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PHYLOGENETIC STUDY OF *SALACCA* SPP. BASED ON *TRN*L-F INTERGENIC SPACER SEQUENCES OBTAINED FROM ACEH, INDONESIA

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SUMMARY

Snake fruit (*Salacca* spp.), also called Salak, is a unique tropical fruit. The salak wild germplasm is distinct in Aceh, Indonesia, and with its larger variation, it has the potential for further development through breeding. Therefore, in Aceh, the identification and relationship of various salak accessions are necessary in producing superior genotypes; however, complete information is still unavailable. The presented study aimed to construct the relationship about the salak found in Aceh using the *trn*L-F intergenic spacer sequence. The salak different accessions collected from several areas in Aceh included the Seulawah Valley, Montasik, Sabang, Kutacane, and Leuser Ecosystem. In the phylogenetic tree construction, using the maximum parsimony (MP) and neighbor-joining (NJ) methods helped in the Phylogenetic Analysis Using Parsimony (PAUP). The results showed there are 206 parsimony informative characters to construct a phylogenetic tree. The MP cladogram separated the 18 salak wild and cultivated accessions into two groups. Based on the NJ analysis, evolutionarily wild type salak is assumedly the most primitive accession and could have genes for the various traits that may disappear in cultivated types due to domestication pressure. The same was also valid from the neighbor-joining cladogram with the shortest clade branch.

Keywords: Salak, cultivated types, wild type, maximum parsimony method, neighbor-joining method, PAUP, *trn*L-F intergenic spacer

Key findings: Relationship information of the salak (*Salacca* spp.) is crucial in developing future superior genotypes. Based on molecular data, the cladogram was able to separate the salak wild and cultivated types. Evolutionarily, the wild type could be the most primitive accession and seemed to have viable genes for the various traits.

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INTRODUCTION

Salak (*Salacca* spp.) is a group of endemic palms found in tropical areas (Zumaidar and Miftahuddin, 2018). The use of salak is generally as fresh fruit and processed product by the community. The said fruit belongs in the underutilized fruit group; however, it has the potential in pharmacology because of its high antioxidant content and as the best source of carbohydrates and dietary fibers (Saleh *et al*., 2018). Concerning salak fruit production, especially *Salacca sumatrana,* the peak production is in May-August, previously marked by massive flowers in January to April. The fruit formation period is around 5–6 months (Adelina *et al*., 2021). Ecologically, salak plays a vital role as a phytoremediation for heavy metals and can save the soil from environmental pollution (Mazumdar *et al*., 2019).

Based on morphology and molecular aspects, salak genetic diversity is high, with the discovery of more than 40 cultivars in Indonesia (Zumaidar *et al*., 2015). Around 19 species of salak were prevalent in lowland tropical rainforests up to 800 meters above sea level (Budiyanti, 2016). The salak distribution can be prominent in three different regions—Borneo, the Malay Peninsula, and Sumatra worldwide. The salak two species most commonly found in Indonesia are *S. sumatrana* and *S. zalacca*. The Tropical Fruit Research Institute, Indonesia divided the salak genotypes into two, i.e., cultivated and wild (Annisaurrohmah *et al*., 2014; Zumaidar and Miftahuddin, 2018).

Salak's three wild species found in Indonesia rarely consumed consisted of *S. wallichiana, S. affinis*, and *S. magnifica* (Budiyanti, 2016; Zumaidar and Miftahuddin, 2018). The leaflet size is one of the vital morphological characteristics that can distinguish the salak species, especially in the cultivated group (Hadiati *et al*., 2023). Based on observations in the field, the Aceh Province, Indonesia, has a diversity of wild and cultivated salak varieties. Cultivated salak is predominant in fruit plantations in the Lembah Seulawah, Montasik, Sabang, and Kutacane areas, and the wild species are prominent in the Gunung Kemiri area and Southeast Aceh. The salak three species found in Aceh are *S. zallaca*, *S. sumatrana*, and *S. acehensis,* observed with the highest variation due to the genetic mixing of these species. Wild salak has the disease resistance potential, and incorporating such disease tolerance is possible in cultivated types through breeding. However, its identity and relationship are still unknown. Currently, the wild salak from Aceh is typically the *S. acehensis,* enlisted in the endangered species (Zumaidar *et al*., 2014).

