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Full Length Article

Diversity and Conservation of Kenari Nuts (*Canarium* spp.) in Makean Island, North Maluku Evidenced by Morphologies and rbcL Sequences

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Abstract

The Island of Makean in North Maluku (Wallacea region) is recognized as a biodiversity hotspot for kenari nuts (*Canarium* spp.), but studies on this subject have rarely been explored. This study aimed to examine the diversity of six local kenari in Makean Island based on morphologies and molecular using the rbcL gene, and also to propose its conservation strategy by gap analysis. Results showed that the phenetic dendrogram was divided into three clusters (SI 0.694 to 0.878). Kenari Batu and Alus were placed in clusters 1 and 2, respectively. Meanwhile cluster 3 contained Kenari Kusum, Laka, Poda, and Towas. The fruit and seed kernel were the distinguishing features among the clusters. Molecular characterization showed a high conservation level of 99.47%, which consisted of 562 monomorphic and 3 polymorphic nucleotides. The nucleotide composition exhibited high levels of A+T bases at 55.35%. Point mutations of transversion and transition were found in Kenari Alus and Batu. The phylogenetic tree resulted in two clusters. Kenari Batu and Alus were clustered in clade 1 (SI 0.998) and were closely related to *C. indicum* (SI 0.994 to 0.996). Kenari Kusum, Towas, Poda and Laka were clustered in clade 2 (SI 1.00) and were closely related to *C. vulgare* (SI 0.986 to 0.988). Three conservation strategies were proposed consisting of on-farm, *in situ*, and *ex situ* approaches. This study findings may become a noteworthy basic referrence for broader and further studies on systematic, conservation actions for stakeholders, and sustainable utilization of kenari nuts by communities in tropical regions, particularly on small and remote islands that are vulnerable to natural and anthropogenic threats.

Keywords: *Canarium*; Diversity; Germplasm; Morphology; Genetic; RbcL

Introduction

Genus *Canarium* L. is locally known as canarium nuts in English, pili or galip nuts in Papua New Guinea and The Pacific and kacang kenari or kenari in Indonesia. It is a tropical plant producing potential edible nuts, commercial timber and some minor products belonging to the torchwood family of Burseraceae (Nevenimo *et al*. 2007; Rashid *et al*. 2021). The native range of this genus is Tropical Africa to West Pacific, including 121 accepted species that grow primarily in the wet tropical biome (POWO 2023). In Eastern Indonesia, some species are recognized including *Canarium lamii*, *C. vulgare*, *C. indicum* and *C. hirsutum* (Ellen 2019; Endewip *et al*. 2020). Furthermore, *C. indicum* and *C. vulgare* are considered to have the highest commercial value of kenari nuts production. Fruits and seeds contained high fatty acids of 65–75% for medical, nutraceutical and food applications (Rahman *et al*. 2015).

Makean Island belongs to the Wallacea biodiversity hotspots, containing terrestrial and marine species with high endemism. It is worth noting that Makean (or Machian) is a volcanic island situated among the Halmahera Islands, within the province of North Maluku in Indonesia. It is considered kenari biodiversity hotspot, then also known as Kenari Island (Sagaf and Fatmawati 2021; Sundari *et al.* 2021). Furthermore, kenari fruits and nuts are part of the traditional diets of indigenous people, and archaeological evidence suggests the interaction between humans and the plant has been long-standing (Weeks 2009; Ellen 2019). In Makean Island, the plants are spread naturally from seeds with hereditary ownership, aged on average 45–65 years old (Sagaf and Fatmawati 2021). There are several local names of kenari given by local communities on Makean Island. Local naming's are mostly based on morphological variations, the name of the owner and locality of the plants (Manui *et al.* 2022). Therefore, the scientific nomenclature

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of the local kenari germplasm from the island of Makean needs to be clarified.

Characterization is a fundamental step in germplasm collection management for identification and providing information on traits supporting their sustainable conservation strategy and optimum utilization (Singh *et al*. 2020; Hapsari *et al*. 2022). Morphological-based characterization has long been used and is still relevant to distinguish between species. It is considered more subjective and influenced by environmental factors, hence a molecular approach is needed to confirm the results (Wahyudi *et al*. 2022). The use of DNA barcoding is recommended due to some advantages in studying the genetic diversity, identifying specimens with high accuracy, fast, relatively inexpensive and requires a small part of the plant. In addition, there are other applicable functions, such as ecological surveys (Kress *et al*. 2015), identification of cryptic taxa (Jamdade *et al*. 2022) and species confirmation (Le *et al*. 2020). Some universal DNA barcodes recommended for plants include Internal Transcribed Spacer (ITS) of the nuclear genome and two plastid genes, matK and rbcL, as well as non-coding regions of psbK-psbI, trnH-psbA, rpoC1 and rpoB (Hollingsworth *et al*. 2009; Jamdade *et al*. 2022).

The morphological characteristics and genetic diversity of local kenari on Makean Island have not been scientifically explored and reported. In fact, kenari nuts from this island are a valuable commodity for food and agriculture that needs to be conserved and sustainably used for further breeding and development programs. Therefore, this study aims to analyze the diversity of local kenari germplasm in Makean Island based on morphologies and molecular using the DNA barcode of Ribulose bisphosphate carboxylase large chain (rbcL) from the plastid genome. The rbcL barcode has proven to be moderately informative to show the species-level phylogeny of *Canarium sensu stricto* (Weeks 2009).

