Australian Journal of Crop Science AJCS

AJCS 16(03):424-430 (2022) ISSN:1835-2707
doi: 10.21475/ajcs.22.16.03.p3569

Molecular identity of native coconut (*Cocos nucifera* L.) germplasm from South Kalimantan, Indonesia

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Abstract

Coconut (*Cocos nucifera* L.) is an agricultural commodity that is very prospective to be developed in Indonesia and other tropical countries, but its development is constrained by various factors. This study aimed to determine the genetic identity, as well as diversity and relationships of native coconut germplasm from South Kalimantan, Indonesia, using a cpDNA (*mat*K) marker. The results show that this germplasm has a low-level genetic diversity, π% = 0.0258. However, the phylogenetic analysis revealed that native coconut germplasms from this region have separated into different clades, two for Maximum Likelihood and three for Neighbor-Joining, where *Genjah Kuning 3* has closely related to an outgroup. Thus, this information is important as a fundamental reference in developing new high-yielding coconut in the future.

Keywords: Coconut; genetic diversity; breeding program; cpDNA.
Abbreviations: *mat*K _ *Maturase* K; ML _ Maximum Likelihood; NJ _ Neighbor-Joining.

Introduction

Coconut (*Cocos nucifera* L.) is one of the most prospective commodities in Indonesia and other tropical countries. It corresponds to the high world market demand for these commodities and their derivative products, such as coconut meat, water, coconut oil, coconut shells, and coconut fiber (DGNED, 2017). For example, in Indonesia, until the second quarter of 2020, the coconut exports were recorded at 988.3 thousand tons or worth USD 519.2 million. It records to have increased by 16% and 17% compared to the same period in the previous year (DGP, 2020). In 2019, specifically for coconut derivatives or processed products, Indonesia's export value reached USD 2.17 billion, consisting of processed coconut meat of USD 663.8 million, coconut shells of USD 209.6 million, coconut water USD 35.3 million, and coconut fiber USD 12.6 million (Trademap, 2021).

Taxonomically, coconut is the single species that belong to the *Cocos* genera and presents two varieties: tall (var. *typica*) and dwarf (var. *nana*) (Azevedo et al., 2018, Geethanjali et al., 2017). The tall varieties are outcrossing, and the dwarfs are mostly autogamous (Geethanjali et al., 2017). Based on the color forms, the dwarf variety of this germplasm can be divided into three subvarieties, i.e., green dwarf, red dwarf, and yellow dwarf. Furthermore, the red dwarf can be divided into two phenotypically distinct types, namely Malayan and Cameroon (Azevedo et al., 2018).

In South Kalimantan, one of the largest coconut-producing areas in Indonesia, there is a native coconut cultivar with unique characteristics, namely *Genjah Salak* (Figure 1). Generally, this coconut is characterized by small fruits, slender stems, relatively short leaves, fast flowering, and mostly self-pollinating. Plants can flower at the age of 1.5 years and initially harvest for three years. Fruit production per stem can reach 80-120 fruit/tree/year (Anggreany, 2020). However, this coconut has not been characterized in depth and utilized optimally in breeding programs.

Nowadays, the coconut breeding program is directed not only to the number of fruits per plant or their production but also in increasing the albumen content, sensorial characteristics, and resistance to pests, diseases, and drought. In this case, one of the breeding strategies is intervarietal hybrids crossing between dwarf x tall coconuts varieties. Generally, these present hybrids show heterosis for fruits production, earliness, resistance to pests and diseases, number of leaves, and stem girth (Azevedo et al., 2018). From 1950 to 1993, more than 400 hybrids were developed worldwide under established coconut improvement programs using various breeding strategies. There were also instances were 'accidental' ones due to natural out-crossing were identified and selected by the farmers (Batugal et al., 2009).

In line with the development of high-yielding coconut seeds, selection and genetic characterization of germplasm are necessary. According to Acquaah (2012), genetic characterization is an urgent step in plant breeding programs. Ríos (2015) also stated that an in-depth study of the genetic status of germplasm is the key to success in breeding efforts, especially the development of new superior cultivars in the future. Especially in South

Kalimantan, genetic characterization and evaluation of coconut germplasm have not been widely carried out (Nazari 2011), unlike in other areas, such as Java, Bali, and Sulawesi (Mahayu and Taryono, 2019).