The land conversion policy implemented in Sumatra, Indonesia, has also caused threatening salak species' extinction in its natural habitat. Therefore, its conservation and expansion in cultivation are absolutely necessary to prevent this valuable fruit from vanishing. The initial step in determining conservation strategies and cultivation techniques is to define a well-constructed evolutionary relationship of the salak species (Hidayat and Pancoro, 2008; Wallinger *et al*., 2012; DeSalle and Goldstein, 2019). The morphological characteristics of salak has been a focus in various phylogenetic study; however, the continuity of morphological characteristics makes it difficult to define evolutionary relationships. Therefore, the informative DNA sequence-based molecular markers are beneficial to support and strengthen the data based on morphological characteristics (Zumaidar *et al*., 2015).

The *trn*L-F intergenic spacer sequence is one of the molecular markers used to analyze the phylogeny of salak species in Aceh. This marker is conservative, with a low rate of evolution (Kajita *et al*., 1998; Coissac *et al*., 2016; Fitmawati *et al*., 2017), and its sequences vary in height, short, and are easy to amplify (Small *et al*., 2005). Therefore, based on the previous discussion, the promising study aimed to analyze and construct the phylogenetic relationships of salak from Aceh, Indonesia, using the *trn*L-F intergenic spacer sequence.

MATERIALS AND METHODS

Salacca spp. DNA extraction

Eighteen salak accessions were samples used in the latest study, collected from Aceh, Indonesia (Figure 1, Table 1). Outgroups from the GenBank data used were the species *Salacca ramosiana* (AM113614.1) and *Oncocalamus manii* (AJ241280.1). Genomic DNA extraction proceeded from 0.15 g of fresh young leaf samples using the CTAB method with some modifications (Doyle and Doyle, 1987). Checking the quality of the extracted DNA used an electrophoresis machine, with a 1% agarose gel concentration in 1X TBE buffer.

Amplification and sequencing

The barcoding primers used were derivatives of the plastid *trn*L-F. The applied *trn*L-F sequence was the cpDNA forward primer 5'-5'ATT TGA ACT GGT GAC ACG AG -3' and the cpDNA reverse primer 5'- GGT TCA AGT CCC TCT ATC CC-3' (Small *et al*., 2005). Each PCR reaction received amplification in a volume of 50 µL, consisting of 5 µL DNA (0.5–2.0 ng), 1 µL E primer, 1 µL F primer, 25 µL DreamTaqTM Hot Start Green PCR Mix (containing DreamTaq Hot Start DNA polymerase, 2X DreamTaq Green Buffer, 0.4 mM dNTPs, and 4 mM MgCl2), and 18 µL nuclease-free water. The PCR reaction temperature profile had pre-denaturation at 95 °C for 5 min, followed by 35 cycles; each cycle consisted of denaturation at 95 °C for 45 s, annealing at 50.6 °C for one minute, initial extension at 72 °C for 45 s, final extension at 72 °C for 10 min, and cooling at 15 °C. The PCR products' electrophoresis ensued on 1% agarose gel in 1X TBE buffer. The PCR results attained sequencing at the First BASE Laboratories through PT Genetika Science, Indonesia.

Phylogenetic analysis

The sequence data identification for sequence similarity used the BLAST analysis via online data mining based on the database available at the National Center for Biotechnology Information (NCBI). The DNA sequences of the *trn*L-F intergenic spacer region and out-group reached alignment with the ClustalW Multiple Alignment in the UGENE program (Golosova *et al*., 2014). Phylogenetic tree construction using the maximum parsimony (MP) and neighbor-joining (NJ) methods engaged the PAUP* program version 4.0b10 (Swofford, 2002). The obtained branch support values utilized 1000× bootstrap, with categories of strong (> 85%), moderate (70%–85%), weak (50%–69%), and poor (< 50%) (Kress *et al*., 2002).