In addition, this study also aims to build its conservation strategy by gap analysis (Maxted *et al.* 2008). The inhabiting species on a small island are considered more vulnerable to natural and anthropogenic factors such as habitat destruction, over-exploitation, climate change, alien species invasion, natural catastrophes and limited natural resources (Leunufna *et al*. 2022). Makean Island is a natural disaster-vulnerable area from volcanic eruptions; hence conservation efforts should be concentrated. The results of this study may become noteworthy basic information for broader and further studies on systematic, conservation, and sustainable utilization of kenari nuts in tropical regions, particularly in small and remote islands.

Materials and Methods

Site and population study

Administratively, Makean Island is located in South Halmahera Regency, North Maluku, Indonesia near the southern end of volcanic islands off the western coast of Halmahera. It lies between the island of Moti and Tidore to North and Kayoa and the Bacan Group to the south. Island spans a width of approximately 10 kilometers and is divided into two districts, namely Makean and West Makean. Its total land area measures 84.36 square kilometers and as of 2020, it is home to a population of around 14,000 people. The land topography ranges from slightly steep to steep, with a slope of 15–40% and > 40%. Only 38.9% of the land is classified as flat and sloping, which is mostly found in coastal areas. Furthermore, the island has an active volcano of Mount Kie Besi with a 1.5 km wide summit crater at a peak of 1,300 meters and a small lake on its Northeast side. Mount Kie Besi often erupts, making Makean Island a natural disaster-vulnerable area due to lava floods and hot clouds (Fig. 1; BPS 2021).

Kenari plants are found on Makean Island at altitudes ranging from 0 to 206 m above sea level, as shown in Fig. 1. Population characteristics are considered as sociability type 2, periodicity type 3, and vitality type 1 (Sundari *et al*. 2021). They formed small groups, flowering and fruiting, and reproduced with complete floristic stratification including seedlings, saplings, poles, and trees. Some of the plants are known to be decades (> 10) to hundreds of years old, and the environmental factors on Makean Island are suitable for the growth and reproduction of kenari plants. It has a tropical climate with low to medium rainfall intensity with an average of 0 mm to 200 mm (BMKG 2023). Based on the actual environmental factor measurements, the air temperature ranges from 28°C to 31°C. Soil pH neutral to slightly alkaline ranges from 7 to 8.5 with 10 to 40% moisture, respectively.

Plant materials

The plant materials examined were six local kenari in Makean Island comprised of Kenari Kusum, Towas, Poda, Laka, Alus, and Batu. Kenari plants were observed and sampled from eight locality covering all of the kenari hotspots in Makean Island, including Ngofakiaha, Ngofagita, Sebelei, Talapao, Tegano, Ngofabebawea, Bobawea and Samsuma, as shown in Fig. 1 and Table 1. The criteria and selection of plant materials observed were healthy trees with a productive age of > 10 years.

Morphological observation and clustering analysis

Morphological observation was conducted following the description of *Canarium* by Thomson and Evans (2006). The observed morphological features include vegetative and generative organs. The characters observed are tree growth pattern, diameter, color and texture, leaf with flushing and abscission period, leaf number per shoot, size, and petiole length, flower panicle number per shoot, size and color, fruit bunch per shoot, fruit number per bunch, fruit shape, fruit

size and weight, peel color, pulp color and seed kernel shape, color, weight and size.

Morphological characters observed were scored using a reference number of "0" or "1" for characters that were present or not present in the plant samples. The data were then analyzed using the clustering method of Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in Multivariate Statistical Package program (MVSP) v. 3:22 (Kovach 2007) to generate phenetic dendrogram and Similarity Index (SI). The result of the dendrogram was analyzed with synapomorphy characters to determine the distinguishing characters among local kenari in Makean island. Synapomorphies are characters shared by a group of taxa due to inheritance from a common ancestor (Novick *et al*. 2010).

DNA isolation and PCR amplification

The molecular analysis was conducted in Laboratory of Genetika Science Indonesia (Tangerang, Indonesia) and the material samples were desiccated young leaves. Whole genomic DNA isolation was conducted using Quick DNA Plant/Seed Miniprep Kit (Zymo). For the PCR amplification process, rbcL-F as the forward (5'-ATGTCACCACCAACAGAGACTAAAGC-3') and rbcL-R as the reverse primer (5'-GTAAAATCAAGTCCACCRCG-3') were used, as previously described by CBOL (2009). The PCR amplification was performed in an Agilent Surecycler 8800™ with 25 μL total volume of PCR reaction comprising of 9.5 μL dd H_2O, 12.5 μL 2x MyTaqHS Red Mix (Bioline), 1 μL of 10 μM rbcL forward primer, 1 μL of 10 μM rbcL reverse primer and 1 μL DNA template. PCR thermal cycle condition comprised of initial denaturation at 95°C for 1 min (1 cycle), followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 53.5°C for 30 s, extension at 72°C for 45 s and post extension at 72°C for 7 min (1 cycle). Amplified products were then purified and sequenced at 1st BASE Laboratories Sdn Bhd, Malaysia using ABI PRISM 3730xl Genetic Analyzer developed by Applied Biosystems, USA.