In addition, genetic characterization of the coconut germplasm was many carried out by morphological and agronomic markers (Geethanjali et al., 2017). Even these markers are strongly influenced by environmental factors. Besides, it takes a long time because the observations are conducted until the plants are mature (Mursyidin and Khairullah, 2020). Several molecular markers have been applied to characterize coconut plants, such as RAPD (Kandoliya et al., 2018, Masumbuko et al., 2014, Rajesh et al., 2014), SSR, or microsatellites (Geethanjali et al., 2017, Loiola et al., 2016, Mahayu and Taryono, 2019, Rasam et al., 2016, Wu et al., 2019), and other DNA fingerprinting markers (Azevedo et al., 2018, Rajesh et al., 2015). However, these markers also have limitations, such as the analysis results being very subjective and relatively unstable.

Currently, DNA barcoding is one option of molecular technique for analyzing the genetic diversity and relationship of germplasm (Hollingsworth et al., 2011, Kress, 2017). In general, this technique utilizes standardized short DNA sequences, such as chloroplast DNA which has a simple structure, high genetic stability, and uniparentally. Consequently, the analysis process becomes faster and more accurate (Galimberti et al., 2014). According to Singh et al. (2017), these markers also have the advantage of determining the diversity and relationship status of germplasm that has a very close relationship, such as coconuts.

This study aimed to determine the genetic identity, diversity, and relationships of native coconut germplasm in South Kalimantan, Indonesia, using a cpDNA (*mat*K) marker. The results of this study are usable as a fundamental reference in developing new high-yielding coconuts in the future.

Results

Genetic diversity and sequence characteristics

The *mat*K sequence of native coconuts shows different lengths, ranging from 844 to 863 bp. However, the BLAST results (Table 3) showed and validated that all *mat*K sequences of this germplasm with a percent identity of 100. In this case, *Genjah Kuning 1* has the highest query cover of 100% with the GenBank database. Further analysis shows that three polymorphism events are present in this sequence (Fig 3, Table 4), and all represent substitution-transversion mutation (Table 5). As a result, this Indonesian germplasm has a low genetic diversity, $\pi\% = 0.0258$ (Table 4). Following Table 4, the *mat*K gene of local coconut has a GC content of 33.30%. According to Table 4, a specific mutation of coconut was found on *Genjah Kuning 2* (introduction cultivar), where the Thymine (T) changed with Cytosine (C) in the second nucleotide position. A similar change (from T to C) also occurred in two coconut cultivars, namely *Dalam Kalumpang* and *Genjah Entok Super*, at the nucleotide position of 862 bases.

Genetic relationship

Based on the *mat*K sequence, the coconut germplasms natively from the South Kalimantan, Indonesia, have separated into different clades, two for Maximum Likelihood (Fig 4) and three for Neighbor-Joining (Fig 5). Specifically for Neighbor-Joining, *Genjah Kuning 3* has separated from the most coconut germplasm and has closely related to an outgroup (Fig 5).

Discussion

Identification and characterization, including determination of genetic diversity and relationship, are the main activities in supporting coconut development in the future. In this study, we used the cpDNA (*mat*K) marker to determine the genetic identity, diversity, and relationship of thus germplasm from the South Kalimantan, Indonesia. Conceptually, *maturase* K (*mat*K) is a chloroplast intron-encoded gene with unique characteristics. This gene is characterized by various lengths, both in the complete and partial regions. The total sequence of this gene is reported as 1,536 bp length (Mustafa et al., 2018). Thakar et al. (2016) also reported that the chloroplast *mat*K gene contains about 1,500 bp. In this study, the partial sequence of *mat*K of local coconuts shows different lengths, ranging from 844-862 bp. These length differences of *mat*K genes are also reported by several researchers, for example, Tosh et al. (2016) in Angiosperms with 830 bp–857 bp length, as well as Li et al. (2012) in *Ficus*, Căprar et al. (2017) in tomato, Habib et al. (2017) in *Tetrastigma*, Immanissa et al. (2020) in cacao, Suriani et al. (2021) in *Zanthoxylum*, and Mursyidin et al. (2021) in local rice landraces (*Oryza sativa* L.).