RESULTS AND DISCUSSION

Sequencing and phylogenetic analysis using the Maximum Parsimony method

The obtained sequencing of *trn*L-F intergenic spacer and outgroup's aligning used the ClustalW Multiple Alignment in the UGENE program. Lengths of nucleotide bases ranged from 356 to 418 bp in the Aceh salak accessions. The G+C content ranged from 29.04% to 33.49%. Based on Chargaff's law, the DNA base content's expression was G+C in the nucleotide arrangement, with varying values ranging from 26% to 74%.

The sequence alignment of salak accessions used 468 characters, with 208 constant characters, 54 parsimony uninformative characters, and 206 parsimony informative characters. A site is parsimony informative if it contains at least two types of nucleotides, occurring with a minimum frequency of two. This site showed the differences in the number of substitutions in the branching pattern to explain the variation at the site that occurs due to evolution over a long period. Parsimony informative sites proved useful for reconstructing parsimony phylogenetic trees, while parsimony uninformative characters were those that do not provide information about the presence of variation (Kumar *et al*., 2018).

The parsimony analysis of 18 salak accessions revealed a consistency index (CI) value of 0.95 and a retention index (RI) value of 0.99, with a tree length of 330 bp. The CI

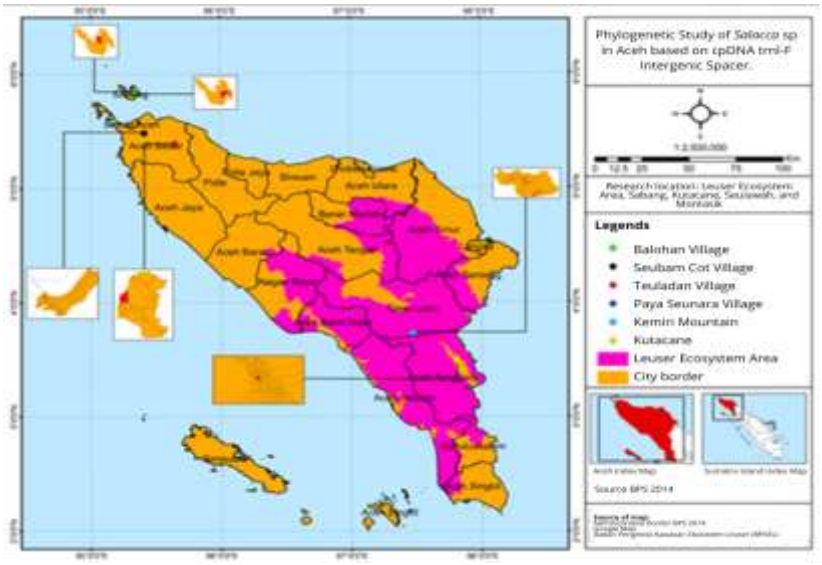

Figure 1. Locations of *Salacca* spp. accessions in Aceh, Indonesia.

Table 1. Nucleotide base BLAST of *Salacca* spp. on the GenBank from Aceh, Indonesia.

No.	Accession code	Sample Location	Type	Sequence similarity on GenBank	Sample code on GenBank	Query cover (%)
1	NH.05	Seulawah Valley	Cultivated	*Salacca ramosiana*	KT312921.1	98.40
2	NH.06	Seulawah Valley	Cultivated	*Salacca ramosiana*	KT312921.1	99.43
3	NH.07	Seulawah Valley	Cultivated	*Salacca ramosiana*	KT312921.1	98.92
4	NH.08	Montasik, Greater Aceh	Cultivated	*Salacca ramosiana*	KT312921.1	99.72
5	NH.09	Montasik, Greater Aceh	Cultivated	*Salacca ramosiana*	KT312921.1	98.59
6	NH.10	Montasik, Greater Aceh	Cultivated	*Salacca ramosiana*	KT312921.1	99.39
7	NH.11	Balohan, Sabang	Cultivated	*Salacca ramosiana*	KT312921.1	93.96
8	NH.12	Balohan, Sabang	Cultivated	*Salacca ramosiana*	KT312921.1	99.75
9	NH.14	Paya Seunara, Sabang	Cultivated	*Salacca ramosiana*	KT312921.1	99.75
10	NH.TM	Kutacane, Southeast Aceh	Cultivated	*Salacca ramosiana*	KT312921.1	98.90
11	NH.JO	Kutacane, Southeast Aceh	Cultivated	*Salacca ramosiana*	KT312921.1	99.43
12	NH.MA	Kutacane, Southeast Aceh	Cultivated	*Salacca ramosiana*	KT312921.1	99.73
13	NH.R1	Leuser Ecosystem	Wild	*Salacca ramosiana*	KT312921.1	98.53
14	NH.R2	Leuser Ecosystem	Wild	*Salacca ramosiana*	KT312921.1	99.75
15	NH.R3	Leuser Ecosystem	Wild	*Salacca ramosiana*	KT312921.1	96.54
16	NH.R4	Leuser Ecosystem	Wild	*Salacca ramosiana*	KT312921.1	98.54
17	NH.R5	Leuser Ecosystem	Wild	*Salacca ramosiana*	KT312921.1	99.75
18	NH.R6	Leuser Ecosystem	Wild	*Salacca ramosiana*	KT312921.1	98.54