Genetic variability and phylogenetic analysis

The rbcL sequences results were evaluated using ABI sequences Scanner v.10, and genetic variability including nucleotide mutations was analyzed with DnaSP 6.12.03. Basic Local Alignment Search Tool (BLASTn) program in GenBank National Center for Biotechnology Information (NCBI) was employed to search the potential references of homologous sequences. Subsequently, they were retrieved for phylogenetic analysis and species identification, as shown in Table 2. The phylogenetic tree was constructed using MEGA 7.0.26 software, both for the six local kenari and for the combined data obtained from NCBI based on the evolution model of Kimura 2 Parameter (K2P) using Neighbor-Joining (NJ) algorithms with 1000 bootstrap replications. Bootstrap Support (BS) was categorized as strong, moderate, low, and very low when BS > 85, 70–85, 50–69 and < 50 (Kress *et al*. 2002). A pairwise distance analysis was also performed to generate a genetic SI.

Conservation strategy analysis

To build the suitable conservation strategy for local kenari in Makean Island, gap analysis was employed referring to Maxted *et al.* (2008). The gap analysis was carried out with the following steps: (1) circumscription of target taxon and target area; (2) assessment of natural diversity through a review of intrinsic taxonomic, genetic and ecogeographical diversity combined with threat assessment; (3) assessment of current complementary *in situ*, on-farm and *ex situ* conservation strategies and (4) reformulation of the conservation strategy through analysis of the differences between the pattern of natural, intrinsic diversity and the elements of that diversity already effectively represented by existing *in situ*, on-farm and *ex situ* conservation actions.

Results

Morphological characteristics and clustering

Based on observation data in the field, it was noted that all six local kenari have synapomorphy characteristics in the tree growth, main stem, leaf shape, and color. The tree growth is monopodial, main trunk cylindrical shape with a surface texture rough and greenish-gray color, as shown in Fig. 2. The leaves of kenari plant are typically oblong-obovate to oblong-lanceolate in shape and arranged in a pinnate manner, with stipules present. They exhibit bright to dark green coloration and have dimensions ranging from 5.2 x 12.8 to 7.7 x 17.0 cm. The flowers are small (ca. 1 cm across) and yellowish-white, and arranged in terminal panicles with 3 to 21 flowers per panicle, as shown in Fig. 3.

Clustering analysis in MVSP v. 3:22 based on morphological data of six local kenari in Makean Island resulted in a phenetic dendrogram, with a SI of 0.694 to 0.878, as shown in Fig. 4 and Table 3. Kenari Batu was separated in cluster 1 with specific morphological characters for having medium fruit as well as a kernel with mesocarp and exocarp. Furthermore, Kenari Alus was separated in cluster 2 characterized for having small and rounded fruit with kernels. The SI between clusters 1 and 2 was 0.735, as shown in Table 2. Cluster 3 consists of Kenari Kusum, Laka, Poda and Towas with a high SI of 0.816 to 0.878, as shown in Fig. 2 and Table 2. They have synapomorphy characteristics of medium to large fruit and seed kernels with elliptical to ovoid shapes, as shown in Fig. 5 and 6. Kenari Towas and Batu were the farthest pair with an SI of 0.694. Meanwhile, Kenari Poda and Laka were

Table 1: Plant materials of local kenari in Makean Island examined

No.	Local name	Synonim	Locality source
1	Kenari Kusum		Ngofakiaha, Sabalei and Talapoa
2	Kenari Towas		Sabalei, Samsuma, Ngofabebawea and Tegano
3	Kenari Poda	Tuwa	Ngofakiaha, Sabalei, Ngofagita and Talapoa
4	Kenari Laka	Ungu	Bobawea, Ngofakiaha, Sabale and Samsuma
5	Kenari Alus		Samsuma, Bobawea, Ngofabobawea, Sabalei and Ngofagita
6	Kenari Batu	Ngelalai	Sabalei, Samsuma, Tagano, Talapoa and Bobawea

Table 2: List of *rbcL* sequences of *Canarium* spp. and outgroup retrieved from NCBI

No.	Accession number	Species name	Section	Native range (POWO 2023)
1	FJ466632	*Canarium indicum*	*Canarium*	Maluku to Vanuatu
2	FJ466640	*Canarium vulgare*	*Canarium*	Java to Vanuatu
3	FJ466636	*Canarium ovatum*	*Canarium*	Philippines
4	FJ466642	*Canarium zeylanium*	*Canarium*	Sri Lanka
5	FJ466639	*Canarium pimela*	*Pimela*	China to Indo-China
6	FJ466628	*Canarium bengalense*	*Pimela*	India to China and Indo-China
7	FJ466626	*Canarium album*	*Pimela*	China to Indo-China
8	FJ466638	*Canarium strictum*	*Pimela*	India to China and Thailand
9	FJ466637	*Canarium pilosum*	*Pimela*	Peninsula Malesia to Fiji.
10	GU246029	*Canarium muelleri*	*Canariellum*	Queensland
11	FJ466641	*Canarium whitei*	*Canariellum*	New Caledonia
12	FJ466635	*Canarium oleiferum*	*Canariellum*	New Caledonia
13	FJ466643	*Protium madagascariense*	Outgroup	Madagascar
14	FJ466625	*Bursera tecomaca*	Outgroup	Mexico
15	FJ466630	*Commiphora edulis*	Outgroup	South to East Africa