According to Chen and Shiau (2015), the differences in length of *mat*K genes are due to separate base substitution and single nucleotide insertions-deletions (indels) events. In this case, only three polymorphic sites are shown in this sequence (Table 4), and all represent substitution-transversion mutation (Table 5). In *Anoectochilus*, Chen and Shiau (2015) reported 11-23 SNP found in the *mat*K region. In concept, *mat*K is a plant intron-encoded protein located in the large single-copy regions of the chloroplast genome with a relatively high mutation rate (Barthet et al., 2020, Kar et al., 2015). However, the mode and tempo of *mat*K evolution are distinct from other chloroplast genes. Kar et al. (2015) reported substitution rate in *mat*K is three times higher than the large subunit of Rubisco (*rbc*L) and six-fold higher at the amino acid substitution rate, establishing it as a fast- or rapidly-evolving gene.

In addition to the high substitution rate, *mat*K also displays varying numbers and sizes of indels, and most have been found in multiples of three, conserving the reading frame. However, the presence of indels found in some plant families raises the question, of whether a gene with such a feature can maintain stable protein structure and function (Kar et al., 2015).

Due to the low level of polymorphism (SNP) found in this region, only three sites (Table 4), the *mat*K sequence of local coconuts are very high conserved. Consequently, this germplasm has a low-level genetic diversity, $\pi\% = 0.0258$. According to Gao et al. (2017), low-level genetic diversity is influenced by several factors, such as a founder effect, natural selection, and inbreeding. For plant breeding, genetic diversity is urgent, particularly in developing new cultivars with desired traits (Govindaraj et al., 2015).

In general, genetic diversity in cpDNA, including *mat*K, is caused by a single nucleotide mutation that occurs over a very long period (Suriani et al., 2021). Consequently, it provides a high phylogenetic signal for resolving evolutionary relationships among plants at all taxonomic levels (Kar et al., 2015). In other words, as a fast-evolving

Table 1. List of local coconut (*C. nucfera* L.) germplasm used in this study.

Cultivar	Type	Origin	Accession Number	Length (bp)
Dalam	Tall	Barito Kuala, South Kalimantan	OK086747	847
Dalam Hijau Kalumpang	Tall	Hulu Sungai Selatan, South Kalimantan	OK086748	860
Genjah Entok Super[*]	Dwarf	Hulu Sungai Selatan, South Kalimantan	OK086749	856
Genjah Kuning 1	Dwarf	Banjarbaru, South Kalimantan	OK086750	844
Genjah Kuning 2[*]	Dwarf	Barito Kuala, South Kalimantan	OK086751	850
Genjah Kuning 3	Dwarf	Tanah Laut, South Kalimantan	OK086752	856
Genjah Salak 1	Dwarf	Banjarbaru, South Kalimantan	OK086753	858
Genjah Salak 2	Dwarf	Banjar, South Kalimantan	OK086754	847
Genjah Salak 3[*]	Dwarf	Barito Kuala, South Kalimantan	OK086755	859
Genjah Wulung	Dwarf	Barito Kuala, South Kalimantan	OK086756	859
Anonymous[**]	Dwarf	Taiwan	KF285453.1	863

Notes: [*]Introduction; [**]An outgroup, obtained from GenBank

Fig 1. Morphological characteristics of *Genjah Salak*, a native coconut of South Kalimantan, Indonesia (Adopted from Anggreany, 2020).

Table 2. The *mat*K primers were used in this study.

Primer	Position	Sequence (5'-3')	Annealing (°C)	Target (bp)
*mat*K	*Forward*	CGTACAGTACTTTTGTGTTTACGAG	48	900
	Reverse	ACCCAGTCCATCTGGAAATCTTGGTTC		

Source: Le et al. (2020).

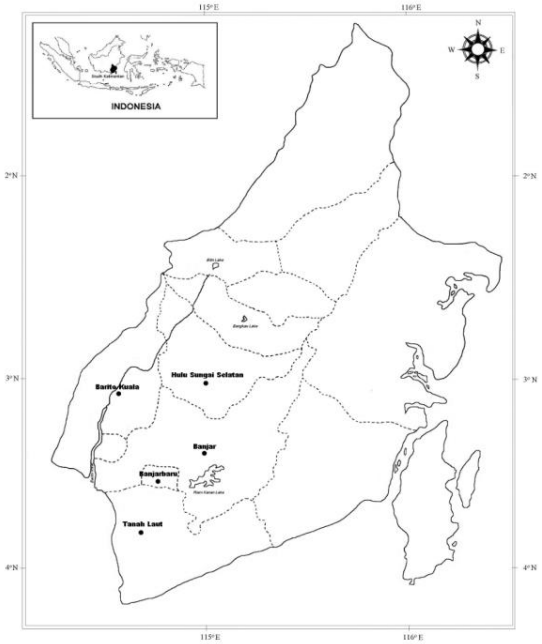

Fig 2. Maps of sampling locations (South Kalimantan, Indonesia), where the native coconut (*C. nucfera* L.) germplasm was collected and used in this study. See Table 1 for details.

