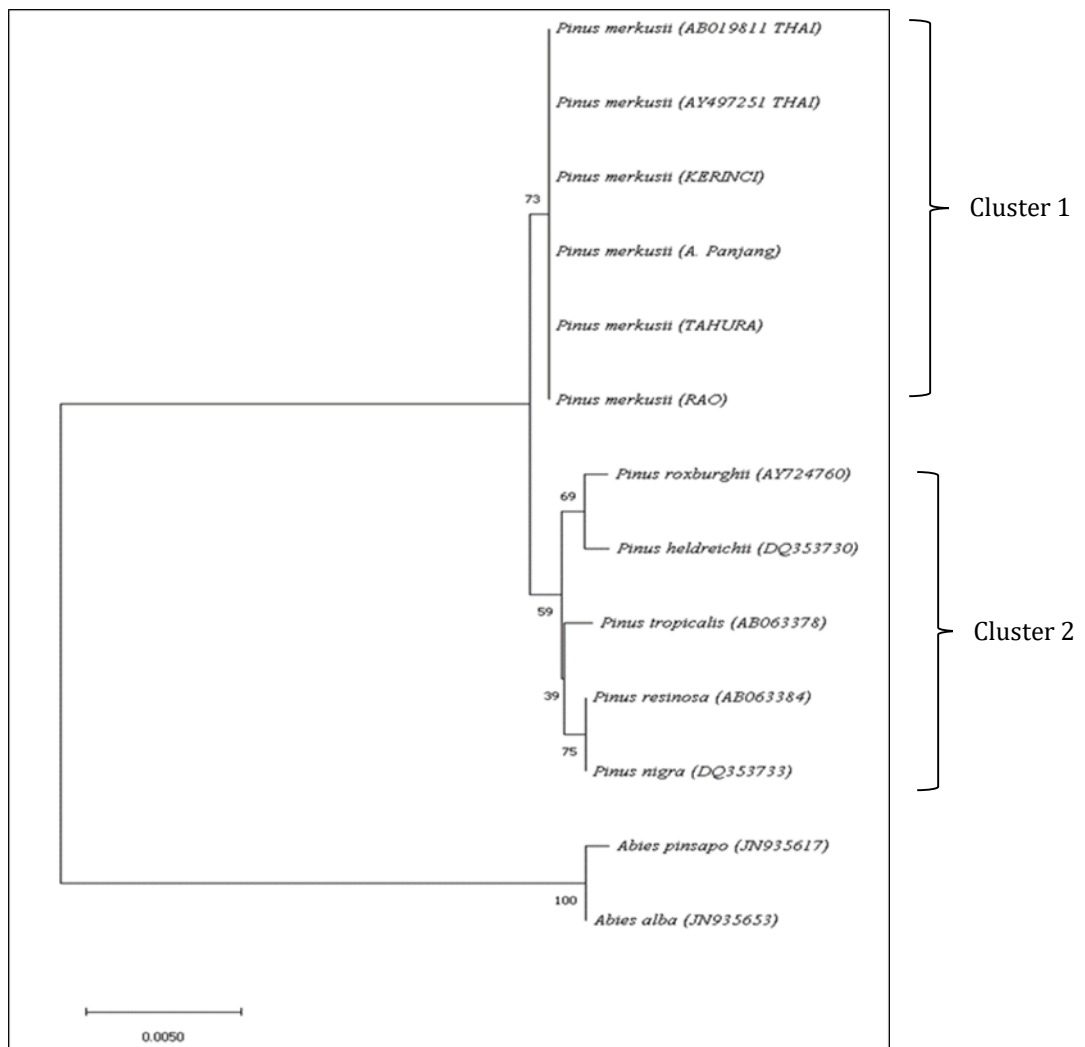


Figure 1 Dendrogram of the *Pinus* genus based on the rbcL marker using NJ Method

Pinus merkusii samples were used in the phylogenetic analysis, representing five populations from South Rao, TAHURA, Alahan Panjang, Kerinci, and two samples from NCBI data from Thailand. These samples were supplemented with three different species from the same genus, *Pinus*, and three different species from the same family, Pinaceae, to serve as an outgroup.

All *P.merkusii* individuals from the five populations are found in the same cluster (cluster 1), according to the results of DNA sequence analysis of the *rbcL* region (Figure 1). This cluster has a bootstrap percentage of 73 percent, and the value of sequence divergence and genetic distance is 0 percent among the five members of the population. This shows the percentage of individual similarity between the five populations. These results are not correlated in morphological studies where there are differences in several characters between *P.merkusii* from Thailand and *P.merkusii* from Sumatra, namely the character of seed size, seedling stage, shape, leaf or needle, size of cones or strobilus, surface and thickness of bark [5]. The morphological, anatomical, and physiological characteristics of *P.merkusii* Native Kerinci and Aceh differ in many ways, including leaf length, skin thickness, and number of seeds per kg, stem shape, presence of an earlywood/latewood boundary, concentration, and latex content composition [10], [8], [18], [16].

It is possible that changes in environmental factors or adaptations do not affect changes in genetic structure, especially in the arrangement of *rbcL* bases due to slow mutation rates, given the lack of regional differences in *rbcL* bases (cpDNA) between *P.merkusii* Kerinci and *P.merkusii* Aceh. Several variables can affect how effective DNA barcodes are at differentiating between taxa. For DNA barcodes to be effective, there must be enough time for gene drift and mutation to result in a set of characteristics in a population of individuals that will distinguish them from other species, especially in woody plants, which have a slow rate of mutation and a long generation time [12].

4. Conclusion

According to the research's findings, the molecular characteristics of the four populations of *P.merkusii* do not differ in nucleotide bases in the *rbcL* region, as shown by the 100% conservative character percentage. Moreover, the four populations of *Pinus merkusii* West Sumatra are all found in one cluster with the same branch lengths, showing that *P.merkusii* native Aceh and Kerinci belong to the same taxa species.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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