Table 3: Similarity index of six local kenari in Makean Island based on morphology characters

Local name	Kusum	Towas	Poda	Laka	Alus	Batu
Kenari Kusum	1					
Kenari Towas	0.837	1				
Kenari Poda	0.857	0.816	1			
Kenari Laka	0.857	0.857	0.878	1		
Kenari Alus	0.796	0.837	0.735	0.816	1	
Kenari Batu	0.857	0.694	0.796	0.755	0.735	1

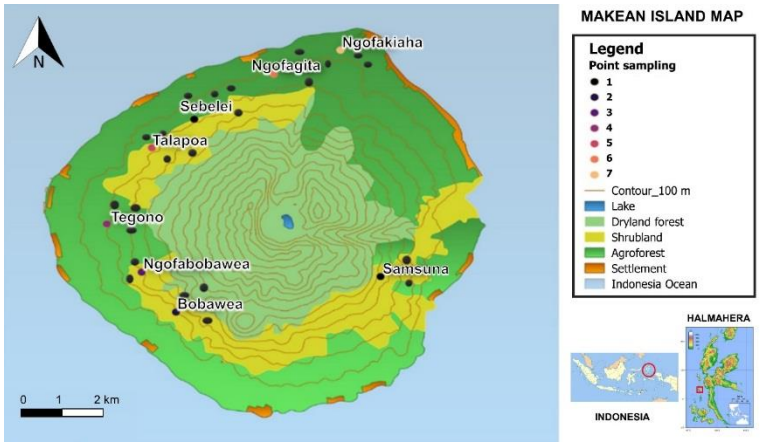

Fig. 1: Map of Makean Island and locality source of kenari plant materials examined

the closest pair with a similarity of 0.878, as shown in Table 3 and 4.

From this current study, Kenari Alus is distinguished by its small size and dark-purple fruit, with dark brown, small, and rounded seed kernels. Kenari Batu is characterized by medium size and dark-purple fruit, with dark brown, small, and rounded seed kernels. Meanwhile, Kenari Kusum has large size and green fruit, with brown, large, and elliptical-ovoid seed kernels. Kenari Poda is characterized by medium size and dark green fruit, with brown, medium and elliptical-ovoid seed kernels. Kenari Laka is characterized by medium size and dark-purple fruit, with brown, medium and elliptical-ovoid seed kernels. Furthermore, Kenari Towas has large size and green fruit, with dark brown, large and elliptical-ovoid seed kernels, as shown in Fig. 5 and 6.

Table 4: Nucleotide composition of *rbcL* gene of six local kenari in Makean Island

OTU	Nucleotide composition (%)					
	T(U)	C	A	G	A+T	G+C
Kenari Kusum	27.10	21.15	28.15	23.60	55.24	44.76
Kenari Towas	27.00	21.08	28.40	23.52	55.40	44.60
Kenari Poda	27.39	21.20	27.74	23.67	55.12	44.88
Kenari Laka	27.35	21.08	28.05	23.52	55.40	44.60
Kenari Alus	27.80	21.33	27.80	23.08	55.59	44.41
Kenari Batu	27.32	21.37	28.02	23.29	55.34	44.66
Average	27.33	21.20	28.02	23.45	55.35	44.65

Remarks: OTU = Operational Taxonomic Unit, G = Guanin, A = Adenin, C = Cytosin and T = Thymin

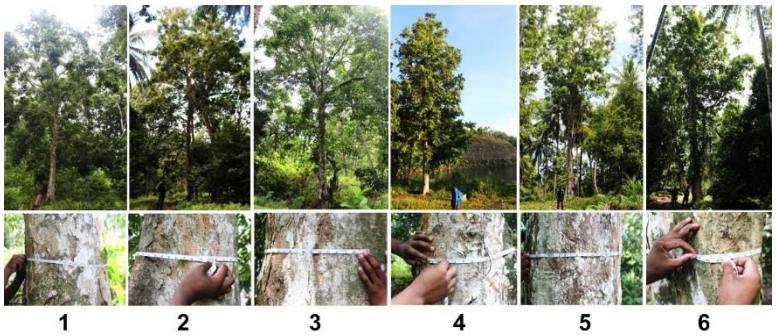

Fig. 2: Synapomorphy characters of tree growth and main stem of six local kenari in Makean Island: **1**. Kusum, **2**. Towas, **3**. Poda, **4**. Laka, **5**. Alus, **6**. Batu

Fig. 3: Sinapomorphy characters of inflorescence and flowers of six local kenari in Makean Island

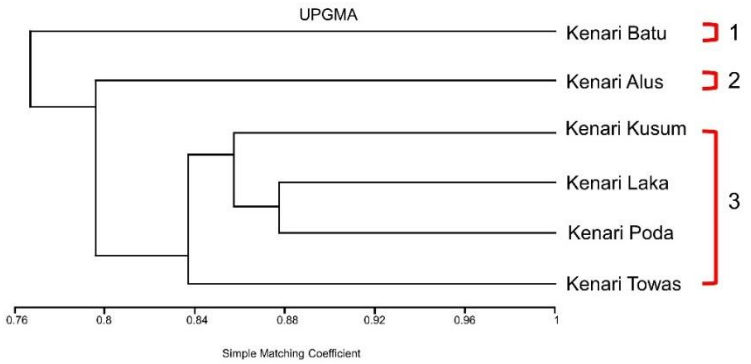

Fig. 4: Dendogram clustering of six local kenari in Makean Island based on morphology characters