and RI values indicate the consistency and retention of the cladogram tree. A CI value = 1 indicates a change in character in the base that is parsimonious, while an RI value = 1 indicates a complete character, consistent with the phylogeny (Nei and Kumar, 2000). High CI and RI values imply the resulting cladogram has a high level of parsimony (Juliantari *et al*., 2018). In general, the phylogenetic tree analysis by maximum parsimony shows a strong category, characterized by branching values, mostly 100% in clade I and clade II.

The salak phylogenetic tree in the Aceh region forms a monophyletic group and a branch originating from the same ancestor. The maximum parsimony cladogram can separate the wild and cultivated snake fruit types into two distinct groups. The

phylogenetic tree divides 18 salak accessions into two clades—I and II, collected from different locations in Aceh, which tend to cluster based on the geographic locations (Figure 2). Clade I comprised the accessions collected from the locations of Lembah Seulawah and Montasik, Aceh Besar Regency and Kutacane, Southeast Aceh Regency. Meanwhile, the clade II consisted of accessions procured from Balohan and Paya Seunara of Sabang City and the Leuser ecosystem area. Cultivated salak accessions proceeded to cluster in clade I, while the wild types grouped in clade II. The salak accessions NH.12 and NH.14 (Sabang) belonged in the cluster with the wild group (Figure 1) because of no significant variations in several characteristics in these wild samples. The grouping of cultivated and wild accessions depended on the variations in numerous nucleotide base sequences. Based on its organoleptic observation, it seemed Sabang salak has a slightly astringent flavor, indicating low tannin content as the wild salak type.

Reconstruction of relationship based on proximity of the evolutionary distance was indicative of the value of the kinship distance matrix between the salak accessions. The evolutionary model does not explicitly describe the mechanisms of mutation and natural selection; however, this model describes the relative rate of variations in the different nucleotides. The difference in genetic distance describes the rate of evolution of each salak accession, and the greater the genetic distance, the more adaptation strategies resulted from the species to survive in its existing environment. Genetic distance also showed the greater variation in the species, resulting in the species trait variations to survive and pass on the traits to the next generation through inheritance. The rate of evolution can be fast or slow, depending on the adaptation mechanism and the environmental conditions of its habitat (Matthews *et al*., 2020).

