Genetic diversity based on molecular analysis for Asam Gelugur (*Garcinia atroviridis* griff. ex t. anders) some districts in North Sumatra

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Abstract. *Garcinia atroviridis* Griff. known as asam gelugur, it is native to Peninsular Malaysia. The distribution area of Asam gelugur in Indonesia is from Aceh to South Sumatra. The use of Simple Sequence Repeats (SSR) markers aims to understanding of the genetic diversity of *G. atroviridis*. This study aims to observed at the genetic diversity of asam gelugur from Deli Serdang, Langkat, Karo, Serdang Bedagai and Asahan Districts, North Sumatera, Indonesia. The research was conducted at the Molecular Genetics Laboratory, Agriculture Faculty, Universitas Sumatera Utara, Medan. The research method used of the molecular marking method using 2 SSR primers (M1s75 and Vsur) based on PCR (Polymerase Chain Reaction) which amplifies the Specific DNA sequence. The results showed that primers M1s75 and Vsur were able to amplify 25 samples. The size of the bands was around 150-285 bp and showed monomorphic bands.

1. Introduction
Asam Gelugur (*Garcinia atroviridis* ex Griff T. Anders) is a tropical and subtropical annual plant originating from South Asia and Southeast Asia which grow widely on the island of Sumatra. Based on the examination of herbarium collection and literature review, there are 77 species of *Garcinia* in Indonesia. The 22 species are found in Kalimantan, 22 species in Sumatera and Sulawesi, 17 species in Moluccas and Papua, 8 species in Java, and 5 species in Lesser Sunda Island of Indonesia. Six species of these are cultivated plants (*Garcinia atroviridis*, *G. beccasi*, *G. dulcis*, *G. mangostana*, *G. nigrolinatta* and *G. parvittora*), while 58 species as the wild plants, 22 species as edible fruits and 21 species as timber plants [1].

Based on the results of the analysis of the diversity from 18 accessions of Asam Gelugur (based on fruit morphological characters) indicated that fruit accessions from Serdang Bedagai, Batubara and Asahan districts, North Sumatera, Indonesia, have low diversity [2]. Based on the dendrogram of fruit morphology showed that 18 accessions were categorized into four groups on a distance scale of 0.796 which had a similarity level around 79%-85%. Another experiment of [3,4], based on the identified morphological characters Asam Gelugur in Deli Serdang, Langkat and Serdang Bedagai Districts, North Sumatera, Indonesia, pointed out that 21 accessions of asam gelugur were found in each location has a relatively close similarity distance.

Identification of the genetic diversity of *G. atroviridis* based on its molecular markers has not been studied in Indonesia. Therefore, molecular studies of asam gelugur are needed to obtain clearer and
more informative genetic relationships of asam gelugur to produce a more precise estimate of genetic similarity in asam gelugur germplasm analysis. This is appropriate with literature [5] which states that the level of diversity analysed by SSR and RAPD in the population is very important information for the evolution of G. xanthocymus in geographic different area.

There are significant morphological and anatomical differences between female and hermaphrodite flower. Hermaphrodite flower has relatively long filaments and produce abundant and fertile pollen, whereas females produceless pollen. They are also differed significantly in reproductive traits. Hermaphrodite flowers have more flowers than female flowers, but fall off gradually before bearing fruit. In contrast, female trees have relatively larger ovules on each flower, producing larger fruit and more seeds per fruit than hermaphrodite trees. The morphological characterization of flowers observed included the position of female and hermaphrodite flowers, flower size (small to large), colour of sepals and flower petals [6].

This learning was designed to getting a better considerate of the genetic assortment of G. atroviridis by using SSR markers.

2. Materials and methods

2.1. Materials
The research was conducted at the Molecular Genetics Laboratory, Agriculture Faculty, Universitas Sumatra Utara, Medan. The materials used in this study was the leaf of G. atroviridis taken from five districts of Deli Serdang, Langkat, Karo, Serdang Bedagai and Asahan. Aluminium foil, liquid nitrogen, Polyvinylpyrrolidone (PVPP), CATB extraction buffer (2 g NaCl, 5 g CTAB, 100 ml aquades), TAE Buffer, TE Buffer, β-mercaptoethanol, KIAA (24 ml Chloroform: 1 ml IsoAmil Alcohol), isopropanol, absolute ethanol, 50 bp Ladder DNA marker, Go taq Green Master Mix, aquabidest, primer M1s75 and Vsur, agarose gel, loading dye, tissue, rubber gloves and masks.

The tools used in this study were scissors, plastic clips, mortar, pestle, 0.5-10 µl size micropipette, 20-200 µl, 100-1000 µl, tip (white, yellow, and blue), tube rack, Eppendorf 2 tube, 0 ml and 0.5 ml, 0.2 ml PCR tube, water bath, thermometer, glassware (erlenmeyer, measuring cup etc.), magnetic stirrer, hot plate, centrifuge, freezer, refrigerator, computer, digital scales, vortex, agarose molds, electrophoresis (Chamber Well), UV Transilluminator PCR, Cambridge UV Gel-Doc, power supply, spatulas, cameras and stationery.

2.2. Research methods
The research method was using of two SSR primers (M1s75 and Vsur) based on PCR (Polymerase Chain Reaction) which amplifies the Specific DNA sequence.

2.3. Implementation of research

2.3.1. Amplification and selection of SSR markers. The amplification was carried out based on 2 SSR primers. Before running PCR, DNA dilution was carried out by taking 3 µl of DNA stock and added 5 µl ddH₂O (8µl in total) of aliquot DNA obtained for each sample. Then a primary dilution was carried out, and centrifuged for 5 min after which ddH₂O was added according to the molar size. The primary aliquot is created by taking 10-15 µl of the primary stock.