RbcL sequences characteristics and variation

The whole genome DNA of six local kenari in Makean Island was easily amplified by rbcL primers at approximately 500–600 bp and the Sanger sequencing to the PCR products resulted in 568–598 nucleotides. Furthermore, NCBI BLASTing showed that DNA sequences were homologous with rbcL of *Canarium* spp.

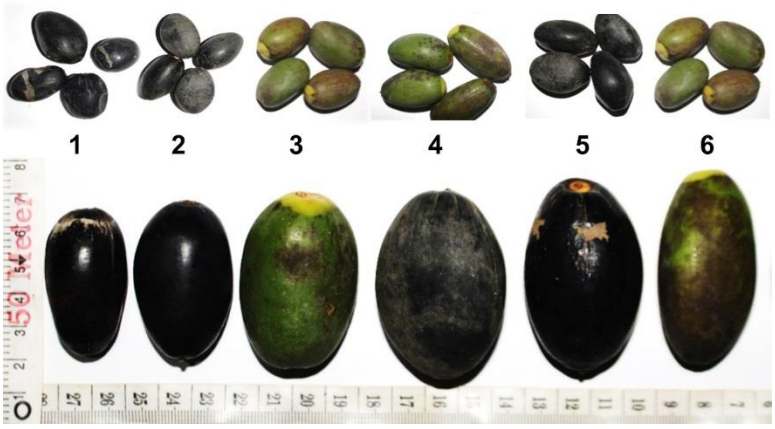

Fig. 5: Variations of fruit shape, size and color of six local kenari nuts in Makean Island: **1**. Kusum, **2**. Towas, **3**. Poda, **4**. Laka, **5**. Alus, **6**. Batu

Fig. 6: Variation of seed kernels (with testa) shape, size, and color of six local kenari nuts in Makean Island: **1**. Kusum, **2**. Towas, **3**. Poda, **4**. Laka, **5**. Alus, **6**. Batu

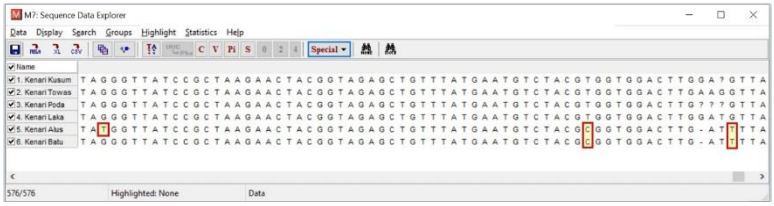

Fig. 7: The polymorphic sites of *rbcL* sequences of six local kenari in Makean Island

and some species from Burseraceae with high query covers and similarity per identity > 98%. About 15 rbcL sequences of *Canarium* spp. and some outgroup species were retrieved for phylogenetic analysis and species identification, as shown in Table 2.

The RbcL nucleotide sequences alignment to the six local kenari in Makean Island showed a high conservation level reached of 99.47%. The total selected sites were 576 comprising 562 monomorphic and 3 polymorphic, with 11 excluded data. Meanwhile, the pairwise genetic SI ranged from 0.994 to 1.00. The nucleotide composition was high and low in A + T and G + C bases at 55.35 and 44.65%, respectively.

The polymorphic sites consisted of 1 singleton variable and 2 parsimony informative sites. Polymorphism occurs due to point mutations considered as single nucleotide SNP. Singleton variation was identified in Kenari Alus at site position 506, with transversion type G → T. Meanwhile, parsimony informative characters were identified in Kenari Alus and Batu at site positions 553 and 567, with transition type T → C as shown in Fig. 7.

Phylogenetic and species identification

The genetic cladogram of six local kenari in Makean Island alone based on rbcL sequences resulted in two clusters supported by strong BS, as shown in Fig. 8. Kenari Batu and Alus were clustered in clade 1, with a genetic SI of 0.998, while Kenari Kusum, Towas, Poda and Laka were in clade 2, with a genetic SI of 1.00.

For further phylogenetic analysis and species identification, 21 Operational Taxonomic Units (OTU) of rbcL sequences were aligned, and the final selected sites were 534 bp as seen in Table 2. This shows that a high