Table 3. The BLAST results of *mat*K sequences of native coconut (*C. nucifera* L.) germplasm from South Kalimantan, Indonesia.

Local name	Max Score	Total Score	Query Cover (%)	E Value	Per. Identity (%)	Acc. Length	Acc. Num.
Dalam	1559	1559	99	0	100.00	1795	HG969989.1
Dalam Hijau Kalumpang	1581	1581	99	0	100.00	1795	HG969989.1
Genjah Entok Super	1580	1580	99	0	100.00	1795	HG969989.1
Genjah Kuning 1	1559	1559	100	0	100.00	1795	HG969989.1
Genjah Kuning 2	1563	1563	99	0	100.00	1795	HG969989.1
Genjah Kuning 2	1581	1581	99	0	100.00	1795	HG969989.1
Genjah Salak 1	1581	1581	99	0	100.00	1795	HG969989.1
Genjah Salak 2	1559	1559	99	0	100.00	1795	HG969989.1
Genjah Salak 3	1581	1581	99	0	100.00	1795	HG969989.1
Genjah Wulung	1581	1581	99	0	100.00	1795	HG969989.1

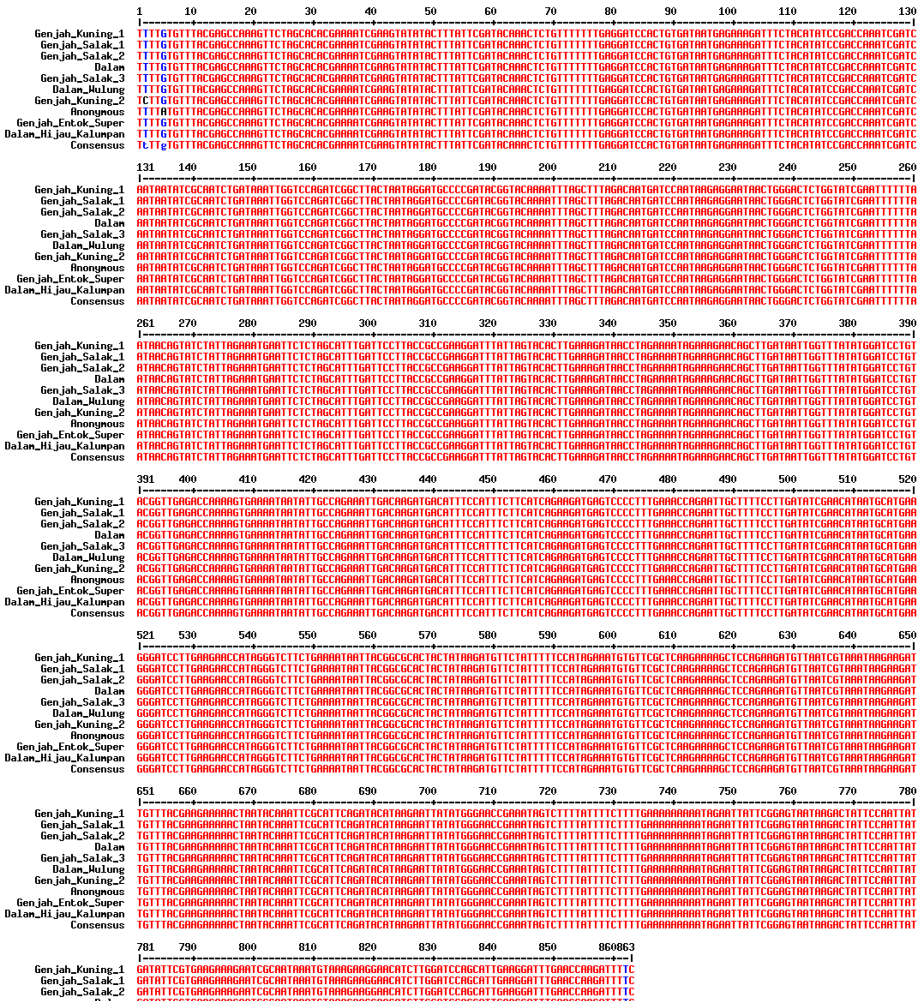

Fig 3. Multiple alignments of *mat*K sequence of the native coconut (*C. nucfera* L.) germplasm showing three mutational events (see Table 5 for details).

