Genetic divergence landrace of langsat (*Lansium parasiticum*) from Siberut Island based on ITS and MatK markers

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**ABSTRACT**

Langsat is a specific potential of tropical fruit, especially in the Southeast Asia region. The center of distribution this species in Indonesia is Sumatra region, including the Siberut Island. There are some local germplasm (landraces) of langsat from Siberut Island namely Seccet, Siamung, Telu toru gokgok, Elakmata, and Langsat padang. Analysis of genetic diversity and genetic divergence is very important for sustainable utilizing of this tropical fruit. Based on analyzed of fourteen accessions of langsat using ITS and MatK markers indicated that landrace of langsat from Siberut Island had the higher diversity of haplotypes (ITS, \(H_d = 0.95\); MatK, \(H_d = 0.80\)) compared to those of the Sumatran mainland (ITS, \(H_d = 0.85\); MatK, \(H_d = 0.28\)). Based on the phylogenetic trees of fourteen accessions analyzed showed that the accessions of langsat from Siberut Island were separated from accessions of Sumatran mainland.

**Key words:** Genetic divergence, Haplotype diversity, ITS, Langsat, MatK, Siberut Island.

**INTRODUCTION**

Langsat (*Lansium parasiticum* (Osbeck) K.C. Sahni & Bennet) included in a group of family Meliaceae is a tropical fruit that has high economic value. This fruit is widely distributed in Southeast Asia countries one of them is Indonesia (Syamsuardi *et al.*, 2018). In addition to being used as a fruit plant, this type is also used as cosmetic ingredients and traditional medicines (Tilaar *et al.*, 2008; Manosroi *et al.*, 2012). Distribution of langsat in Indonesia, including Sumatra, Kalimantan, Java, and Sulawesi.

One area in Sumatra has been cultivating this species since long is Siberut Island in the Mentawai Archipelago. Historical records mention the Mentawai Islands are separated from the mainland of Sumatra since the Pleistocene era around more than 500,000 years ago (Verstappen, 1975). This geographical condition makes the Mentawai Islands have a high and unique biodiversity diversification so that they are different from those found on the island of Sumatra according to Hadi *et al.* (2009). Langsat in Siberut Island is a landrace plant that is intentionally planted in traditional fields (*Pumonean*) as one of the food sources. Indigenous people recognize this species with seccet, siamung, telu toru gokgok and Elakmata where each of these plants has its own characteristics (Indra *et al.*, 2017). Landrace is a cultivated plant that has a historical origin and is often genetically diverse and is adapted locally and is associated with traditional farming systems (Villa *et al.*, 2005), and is very important for the future breeding programs (Cristobal and Herrero, 2016).

Landrace langsat is a source of germplasm that is very important for the sustainable use of Indonesian tropical fruits. Analysis of the level of genetic variation and genetic divergence is very important to determine the potential evolution and genetic conservation of tropical fruits. The Molecular approach using the ITS and MatK markers is a convising alternative to more accurately measure the level of genetic diversity and divergence.

**MATERIALS AND METHODS**

Total fourteen accessions of Langsat (*Lansium parasiticum*) leaf were used for DNA extraction, there were 8 accessions were collected from Siberut Island with a local name Seccet (SC), Siamung (SM1;SM2), Telu toru gokgok (AT1;AT2), Elakmata (EL1;EL2), Langsat padang (LP) and 6 accession were collected from Mainland of Sumatra were Duku Tembung (DT1;DT2), Duku Kumpeh (KP1;KP2), and Langsat Sijunjung (LS1;LS2) with detail collection site were presented in Fig 1.

DNA isolation was carried out based on the CTAB method (Doyle and Doyle, 1987) which had been modified. Amplification using primers recommendation from (Syamsuardi *et al.*, 2018) with the modification of temperature optimization PCR amplification using ITS Primer (Muellner *et al.*, 2003). Also, PCR amplification use

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MatK primer with modification of temperature optimization (Dunning and Savolainen, 2010).