The initial preparation of the PCR was to melt the components for running PCR, namely Promega's Go Taq Green Master Mix PCR package in a box of broken ice. To facilitate the making of the master solution 20 samples will be used, then the master solution consists of: ddH2O 4.5 µl x 25 = 112.5 µl, Go Taq Green Master Mix 7.5 µl x 25 = 187.5 µl, primary aliquot 1 µl x 20 = 25 µl. 13 µl was taken from the tube to another tube so that 25 tubes were obtained for PCR and 2 µl of each aliquot DNA was added. Then the tubes containing DNA stock and master mixtures were included in the sample block in the PCR machine with annealing temperature of 54°C. The Applied Biosystem Gene Amp
PCR amplification reaction was designed for 30 times, temperature and thermal cycle used in Asam gelugur, shown in Table 1 and 2.

**Table 1.** Cycle, process, temperature and time of amplification.

| No. | Stages            | Temperature | Time | Number of cycles |
|-----|-------------------|-------------|------|------------------|
| 1   | Pre-Denaturation  | 94°C        | 2 min| 1                |
| 2   | Denaturation      | 94°C        | 45 sec| 30              |
| 3   | Annealing         | T°(opt)     | 45 sec| 30              |
| 4   | Extension         | 72°C        | 1 min| 30              |
| 5   | Final Extension   | 72°C        | 10 min| 1               |
| 6   | Cooling           | 4°C         | 30 min| 1               |

T°(opt) : annealing temperature was optimized of each primer (48-50°C)

**Table 2.** Annealing temperature of SSR.

| Primer | Stages                  | Total base pair | Annealing temperature |
|--------|-------------------------|-----------------|-----------------------|
| MIs75  | F: AAGAAAATGGGGGCAAGCCT | 45              | 60°C                  |
|        | R: TGCTGTATTTCTCTCTCTGAT |                 |                       |
| Vsur   | F: CTagTTGATTTTCAATTCCAC | 40              | 52°C                  |
|        | R: TGGTATGACTAGCACA    |                 |                       |

Before agarose electrophoresis was prepared with a concentration of 1.3%, agarose weighed 1.04 g and then dissolved by adding 80 ml of TAE buffer to 1x. The solution is inserted into the erlenmeyer, then heated and stirred with a magnetic stirrer until the solution becomes clear. After that the solution is added with 1 µl solution of red gel. Then reheated and then cooled in the same way. After the solution has cooled a bit (60°C) the solution is put in the agar mold that has been installed with a well-forming comb and allowed to solidify for 40 min or until the gel hardens. Well-forming combs are slowly released and agarose gels are ready to be used for electrophoresis.

The electrophoresis tray containing agarose gel placed into an electrophoresis tank and 1x TAE buffer solution poured into the tank ± 670 ml (until submerged). Inserted 50 bp Ladder as much as 1.5 µl in the first gel well as a measure of DNA tape. Prepared DNA samples were inserted as much as 4 µl into the well in the next gel. After all the samples are inserted in the well (well), the electrophoresis tank is closed and connected to an electric current. Then the electrophoresis process is ready to run. Electrophoresis was carried out at 46 volts for 120 min. After the electrophoresis is complete, the electric current is turned off and the tray is taken using a glove. DNA visualization that has been electrophoresed was carried out with UV transilluminator by placing the gel on the UV transilluminator and if the band DNA molecule looks bright it is documented.

2.3.2 Base pair size determination. The base fragment size (bp) of the PCR product was determined by using the UVITEC Cambridge Fire Reader software. The DNA fragment used is 50 bp Ladder. The size of the DNA band (bp) will be aligned from the ladder we use. The measurement of the formed band pattern with the DNA glow formed during the electrophoresis process with UV light.

3. Results and discussion

The amplification of 25 samples using 2 SSR primers namely MIs75 and Vsur where both primers showed monomorphic bands. The results of observing the number of DNA fragments and the polymorphic percentage of each primer can be seen in the Table 3.

Based on Table 3, it can be seen that the banding pattern produced by the 2 primers used produces the same banding pattern. The sizes of the resulting ribbons varied between 150-285 bp, with 0% polymorphic band.
Table 3. Polymorphic presentation.

| No | Primer | Size band (bp) | Total Band | Total Polymorphic Band | Total Monomorphic Band | Polymorphic Presentation |
|----|--------|----------------|-------------|------------------------|------------------------|--------------------------|
| 1  | M1s75  | 200-285        | 25          | 0                      | 25                     | 0%                       |
| 2  | Vsur   | 150-240        | 25          | 0                      | 25                     | 0%                       |

The primer M1s75 was able to amplify of 25 plant DNA. The banding pattern is homozygous, the number of bands is 25 which are monomorphic bands, and the size of the bands is around 200-285 bp. The percentage of polymorphic bands is 0% (figure 1).

![Figure 1](image1.png)

**Figure 1.** Amplification of asam gelugur DNA with SSR markers with M1s75 primer

Primer Vsur was able to amplify 25 plant DNA. The banding pattern is homozygous, the number of bands is 25 which are monomorphic bands, and the size of the bands is around 150-240 bp. The percentage of polymorphic bands is 0% (figure 2). The difference in the intensity of the DNA bands is powered by the scattering of the original attachment sites on the genome, genome purity and strength of solution in the reaction.
4. Conclusions

The results showed that primers M1s75 and Vsur were able to amplify 25 samples, with monomorphism band and the banding pattern are homozygous. The band scope of M1s75 is around 200-285 bp, and the band size of Vsur is around 150-240 bp. The required a testing by using more primers to increase polymorphism so that higher and more correct genetic diversity can be identified.

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