Table 4. Genetic information of *mat*K sequences of native coconut (*C. nucifera* L.) germplasm from South Kalimantan, Indonesia.

Parameter	*mat*K
Range of sequence length (bp)	844-863
Number of polymorphic sites (*S*)	3
Maximum Likelihood Value (ln*L*)	-1215.080
Transition/Transversion (Ti/Tv) bias value (*R*)	235967.41
GC content (%)	33.30
Nucleotide diversity (π%)	0.0258

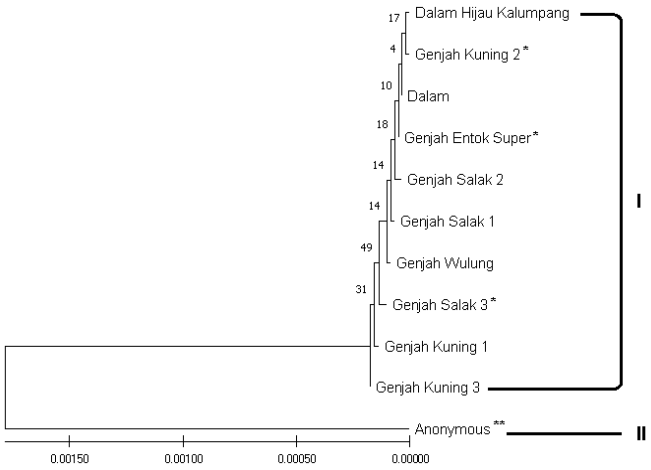

Fig 4. Genetic relationship among coconut (*C. nucifera* L.) germplasm natively from South Kalimantan, Indonesia, generated by Maximum Likelihood (ML) with a bootstrapping of 1,000 replicates. Notes: *Introduction; **An outgroup, obtained from GenBank.

Table 5. Polymorphic sites found on *mat*K sequences of native coconut (*C. nucifera* L.) germplasm from South Kalimantan, Indonesia.

Cultivar	Nucleotide Position		
	2[a]	5[a]	862[a]
Dalam	-	G	-
Dalam Hijau Kalumpang	-	G	C
Genjah Entok Super[*]	-	-	C
Genjah Kuning 1	-	-	-
Genjah Kuning 2[*]	C	G	-
Genjah Kuning 3	-	-	-
Genjah Salak 1	-	G	-
Genjah Salak 2	-	G	-
Genjah Salak 3[*]	-	G	-
Genjah Wulung	-	G	-
Anonymous[**]	-	-	-
Consensus	T	A	T

Notes: *Introduction; **Outgroup; [a]Substitution-transversion.

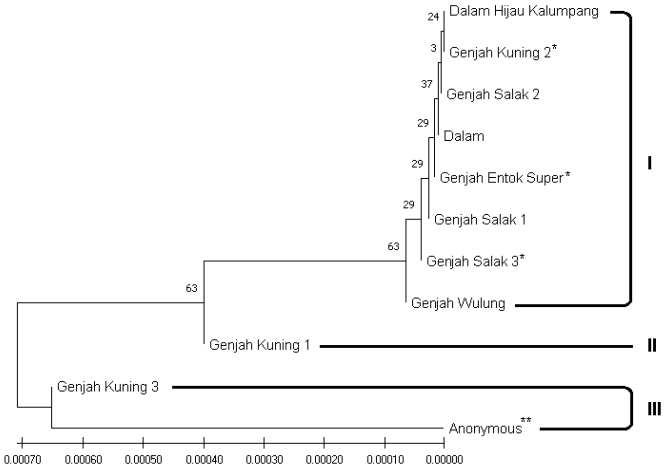

Fig 5. Genetic relationship among coconut (*C. nucifera* L.) germplasm natively from South Kalimantan, Indonesia, generated by Neighbor-Joining (NJ) with a bootstrapping of 1,000 replicates. Notes: *Introduction; **An outgroup, obtained from GenBank.

gene, *mat*K is provide enough revenues for evolutionary analysis at the family level and below (Kar et al., 2015). In phylogenetic analysis, phylogenetically informative traits are those which are variable and not the product of homoplasy (parallel evolution). However, these traits are not so variable that alignment between specific taxonomic levels can be accomplished. According to Kar et al. (2015), *mat*K provides many informative traits in regions that do not have excessive variability nor excessive conserved sequence and can be aligned to determine evolutionary relationships from the species to the divisional or even higher taxonomic levels.