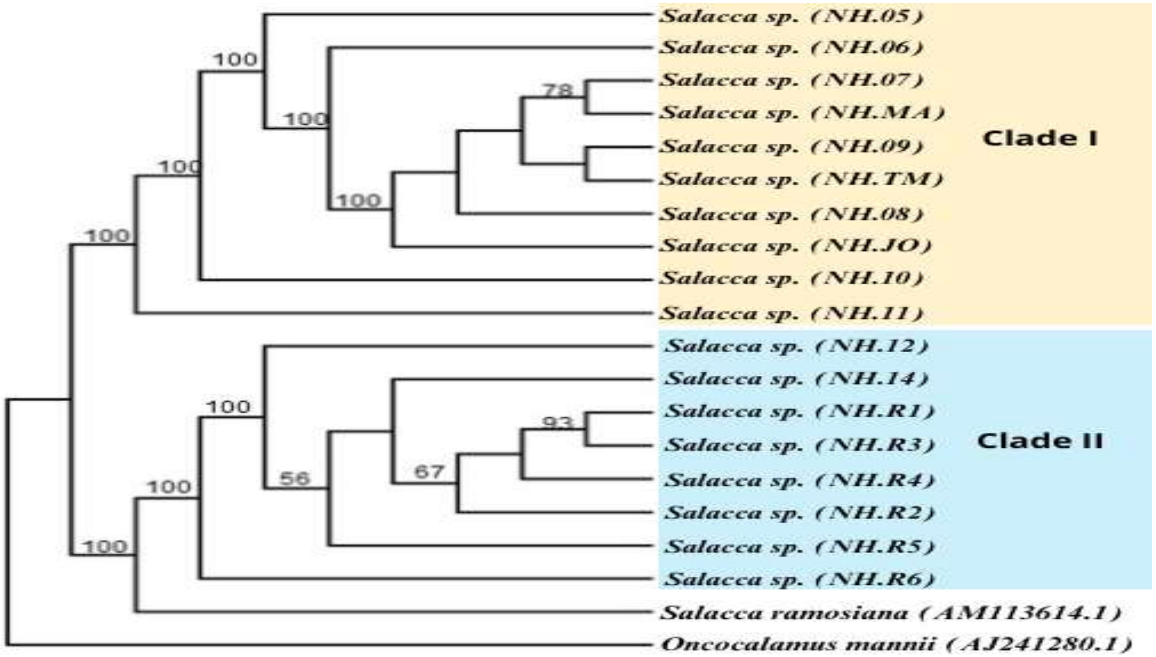

Figure 2. Phylogenetic tree of *Salacca* spp. from Aceh based on trnL-F intergenic spacer by maximum parsimony with bootstrap values 1000×. Bootstrap value on node < 50% does not appear. Yellow color signed salak cultivated type and blue color signed salak wild type.