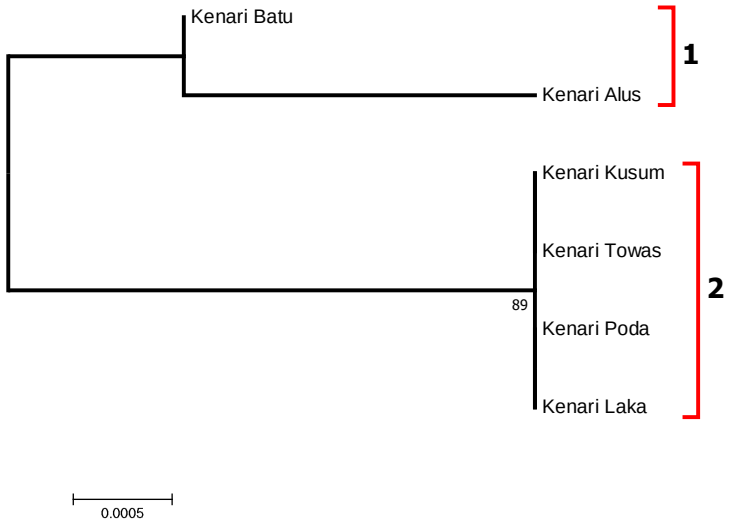

Fig. 8: NJ genetic cladogram of six lokal kenari in Makean Island based on RbcL sequences

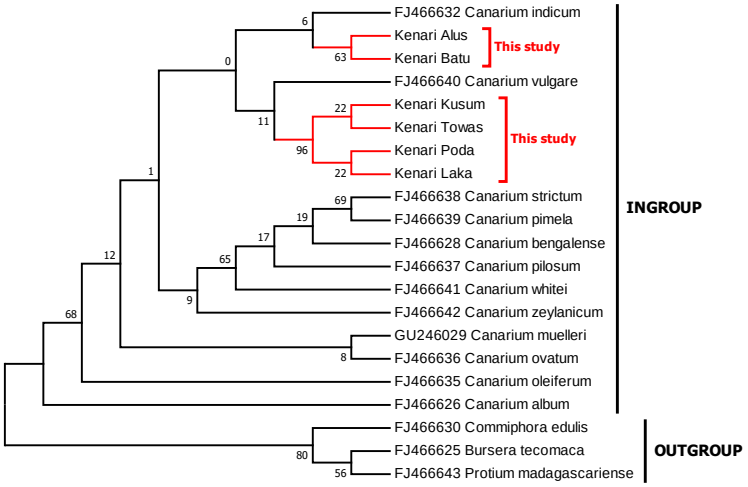

Fig. 9: Bootstrap consensus NJ phylogenetic tree of six local kenari with other *Canarium* spp. and outgroup species (Burseraceae) from NCBI based on RbcL sequences

conservation level with 506 or 94.76% nucleotides was monomorphic, and 28 or 5.24% positions of DNA sequences were polymorphic, comprising 10 and 18 singleton and parsimony variable sites at 3.37 and 1.87%. The nucleotide composition was high and low in A + T and G+C bases at 54.70 and 45.20%, respectively. Meanwhile, sequences pairwise similarity of all OTU ranged from 0.967 to 1.00.

Phylogenetic analysis using the NJ algorithm of 21 OTU rbcL sequences resulted in a tree topology of two clades, as shown in Fig. 9. *Canarium* spp. as an ingroup was separated from other genera from Burseraceae as an outgroup and supported by 80 moderate BS. Meanwhile, the section separation within the ingroup of *Canarium* spp. (*Canarium*, *Pimela* and *Canariellum*) was unclear and supported by very low to low BS.

Matrix of gap analysis to build the conservation strategy

The matrix of gap analysis to build the suitable conservation strategy of local kenari on Makean Island was presented in Table 5. The results of this study were proven to fill the gaps of the current state to desired state of conservation strategy prioritization process, including species identification (scientific name), morphology and genetic diversity. Based on this gap analysis, three conservation actions are proposed consisting of *in situ*, on-farm and *ex situ* strategies. On-farm conservation is considered as the most suitable strategy since it has been implemented for a long time by local farmers, however it needs some improvement efforts. *In situ* conservation for the most valuable of local kenari is also proposed, as well as *ex situ* conservation approaches need to be established to avoid loss from natural disasters.

Table 5: The gap analysis matrix for conservation strategy of local kenari in Makean Island