In this study, although the native coconut germplasms from the South Kalimantan, Indonesia, have separated into different clades, two for Maximum Likelihood (Fig. 3) and

three for Neighbor-Joining (Fig 4), this germplasm cannot differentiate precisely. It is strongly related to the low mutation rate that occurs in the *mat*K sequence used. However, using both approaches (ML and NJ), the outgroup sample is separated from the samples studied. Referring to Wilberg (2015), the outgroup is of primary importance samples in phylogenetic analyses because it can be affecting ingroup relationships and, in placing the root, polarizing characters. In this case, following the NJ method, *Genjah Kuning 3* has closely related to an outgroup (Fig 4).

Materials and Methods

Plant materials

A total of eleven samples of native coconut (*C. nucifera* L.) germplasm comprised of ten from the South Kalimantan, Indonesia, collected by a purposive sampling method (Fig 2) and one as an outgroup (obtained from GenBank) were used in this study. The list of all coconut samples and their origins were presented in Table 1.

Molecular assay

The DNAs of coconuts were extracted from young leaf samples using a commercial DNA extraction kit (Geneaid Biotech Ltd., Taiwan). These genetic materials were then quantified by a NanoVue of UV-VIS spectrophotometer (GE Healthcare, UK) and amplified using the *mat*K primers (Table 2). Amplification was done with a total volume of 25 µL, consisting of 22 µL MyTaq HS Red Mix PCR (Bioline, UK), 2.0 µL (10 µM) primer forward and reversed, and 1 µL (10× diluted containing ca. ten ng DNA) template. This reaction was employed by a SimpliAMP Thermocycler PCR (Applied Biosystem, USA) and programmed by following conditions: initial denaturation of 94^{o}C for 4 min; denaturation of 94^{o}C for 30 sec, annealing (48^{o}C, 30 sec); extension (72^{o}C, 1 min); and final extension (72^{o}C, 7 min) (Mursyidin et al., 2021). The amplified DNA fragments were then separated by 1.5% agarose gel with a 1X TBE buffer solution, stained with a FluoroVue dye (SMOBiO Technolgy, Taiwan), and observed by UV transilluminator. The amplified DNA was then purified and sequenced bidirectionally using the Sanger method by the 1st Base Ltd., Malaysia. All sequences of *mat*K genes of coconuts were stored in GenBank with accession numbers shown in Table 1.

Data analysis

The *mat*K sequences of each native coconuts were checked refined manually and assembled using the MEGA-X software (Kumar et al., 2018). Before further analysis, all sequences were aligned using Clustal Omega and traced with BLAST (Sievers and Higgins, 2018). The genetic divergences were calculated using Kimura 2-Parameter (K2) distances (Kumar et al., 2018). The phylogenetic analysis was carried out using two methods, i.e., Maximum Likelihood (ML) and Neighbor-Joining (NJ). The bootstrap analysis evaluated the phylogenetic trees or phylograms with 1,000 replicates (Lemey et al., 2009).

Conclusion

While the native coconut germplasm from the South Kalimantan, Indonesia has a low-level genetic diversity, π% = 0.0258, information on its relationship is valuable in supporting the development of new coconut cultivars with desired traits in the future. In addition, the application of other cpDNA markers, such as *rbc*L, is highly recommended to further verify the genetic diversity and relationship of this coconut germplasm.

Acknowledgment

The authors wish to thank the Director of Plantation and Livestock Service of South Kalimantan, Indonesia, for providing information about the existence of native coconut germplasm used in this study. This study was funded by the internal research grant of Universitas Lambung Mangkurat, Indonesia, No. SP DIPA – 023.17.2.677518/2021 and No. 697/UN8/PG/2021.

References

Acquaah G (2012) Principles of plant genetics and breeding. Second Edition. John Wiley and Sons Inc., New York, USA.