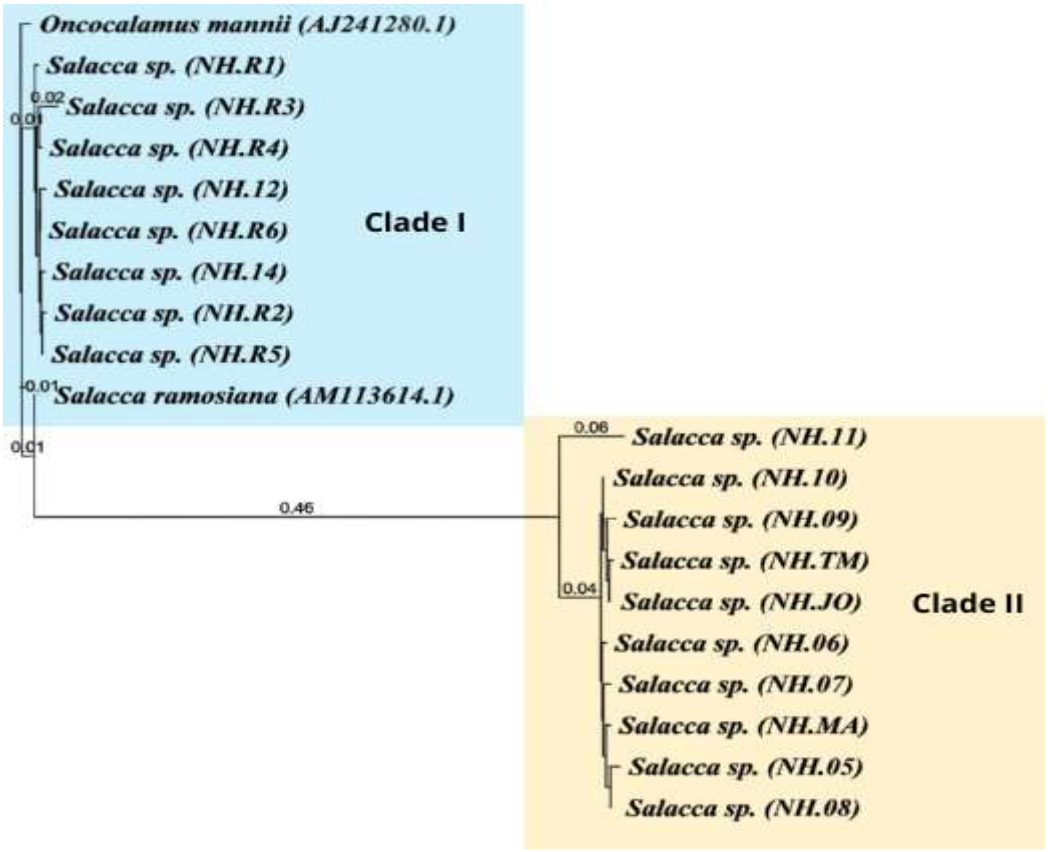

Figure 3. Phylogenetic tree of *Salacca* spp. from Aceh based on trnL-F intergenic spacer by neighbor-joining with bootstrap values 1000×. Yellow color signed salak cultivated type and blue color signed salak wild type.

Phylogenetic analysis using the Neighbor-Joining method

Clade I comprised the salak wild type accessions from Aceh, which were assumedly the first most primitive type of accession to appear (Figure 3). The salak wild type had basis from the neighbor-joining cladogram with the shortest clade branch. However, the branch of the cultivated salak (clade II) was longer (0.46 branch value) than the wild type (clade I) (Figure 3) because of the many variations appearing in nucleotide bases due to domestication and selection pressure, and evolutionarily, it showed more advanced traits. Genetic distance describes the shape of the branch length as the length of the evolutionary journey, describing the sequence distance and considering molecular age. The longer the branch formed, the more variations occur in the characters of the species (Fitmawati *et al*., 2018).

Evolutionarily, wild snake fruit seemed to be the most primitive accession based on the NJ analysis, and the wild salak type still has genes for traits that could be lost in cultivated accessions due to domestication and selection pressure. However, this description of kinship relationship will have a major impact on the variations produced in the cultivated salak group. Crosses between cultivated salak accessions turned out to produce highly significant variations in fruit weight (Budiyanti *et al*., 2019). The variations in the nucleotide base content can affect evolution in the species. Nucleotide variations caused by mutations resulted in genetic diversity (Maloukh *et al*., 2017; Sanjuan and Calap, 2021).

The NJ method was able to describe the evolutionary journey of salak accessions collected in Aceh by providing the best estimate based on the branch length. The NJ method relied on the principle of minimum evolution with an estimate of the branch length (Nei and Kumar, 2000). The pair of sequences with the smallest number of variations among the accessions, called neighbors, indicated0.01 branches value on clade I as the salak wild type. This method aims to correctly identify trees in the neighbor position and also have branches that will produce original data as close as possible (Dharmayanti, 2011; Azouri *et al*., 2021).

Sequence similarity based on NCBI database

Salak accessions in the Aceh area has a sequence similarity to *Salacca ramosiana* based on the Genbank NCBI. The similarity values of 18 samples succeeded amplification, with a range from 96.54% to 99.75% (Table 1). *Salacca ramosiana* Mogea is a species of Balinese salak, distributed in Malaysia and the Philippines (Gari, 2005). The BLAST analysis used online data mining to identify the sequence similarity based on the database available at NCBI by showing the percent query cover value. Query Cover is the percentage of sequences aligning with the sequences in the GenBank database (Ainiyah *et al*., 2020).

Accessions from the Leuser Ecosystem area were the most primitive type of salak in the Aceh area as compared with other samples. Zumaidar and Miftahuddin (2018) explained geographically, the regions of Kalimantan, the Malay Peninsula, and Sumatra in Indonesia, referred to as adjacent triangle regions. They also have the higher level of salak diversity than other regions. Even Sumatra, Indonesia, has diverse habitats and morphology of the salak fruit.

CONCLUSIONS

Salak germplasm will serve as a base material for developing the high quality salak in the future. The plastid *trn*L-F data has 206 parsimony informative characters, which can be useful for constructing a phylogeny tree of salak from Aceh, Indonesia. The maximum parsimony analysis can separate the wild and cultivated types of salak. However, the neighbor joining analysis can group the wild types as primitive and cultivated types as more advanced, evolutionarily.