Step	Indicator	Current state	Desired state	Gap analysis	
				+	-
1	Identification and classification of species and area target	Species target: six local kenari (*Canarium* spp.). Target area: Makean Island, North Maluku, Indonesia. The species identification and classification of local kenari in Makean Island are still unclear.	The diversity of local kenari in Makean Island must be clear about their taxon identity.		✓
2	A review of intrinsic taxonomic, genetic and ecogeographical diversity combined with threat assessment	Six local kenari in Makean Island are recognized with local name *i.e.*, Kenari Kusum, Towas, Poda, Laka, Alus and Batu. No scientific name available.	The scientific name of the local kenari in Makean Island should be determined. This current study showed that Kenari Alus and Batu were identified as *C. indicum.* While Kenari Kusum, Towas, Poda, and Laka were identified as *C. vulgare.*		✓
		The morphological characteristics of local kenari in Makean Island are not yet evaluated.	The morphological diversity of local kenari in Makean Island should be studied. This current study result showed high morphological diversity, divided into 3 clusters with SI 0.694 to 0.878.		✓
		The genetic diversity of local kenari in Makean Island are not yet evaluated.	The genetic diversity of local kenari in Makean Island should be studied. This current study showed high conservation level *rbcL* sequences of 99.47%, divided into 2 clusters. Three point mutations were identified.		✓
		Makean Island is a small and remote island in North Maluku islands with an active volcano which vulnerable to natural disaster threats such as volcanic eruptions and tsunamis.	The conservation strategy of local kenari in Makean Island must consider the ecogeographical condition and all potential threats both natural and anthropogenic.		✓
		The population of local kenari is in healthy condition, but the plants are mostly at old ages and owned by local farmers which passed down generation to generation. Threats due to unsustainable harvesting may occur.	The population of local kenari in Makean island must be maintained, particularly the mother plants. Plant rejuvenation effort is necessary and sustainable harvesting practices must be implemented.		✓
3	Assessment of current complementary in-situ, on-farm and ex-situ conservation strategies	In-situ conservation assessment: there is no reserve area specifically established to conserve local kenari in Makean Island.	In-situ conservation should be proposed at the location of the valuable germplasm of local kenari in Makean Island.		✓
		On-farm conservation: farmers in Makean Island have been conserving local kenari on their owned land for millennia by preserving the mother plants and harvesting in a traditional manner.	On-farm conservation of local kenari has been applied by farmers in Makean Island. However, some improvements should be made.	✓	
		Ex-situ conservation assessment: so far there has been no report on efforts to conserve local kenari in Makean Island outside their habitat (out of the island).	Ex situ conservation approaches urgently need to be established to avoid loss from natural disasters.		✓
4	Priority setting for conservation actions	In situ conservation priorities: not yet applicable.	In-situ conservation should be proposed at the location of the most valuable germplasm. It would be preferable to select location that contain high genetic variability species/varieties, superior varieties and old mother plants.		✓
		On-farm conservation priorities: farmers are cultivating and conserving local kenari in a traditional manner.	Some improvements are required to the on-farm conservation including maintenance and cultivation of the old mother plants also plant rejuvenation action. For sustainable harvesting and agribusiness purposes, farmers are encouraged to conduct selection and breeding.		✓
		Ex-situ conservation: not yet applicable.	Ex situ conservation approaches include conservation in botanical gardens, field gene banks, seed banks, preservation of in-vitro cultures, and gene libraries.		✓

Discussion

In general, all six local kenari in Makean Island have a similarity in morphological characters of vegetative parts in the tree growth, main stem, leaf shape, and color. *Canarium* spp. are medium to broad buttressed trees that grow up to 40 m in height and 30 m in canopy diameter, and have oblong-obovate to oblong-lanceolate shaped, pinnate, spiral and stipulated leaves (Thomson and Evans 2006; Rashid *et al*. 2021). Leaf flushing in Makean Island occurs between March and June, while leaf abscission occurs from May to August. Furthermore, the inflorescence and flowers characteristics observed in this study were also found similar. Most of the *Canarium* spp. are dioecious, with separate male and female flowers on different trees. However, some trees may also bear hermaphrodite and female or male flowers (Thomson and Evans 2006; Rashid *et al*. 2021). Kenari plant in Makean Island typically flowers from March to June and bears fruit from May to July.

The phenetic dendrogram was divided into three distinct clusters. Kenari Batu and Alus were placed in clusters 1 and 2, respectively. Meanwhile, cluster 3 contained Kenari Kusum, Laka, Poda and Towas (Fig. 4). The fruit and kernel are important characteristics to distinguish between local kenari (Fig. 5 and 6) and are strongly related to the preference of consumers (Sundari

et al. 2021; Manui *et al.* 2022).

The rbcL nucleotide sequences length of six local kenari from Makian Island was considered short or partial (568–598 bp), with a maximum of 1,400 bp (Newmaster *et al*. 2006). Sequences alignment of the six local kenari alone showed high conservation level and high in A+T bases. RbcL barcode encoded the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) in the first important step of carbon dioxide fixation (Ichikawa *et al*. 2008). A coding region or exon had high AT base content in association with the functions in transcription and protein translation. Low GC content signified reduced spots of mutation and recombination rates (Hariyanto *et al*. 2021).

The tree topology of genetic cladogram based on rbcL sequences in six local kenari alone was slightly different from morphology's approach (Fig. 4 and 8). Kenari Batu and Alus were clustered in clade 1, while Kenari Kusum, Towas, Poda, and Laka were in clade 2. The points of mutation occurred in Kenari Alus and Batu both transition and transversion made them separated in different clusters from the others (Fig. 7 and 8). Transversion changes were considered to be more drastic than transitions because the substitution of one ring into two requires more energy than the reaction without a change in a ring structure. This can be caused by high energy sources such as radiation, chemical and high environmental stress, or during DNA replication (Duchêne *et al*. 2015). Conservation genetics providing tool to assist the management of threatened species (Willi *et al*. 2022). Hence, assessing genetic variation present within a population of local kenari in Makean Island as natural disasters prone area is a crucial step towards developing effective conservation and breeding strategies, as well as preparing for potential challenges posed by changing environmental conditions and climates.

The phylogenetic tree topology of six local kenari with other *Canarium* spp. and outgroup species (Burseraceae) from NCBI based on RbcL sequences resulted in this study (Fig. 9) supported the polyphyletic of the genus as proposed by Weeks (2009), but needs thorough taxonomic revision. The branching pattern within *Canarium* spp. has a high possibility of changing position. However, the tree topology and similarity matrix can explain the relationship among species.