Sequence analysis obtained by alignment using the ClustalX version 2.0 program (Larkin et al., 2007). Nucleotide base changes (haplotypes) were analyzed using the DNA Sequence Polymorphism 5.10 program (Rozas et al., 2003). Phylogenetic trees were made based on Neighbor-joining algorithms (Saitou and Nei, 1987). The application using MEGA 6 (Tamura et al., 2013) with the addition of GenBank data as an outgroup namely Aglaia elliptica, Aglaia sexipetala, and Aglaia rafinervis.

RESULTS AND DISCUSSION

DNA isolation of 14 Langsat accessions was collected from several locations on Siberut Island and Mainland Sumatra was successfully carried out. The success of amplification with two markers used for all samples were visualized with bright bands on the results of electrophoresis. The sequence results obtained have a base length range of 712 - 787 bp based on ITS and 930 - 1367 on MatK (Fig 2). BLAST analyzed obtained the percentage of similarity (homology) sequence with Lansium domesticum (accepted name Lansium parasiticum) which was more than 95% in the two markers used.

In addition, analysis of haplotype diversity was also carried out to determine the level of polymorphism in L. parasiticum sequences. There were 10 haplotypes on ITS markers and 3 haplotypes on MatK from 14 sequences analyzed (Table 1). The haplotype diversity (Hd) values for ITS are 0.96 and 0.30 for MatK. Meanwhile, the haplotype diversity value between landrace langsat from both locations between Siberut Island and Sumatra mainland was 0.95 and 0.85 based on ITS and 0.80 and 0.28 based on MatK. Based on this, it can be said that the diversity value of L. parasiticum haplotype from Siberut Island was higher than that of the Sumatra mainland. This result refers to Nei and Tajima (1981), the range of haplotype diversity values from 0-1 has high diversity if the value is > 0.5 and low if the value is < 0.5. According to Syamsuardi et al. (2018), if there were many haplotypes in a population, the genetic variation in them was also high. Therefore, genetic variation (based on haplotype) must be maintained as a germplasm source for genetic conservation of local fruits on Siberut Island, Mentawai.
The grouping analysis based on phylogenetic trees showed that accession of the landrace langsat from Siberut Island were separated from those of accession from Mainland of Sumatra (Fig 3). Analysis phylogenetic trees of fourteen accessions three outgroup also indicated that *Langsat padang* was not included in the Langsat from Pulau Siberut but joined the Sumatran mainland group. According to information obtained from the local people of Siberut Island, *Langsat padang* was an introduction plant originating from Sumatran mainland.

The value of sequence divergence for the Langsat of Siberut Island based on ITS markers was ranged from 0.0% to 1.2% with the average value to 0.88% and 0.0% to 0.9% with the average value of 0.48% for langsat varieties on the Sumatran mainland. The sequence divergence value of Langsat based on the MatK marker for the Siberut Island was ranged 0.0% to 0.1% and Sumatra mainland was 0.0% based on Table 2.

The differentiation of accessions langsat from Siberut Island was the initial detection of langsat genetic divergence in the region. This fact suggested that the genetic divergence of langsat was a concordance to their geographical distribution. The different pattern revealed from other studies. According to Panwar *et al.*, (2017), there was no relationship between genetic divergence and geographical divergence in Indian fenugreek (*Trigonella foenum-graecum*

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**Table 1:** Thirteen haplotypes from two DNA marker detected in 14 landrace of langsat (*Lansium parasiticum*) from Siberut Island and Mainland of Sumatra.

| DNA Marker | Haplotype | Location          | Sample               | Number of samples |
|------------|-----------|-------------------|----------------------|-------------------|
| ITS        | H01       | Sumatran mainland | KP1, DT1            | 2                 |
|            | H02       | Sumatran mainland | KP2, DT2            | 2                 |
|            | H03       | Sumatran mainland | LS1, LS2            | 2                 |
|            | H04       | Siberut Island    | LP                   | 1                 |
|            | H05       | Siberut Island    | SC, SM2              | 2                 |
|            | H06       | Siberut Island    | SM1                  | 1                 |
|            | H07       | Siberut Island    | AT1                  | 1                 |
|            | H08       | Siberut Island    | AT2                  | 1                 |
|            | H09       | Siberut Island    | EL1                  | 1                 |
|            | H10       | Siberut Island    | EL2                  | 1                 |
| MatK       | H01       | Sumatran mainland | KP1, KP2, DT1, DT2,  | 11                |
|            |           |                   | LS1, LS2, LP, AT1,   |                   |
|            |           |                   | AT2, EL1, EL2       |                   |
|            | H02       | Siberut Island    | SC                   | 1                 |
|            | H03       | Siberut Island    | SM1, SM2             | 2                 |