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REFERENCES

Adelina R, Suliansyah I, Syarif A, Warnita W (2021). Phenology of flowering and fruit set in Snake Fruit (*Salacca sumatrana* Becc.). *Acta Agrobot.* 74(2): 1–12. doi: 10.5586/aa.742.

Ainiyah RK, Wahyunindita V, Pratama WN, Pratiwi IA, Utami ESW, Hariyanto S (2020). DNA barcoding : Study of bananas (*Musa* spp.) wild and cultivars group from East Java inferred by rbcL gene sequences. *Ecol. Environ. Conserv.* 26: S7–S13.

Annisaurrohmah, Herawati W, Widodo P (2014). Keanekaragaman kultivar salak Pondoh di Banjarnegara. *Biosfera* 31(2): 71–83.

Azouri D, Abadi S, Mansour Y, Mayrose I, Pupko T (2021). Harnessing machine learning to guide phylogenetic tree search algorithms. *Nat. Commun.* 12(1983): 1–9. https://doi.org/10.1038/s41467-021-22073-8.

Budiyanti T (2016). Keragaman salak liar di Indonesia. http://balitbu.litbang.pertanian.go.id/index.php/hasil-penelitian-mainmenu-46/901-keragaman-salak-liar-diindonesia. (Accessed on July 20, 2024).

Budiyanti T, Hadiati S, Prihatini R (2019). Genetic variability on inter and intra population of *Salacca* Pondoh and *Salacca* Jawa crosses. *AIP Conf. Proc.* 2120(1): 030033. https://doi.org/10.1063/1.5115637.

Coissac E, Hollingsworth PM, Lavergne S, Taberlet P (2016). From barcodes to genomes: Extending the concept of DNA barcoding. *Mol. Ecol.* 25(7): 1423–1428. https://doi.org/10.1111/mec.13549.

DeSalle R, Goldstein P (2019). Review and interpretation of trends in DNA Barcode. *Front. Ecol. Evol.* 7(302): 1–22. doi: 10.3389/fevo.2019.00302.

Dharmayanti NLPI (2011). Filogenetika molekuler: Metode taksonomi organisme berdasarkan sejarah evolusi. *Wartazoa* 21(1): 1–10.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19(1): 11–15.

Fitmawati, Fauziah R, Hayati I, Sofiyanti N, Inoue E, Matra DD (2017). Phylogenetic analysis of *Mangifera* from central region of Sumatra using trnL-F intergenic spacer. *Biodiversitas* 18(3): 1035–1040. doi: 10.13057/biodiv/ d180322.

Fitmawati, Hayati I, Mahatma R, Suzanti F (2018). Phylogenetic study of *Mangifera* from Sumatra, Indonesia using nuclear and chloroplast DNA Sequences. *SABRAO J. Breed. Genet.* 50(3): 295–312.

Gari NM (2005). Studies on Bali Salak Cultivars (*Salacca zalacca* var. *amboinensis*) (Arecaceae). Research Thesis. James Cook University, Australia.

Golosova O, Henderson R, Vaskin Y, Gabrielian A, Grekhov G, Nagarajan V, Oler AJ, Quiñones M, Hurt D, Fursov M, Huyen Y (2014). Unipro UGENE NGS pipelines and components for variant calling, RNA-Seq, and ChIP-seq data analyses. *Peer J* 2(8): 1–15. doi: 10.7717/peerj.644.

Hadiati S, Indriyani NLP, Jumjunidang (2023). Review: Population establishment and genetic study of Java, Bali, and Sumatra Salak crossing. *IOPC. Ser. Earth Env.* 1253: 012031. doi:10.1088/1755-1315/1253/1/ 012031.

Hidayat T, Pancoro A (2008). Kajian filogenetika molekuler dan peranannya dalam menyediakan informasi dasar untuk meningkatkan kualitas sumber genetik anggrek. *J. AgroBiogen* 4(1): 35–40. doi: 10.21082/jbio.v4n1.2008.p35-40.