Anggreany S (2020) Potency of genjah salak coconut from South Kalimantan. Report. Assessment Institute of Agricultural Technology. http://kalsel.litbang.pertanian.go.id/ind/index.php? [Indonesian]

Azevedo AON, Azevedo CDO, Santos PHAD, Ramos HCC, et al (2018) Selection of legitimate dwarf coconut hybrid seedlings using DNA fingerprinting. Crop Breed Appl Biotechnol. 18: 409-416.

Barthet MM, Pierpont CL, Tavernier EK (2020) Unraveling the role of the enigmatic MatK maturase in chloroplast group IIA intron excision. Plant Direct 4: 1-17.

Batugal P, Bourdeix R, Baudouin L (2009) Coconut Breeding. In: Breeding Plantation Tree Crops: Tropical Species. Springer, New York, USA.

CBOL (2009) A DNA barcode for land plants. PNAS 106:12794–12797.

Chen JR, Shiau YJ (2015) Application of internal transcribed spacers and maturase K markers for identifying *Anoectochilus*, *Ludisia*, and *Ludochilus*. Biol Plantarum. 59:485–490.

Căprar M, Copaci CM, Chende DM, Sicora O, Şumălan R, Sicora C (2017) Evaluation of genetic diversity by DNA barcoding of local tomato populations from North-Western Romania. Not Bot Horti Agrobotanici Cluj-Napoca. 45: 276–279.

DGNED (2017) Indonesian various coconut products. Export News Indonesia. Directorate General of National Export Development. Indonesian Ministry of Trade. https://djpen.kemendag.go.id/app_frontend/admin/docs/publication/1561519014552.pdf.

DGP (2020) Opportunities to increase market access and added value for coconut main products and by-products through production and marketing partnerships. Directorate General of Plantation, Indonesian Ministry of Agriculture. http://ditjenbun.pertanian.go.id/. [Indonesian]

Galimberti A, Labra M, Sandionigi A, Bruno A, Mezzasalma V, De Mattia F (2014) DNA barcoding for minor crops and food traceability. Adv Agric. 2014: 1–8.

Gao Y, Yin S, Yang H, Wu L, Yan Y (2017) Genetic diversity and phylogenetic relationships of seven *Amorphophallus* species in southwestern China revealed by chloroplast DNA sequences. Mitochondrial DNA Part A. 29: 679-686.

Geethanjali S, Anitha RJ, Rajakumar D, Kadirvel P, Viswanathan PL (2017) Genetic diversity, population structure and association analysis in coconut (*Cocos nucifera* L.) germplasm using SSR markers. Plant Genet Resour. 16: 1–13.

Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Intl. 2015: 1–14.

Habib S, Dang VC, Ickert-Bond SM, Zhang JL, Lu LM, Wen J, Chen ZD (2017) Robust phylogeny of *Tetrastigma* (Vitaceae) based on ten plastid DNA regions: Implications for infrageneric classification and seed character evolution. Front Plant Sci. 8: 590.

Hollingsworth PM, Graham SW, Little DP (2015) Choosing and using a plant DNA barcode. PLoS One. 6: 1–13.

Immanissa S, Faizal I, Salamah A, Sari IA, Susilo AW (2020) Genetic variation in Maturase K (matK) from cacao (*Theobroma cacao* L.) varieties in Indonesia. In: IOP Conference Series: Earth and Environmental Science. 481.

Kandoliya UK, Joshi AK, Mori DS, Marviya GV, Golakiya BA (2018) Genetic diversity analysis of coconut (*Cocos nucifera* L.) genotypes and hybrids using molecular marker. Indian J Agric Biochem. 31: 25-32.

Kar P, Goyal AK (2015) Maturase K gene in plant DNA barcoding and phylogenetics. In: Plant DNA barcoding and phylogenetics. Lambert Academic Publishing, Germany.

Kress WJ (2017) Plant DNA barcodes: Applications today and in the future. J Sys Evol. 55: 291–307.

Kumar S, Stecher G, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 35: 1547-1549.

Le DT, Zhang YQ, Xu Y, Guo LX, Ruan ZP, Burgess KS, Ge XJ (2020) The utility of DNA barcodes to confirm the identification of palm collections in botanical gardens. PLoS ONE. 15: 1–14.

Lemey P, Salemi M, Vandamme AM (2009) The phylogenetic handbook: A practical approach to phylogenetic analysis and hypothesis testing. Second Edit. Cambridge University Press, Cambridge, UK.

Li HQ, Chen JY, Wang S, Xiong SZ (2012) Evaluation of six candidate DNA barcoding loci in *Ficus* (Moraceae) of China. Mol Ecol Resour. 12: 783–790.