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Fig 3: Neighboring joining phylogenetic tree of fourteen accessions of Langsat and three species of *Aglaia* as outgroup based on combined ITS and MatK region.
The similarity was indicated in groundnut (Arachis hypogaea L.) were genetic divergence were not concordance to its geographical distribution (Venkateswarlu et al., 2011).

Genetic divergence of Langsat from Siberut Island to the mainland of Sumatra was reflection the history of geographical isolation of Sumatra and Mentawai Island since 500,000 years ago and result in speciation of flora and fauna due to the geographical isolation that occurred on the Mentawai Island. Landrace langsat from Siberut Island was a plant were planted by Mentawai people on traditional plantations (pumonean), that the germplasm from the Mentawai forest. *Pumonean* is a traditional method used by Mentawai people to make fields that grow like a forest (Indra et al., 2017). Therefore, in *pumonean* they plant main crops, medicinal plants, vegetables, various woods, and fruits included landrace of langsat.

In this study, two molecular markers were used, namely ITS and MatK. ITS markers are found in the core genome and are able to detect polymorphisms variation of taxa very well, in contrast to MatK markers which was more conservative. The MatK was in the chloroplast genome which had more informative characters than other markers in the chloroplast genome. Based on this study, ITS able to detect polymorphism in all accession of *Lansium parasiticum* (Meliaceae) suggested that these markers were useful to detect variation in taxa.

### Table 2: Genetic distance using ITS (low part) and MatK (top) from 14 sample of landrace langsat from Siberut Island and Mainland of Sumatra.

| Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|--------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| DT1    | - | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 |
| DT2    | 0.001 | - | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 |
| KP1    | 0.000 | 0.001 | - | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 |
| KP2    | 0.001 | 0.000 | 0.000 | - | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 |
| LS1    | 0.007 | 0.006 | 0.007 | 0.006 | - | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 |
| LS 2   | 0.007 | 0.006 | 0.007 | 0.006 | 0.000 | - | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 |
| LP     | 0.007 | 0.009 | 0.007 | 0.009 | 0.007 | 0.007 | - | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 |
| SC     | 0.013 | 0.015 | 0.013 | 0.015 | 0.015 | 0.015 | 0.015 | - | 0.002 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 |
| SM 1   | 0.013 | 0.015 | 0.013 | 0.015 | 0.015 | 0.015 | 0.015 | 0.006 | - | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 |
| SM 2   | 0.013 | 0.015 | 0.013 | 0.015 | 0.015 | 0.015 | 0.015 | 0.000 | 0.006 | - | 0.001 | 0.001 | 0.001 | 0.001 |
| AT 1   | 0.010 | 0.012 | 0.010 | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 | 0.12 | - | 0.000 | 0.000 | 0.000 |
| AT 2   | 0.013 | 0.015 | 0.013 | 0.015 | 0.015 | 0.015 | 0.015 | 0.012 | 0.012 | 0.012 | 0.003 | - | 0.000 | 0.000 |
| EL 1   | 0.006 | 0.007 | 0.006 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | 0.007 | - | 0.000 |
| EL 2   | 0.010 | 0.012 | 0.010 | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 | 0.012 | 0.004 |

CONCLUSION

Based on analyzed of fourteen accessions of Langsat from Siberut Island and Sumatran mainland using ITS and MatK markers, we concluded that the landrace of langsat from Siberut Island had the higher diversity of haplotypes compared to those of the Sumatran mainland and the accessions of langsat from Siberut Island were separated from accessions of Sumatran mainland based on the genetic divergence.

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