In this study reported two possible species of local kenari in Makean Island, namely *C. vulgare* and *C. indicum*, with geographic distribution from Java and Maluku to Vanuatu (POWO 2023). In addition, *C. ovatum* and *C. zeylanicum* to the examined six lokal kenari was also considered high in genetic similarity. However, *C. ovatum* and *C. zeylanicum* are native to The Philippines and Sri Lanka (POWO 2023). In Makian Island, the two species are considered outside the range of geographic distribution. Therefore, it can be concluded that Kenari Alus and Batu were identified as *C. indicum*. Meanwhile, Kenari Kusum, Towas, Poda, and Laka were identified as *C. vulgare*.

These local kenari in Makean Island are valuable plant germplasms for food and agriculture that need to be conserved and managed for sustainable utilization. Based on the gap analysis (Table 5), cultivation on the farm is considered the most suitable conservation strategy for the plant. Farmers in Makean Island have been conserving local kenari on their owned land for millennia by preserving the mother plants and harvesting in a traditional manner. The kenari plant is mainly found in lowland rainforests, secondary forests and old garden areas, and is widely planted around villages and settlements. On-farm conservation involves the maintenance of species and traditional varieties by farmers in agroecosystems over time. This dynamic conservation enables the development and evolutionary continuation of plant materials under the influence of the regional environment and the technologies used (Holubec *et al*. 2010; Joshi and Upadhya 2019). However, there are some improvements to the on-farm conservation strategy that need to be carried out including maintenance and cultivation of the old mother plants and plant rejuvenation efforts through selecting seeds and planting (propagation).

Moreover, an *in situ* strategy can also be applied by designating the locations as protected or restricted areas. These methods are necessary only for essential and potentially critical germplasm and cultures (Taryono *et al*. 2020). Therefore, it would be preferable to select locations that contain most valuable germplasms of local kenari such as the high genetic variability species/varieties, the superior varieties and the oldest mother plants. From this study, both Kenari Alus and Batu showed high genetic variability so that it became a priority for conservation. Meanwhile for Kenari Kusum, Towas, Poda and Laka, because they are genetically identical, it is suggested to choose only one as a representative. Further, those sites need to be surveyed to determine which is most appropriate for establishing *in situ* reserves. After completing surveys of candidate reserve sites, target taxon hotspots can be identified and final recommendations made (Maxted *et al*. 2008).

Since Makean Island is vulnerable to natural disasters such as volcanic eruptions and tsunamis, a back up plan for *ex situ* conservation is required. *Ex situ* conservation is the maintenance of species outside their natural habitat and is used to mitigate populations from the dangers of destruction, replacement, and decline. It is designated to maintain genetic material in the state collected, to avoid the loss or degeneration of a species. *Ex situ* conservation approaches include a botanical garden, field genebanks, seed banks, *in vitro* culture preservation, and gene libraries (Maxted *et al.* 2008; Taryono *et al.* 2020). However, to build a new *ex situ* conservation requires high costs and inputs. *Ex situ* conservation requires different types and levels of management intensity, and a multistakeholder approach such as input from multiple experts on botanical views, *ex situ* breeding, gene banking, reintroduction, habitat suitability, *etc*. (Kasso and Balakrishnan 2013). A

cost-effective strategy that could be proposed is by storing plant material (seeds and seedlings) in the existing national botanical gardens and/or university arboretum.

Kenari nut is a promising agribusiness commodity although the market is still limited to certain regions and circles in Sulawesi (Sagaf and Fatmawati 2021; Sundari *et al*. 2021). For sustainable utilization, farmers are suggested to propagate from mother plants and plant in orchards. The species is easily propagated from seed, as nursery-raised seedlings, or by direct seeding into the field (Thomson and Evans 2006). In addition, the selection and breeding of plants to develop individuals which produce high yield, good quality and meet the preferences of the consumers are suggested. A previous study reported that consumers in Ternate Island prefer large, ovoid, thin and white nuts (Sundari *et al*. 2021). Therefore, Kenari Kusum, Towas, Poda and Laka are recommended to be widely propagated. Further bioprospecting on nutritional and essential oils contents, post-harvest handling, and processing of local kenari are also suggested.

Conclusion

This study demonstrated the assessment of morphology and genetic diversity, and the conservation strategy of local kenari germplasms in the small and remote island of Makean, North Maluku which is prone to natural disasters and anthropogenic threats. Based on morphologies and rbcL sequences, Kenari Alus and Batu were identified as *C. indicum*, whereas Kenari Kusum, Towas, Poda and Laka were identified as *C. vulgare*; distinguished by their fruit and seed kernel. The suitable conservation strategies are on-farm to maintain species and varieties in agroecosystems, and *in situ* to secure the most valuable germplasms, as well as *ex situ* to avoid loss from natural disasters. In addition, for sustainable utilization; propagation, selection, and breeding are encouraged.

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Author Contributions

Sundari, L Hapsari and A Mas'ud Conduct fieldwork, labwork and data collection, Sundari, A Mas'ud; Data analysis and interpretation, Sundari, L Hapsari; Writing the original draft manuscript, Sundari, L Hapsari; Review and edit the final manuscript, S, LH; All authors (Sundari, L Hapsari and A Mas'ud) read and approved the final manuscript

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Data is available at the corresponding authors.

Ethics Approvals

No applicable to this study.

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