Loiola CM., Azevedo AON, Diniz LEC, Aragão WM, Carlos CD, Santos PHAD, et al (2016) Genetic relationships among tall coconut palm (*Cocos nucifera* L.) accessions of the international coconut genebank for Latin America and the Caribbean (ICG-LAC), evaluated using microsatellite markers (SSRs). PLoS ONE. 11: 1–11.

Mahayu WM, Taryono (2019) Coconut (*Cocos nucifera* L.) diversity in Indonesia based on SSR molecular marker. AIP Conf Proc. 2099: 1–7.

Masumbuko LI, Sinje S, Kullaya A (2014) Genetic diversity and structure of East African tall coconuts in Tanzania using RAPD markers. Open J Genet. 4: 175-181.

Mursyidin DH, Khairullah I (2020) Genetic evaluation of tidal swamp rice from South Kalimantan, Indonesia based on the agro-morphological markers. Biodiversitas. 21: 4795-4803.

Mursyidin DH, Nazari YA, Badruzsaufari, Masmitra MRD (2021) DNA barcoding of the tidal swamp rice (*Oryza sativa*) landraces from South Kalimantan, Indonesia. Biodiversitas. 22: 1593-1599.

Mustafa KM, Ewadh MJ, Al-Shuhaib MBS, Hasan HG (2018) The in silico prediction of the chloroplast maturase K gene polymorphism in several barley varieties. Agric. 64: 3–16.

Nazari A (2011) Identification of high yielding block of coconut in the district of Hulu Sungai Selatan South Kalimantan. Agroscientiae. 18:1–6. [Indonesian]

Rajesh MK, Jerard BA, Preethi P, Thomas RJ, Karun A (2014) Application of RAPD markers in hybrid verification in coconut. Crop Breed Appl Biotechnol. 14: 36-41.

Rajesh MK, Sabana AA, Rachana KE, Rahman S, Jerard BA, Karun A (2015) Genetic relationship and diversity among coconut (*Cocos nucifera* L.) accessions revealed through SCoT analysis. 3 Biotech. 5: 999–1006.

Rasam DV, Gokhale NB, Sawardekar SV, Patil DM (2016) Molecular characterization of coconut (*Cocos nucifera* L.) varieties using ISSR and SSR markers. J Hort Sci Biotechnol. 91: 347-352.

Ríos RO (2015) Plant breeding in the omics era. Springer International Publishing, Cham, Switzerland.

Sievers F, Higgins DG (2018) Clustal Omega for making accurate alignments of many protein sequences. Prot Sci. 27: 135-145.

Singh J, Kakade DP, Wallalwar MR, Raghuvanshi R, Kongbrailatpam M, Verulkar SB, Banerjee S (2017) Evaluation of potential DNA barcoding loci from plastid genome : intraspecies discrimination in rice (*Oryza species*). Intl J Curr Microbiol Appl Sci. 6: 2746–2756.

Suriani C, Prasetya E, Harsono T, Manurung J, Prakasa H, Handayani D, Jannah M, Rachmawati Y (2021) DNA barcoding of andaliman (*Zanthoxylum acanthopodium* DC) from North Sumatra Province of Indonesia using maturase K gene. Trop Life Sci Res. 32:15–28.

Tosh J, James K, Rumsey F, Crookshank A, Dyer R, Hopkins D (2016) Is DNA barcoding child's play? Science education and the utility of DNA barcoding for the discrimination of UK tree species. Bot J Linn Soc. 181: 711–722.

Thakar SB, Dhanavade MJ, Sonawane KD (2016) Phylogenetic, sequence analysis and structural studies of *maturase K* protein from mangroves. Curr Chem Biol. 10:135–141.

Trademap (2021) List of exported products for the selected product. https://www.trademap.org/Product_SelCountry_TS.aspx?

Wilberg E (2015) What's in an outgroup? The impact of outgroup choice on the phylogenetic position of Thalattosuchia (*Crocodylomorpha*) and the origin of Crocodyliformes. Syst Biol. 64: 621–637.

Wu Y, Yang Y, Qadri R, Iqbal A, Li J, Fan H, Wu Y (2019) Development of SSR markers for coconut (*Cocos nucifera* L.) by selectively amplified microsatellite (SAM) and its applications. Trop Plant Biol. 12: 32-43.