Juliantari E, Fitmawati, Sofiyanti N (2018). Phylogeny of mango (*Mangifera*) in Eastern Sumatra using molecular markers of cpDNA rbc*L*. *Int. J. Sci. Appl. Technol.* 3(1): 17–24.

Kajita T, Kamiya K, Nakamura K, Tachida H, Wickneswari R, Tsumura Y, Yoshimaru H, Yamazaki T (1998). Molecular phylogeny of Dipterocarpaceae in Southeast Asia based on nucleotide sequences of matK, trnL Intron, and trnL-trnF intergenic spacer region in chloroplast DNA. *Mol. Phylogenet. Evol.* 10(2): 202–209.

Kress WJ, Prince LM, Williams KJ (2002). The phylogeny and a new classification of the gingers (Zingiberaceae): Evidence from molecular data. *Am. J. Bot.* 89(10): 1682–1696.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35(6): 1547–1549. doi:10.1093/molbev/msy096.

Maloukh L, Kumarappan A, Jarrar M, Salehi J, El-Wakil H, Lakshmi TVR (2017). Discriminatory power of rbcL barcode locus for authentification of some of United Arab Emirates (UAE) native plants. *3 Biotechnol. J.* 7: 144. doi: 10.1007/s13205-017-0746-1.

Matthews N, Jokela J, Narwani A, Rasanen K, Pomati F, Alternmatt F, Spaak P, Robinson CT, Vorburger C (2020). On biological evolution and environmental solutions. *Sci. Total Environ.* 724: 138194. https://doi.org/ 10.1016/ j.scitotenv.2020.138194.

Mazumdar P, Pratama H, Lau SE, Teo CH, Harikrishna JA (2019). Biology, phytochemical profile and prospects for snake fruit: An antioxidant-rich fruit of South East Asia. *Trends Food Sci. Tech.* 91: 147–158. https://doi.org/10.1016/j.tifs. 2019.06.017.

Nei M, Kumar S (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Saleh MSM, Siddiqui MJ, Meidani A, Ismail NH, Ahmed QU, So'ad SZM, Besbes SS (2018). *Salacca zalacca*: A short review of the palm botany, pharmacological uses and phytochemistry. *Asian Pac. J. Trop. Med.* 11(12): 645–652. doi: 10.4103/1995-7645.248321.

Sanjuan R, Calap PD (2021). Genetic diversity and evolution of viral populations. *Encyclopedia*

of Virology, 53–61. doi: 10.1016/B978-0-12-809633-8.20958-8.

Small RL, Lickey EB, Shaw J, Hauk WD (2005). Amplification of non coding chloroplast DNA for phylogenetic studies in lycophytes and monilophytes with a comparative example of relative phylogenetic utility from Ophioglossaceae*. Mol. Phylogenet. Evol.* 36(3): 509–522. https://doi.org/10.1016/j.ympev.2005.04.018.

Swofford DL (2002). PAUP*, Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b10. Sinauer Associates MA, Sunderland.

Wallinger C, Juen A, Staudacher K, Schallhart N, Mitterrutzner E, Steiner EM, Thalinger B, Traugott M (2012). Rapid plant identification using species and group-specific primers targeting chloroplast DNA. *PLoS ONE* 7(1): e29473. https://doi.org/10.1371/journal.pone.0029473.

Zumaidar Z, Chikmawati T, Hartana A, Sobir S (2015). Keanekaragaman Genetik *Salacca zalacca* Berdasarkan Penanda AFLP. *Flori Bunda* 5(2): 60–70. https://doi.org/10.32556/floribunda.v5i2.2015.130.

Zumaidar, Chikmawati T, Hartana A, Sobir, Mogea JP, Borchsenius F (2014). *Salacca acehensis* (Arecaceae), a new species from Sumatra, Indonesia*. Phytotaxa* 159(4): 287–290. https://doi.org/10.11646/phytotaxa.159.4.5.

Zumaidar, Miftahuddin (2018). Species distribution of genus *Salacca*. *J. Phys. Conf. Ser.* 1116(5): 1–7. doi: 10.1088/1742-6596/1116/5/052083.