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Identification of *Kappaphycus alvarezii* seaweed based on phylogenetic and carrageenan content

Identifikasi rumput laut *Kappaphycus alvarezii* berdasarkan filogenetik dan kandungan karagenan

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ABSTRACT

Increasing seaweed production requires accurate information regarding the genetic sources of seeds used. Identifying the seaweed species *Kappaphycus* molecular is one of the solutions to ensure seaweed cultivators choose seeds for their cultivation businesses. Molecular identification is essential for the system traceability of seaweed products and the creation of databases regarding species variant information *Kappaphycus alvarezii* cultivation as potential data collection for developing and genetically breeding seaweed seeds. To date, there is no information on the genetic potential of *K. alvarezii* cultivated in various seaweed cultivation centers in Indonesia. This study aimed to obtain phylogenetic details based on identification of the genetic source using DNA molecular markers barcoding rbc*L* and analysis of carrageenan content using the Fourier transform infra-red (FTIR) spectrum. The results of DNA sequencing analysis and FTIR testing of 16 varieties of seaweed seedlings obtained from various cultivation centers in Indonesia showed 99% similarity with *K. alvarezii*, a producer of kappa carrageenan.

Keywords: DNA sequencing, haplotypes, kappa-carrageenan, phylogenetics, rbcL

ABSTRAK

Peningkatan produksi rumput laut memerlukan informasi yang akurat mengenai kepastian sumber genetik bibit yang digunakan. Identifikasi spesies rumput laut *Kappaphycus* secara molekuler merupakan salah satu solusi untuk memberikan kepastian pada pembudidaya rumput laut untuk memilih bibit bagi usaha budidaya. Identifikasi molekuler sangat penting dalam sistem *traceability* produk rumput laut dan pembuatan basis data mengenai informasi varian spesies *Kappaphycus alvarezii* budidaya sebagai pendataan potensi untuk pengembangan dan pemuliaan bibit rumput laut secara genetis. Sampai saat ini belum tersedia informasi mengenai potensi genetik rumput laut *K. alvarezii* yang dibudidayakan di berbagai sentra budidaya rumput laut di Indonesia. Penelitian ini bertujuan untuk memperoleh informasi filogenetik berdasarkan identifikasi sumber genetiknya menggunakan penanda molekuler DNA *barcoding* rbc*L* serta analisis kandungan karaginannya menggunakan spektrum Fourier Transform Infra-Red (FTIR). Dari hasil analisis sekuensing DNA dan pengujian FTIR terhadap 16 varietas bibit rumput laut yang diperoleh di berbagai sentra budidaya di Indonesia menghasilkan 99% kemiripan yang tinggi dengan *K. alvarezii* penghasil kappa karagenan.

Kata kunci: DNA sekuensing, filogenetik, haplotipe, kappa-karagenan, rbcL

INTRODUCTION

Seaweed *Kappaphycus alvarezii* are aquatic plants with a low degree of stenohaline with all parts of the plant called the thallus, which cannot be distinguished between the roots, stems and leaves (Kasim *et al*., 2022). The thallus functions to take nutrients in the waters without going through a complicated root system like higher plants (Susanto, 2020). *K. alvarezii* is the only aquaculture commodity that is very economically and ecologically useful (Handayani, 2020) as a producer of commercial bioactive polysaccharides in various industries and the leading provider of ecological services essential primary producer of the world's blue carbon ecosystem. The Siboga Sea Expedition Van Boose 1928 (Basmal, 2021) has identified 782 types of seaweed that live in Indonesian waters (Van Boose, 1928).

Currently, coastal communities use 23 types of seaweed for vegetables and food, and 56 are used as traditional medicine (Handayani, 2020). Long history of seaweed cultivation *K. alvarezii* was initially obtained from the waters of Kalimantan and then developed in various countries as a superior cultivation commodity (Riatiga *et al*., 2017). Cultivation of *K. alvarezii* commercially in Indonesia was first developed in Bali using selected seeds from Tambalang-Philippines. It was the first country to export seaweed *K. alvarezii*, then expanded to other countries, including Indonesia. The cultivation of *K. alvarezii* was only commercially carried out in Indonesia in 1985, far behind the Philippines, which started in 1971 (Parenrengi & Sulaeman, 2004).

In general, the morphology of *K. alvarezii*, the thallus is flat and cylindrical, the branches are elongated irregularly, and the ends of the components are pointed and blunt (Fadilah *et al*., 2016). The trade name for this *cottonii* seaweed species includes *Kappaphycus* and *Eucheuma* (Marquez *et al*., 2015). Different carrageenan types can cause production cost inefficiencies in carrageenan processing in factories that require separating the two types of carrageenan before the extraction process (Tan *et al*., 2017). Observational limitations *K. alvarezii* consequence morphologically plastic accompanied by similar phenotypes of the thallus requires certainty of species identification by utilizing molecular DNA technology (Roleda *et al*., 2021).

According to Madduppa *et al*. (2020) determining a population's genetic diversity level can be done through parameters measuring genetic variability between populations, for example, genetic distance through DNA sequencing. *Cottonii* species include *Kappaphycus* and *Eucheuma*, which produce different carrageenans, each in the form of kappa (κ) and iota (ι) (Porse & Rudolph, 2017). This causes production cost inefficiencies in Factories' carrageenan processing requires separating the two types of carrageenan before the extraction process (Tan *et al*., 2017). Information on the certainty of certain carrageenan-producing *cottonii* species is needed as a guide for the seaweed processing industry to determine its commercial value (Sudarwati *et al*., 2020). This study aims to obtain information on seaweed *K. alvarezii* based on identifying its genetic source using DNA molecular markers barcoding rbc*L* and analysis of carrageenan extract content in various samples of *K. alvarezii* obtained from multiple locations of seaweed cultivation centers *K. alvarezii* in Indonesia.

MATERIALS AND METHODS

Sample collection

This study used dried seaweed thallus samples *K. alvarezii* originating from 16 different locations divided based on the grouping of the four samples origin producing centres *K. alvarezii* in Indonesian territory (Table 1). The samples were rinsed with distilled water to remove debris, followed by crushing or grinding using mortar and pestle set with the addition of liquid nitrogen. Processed samples were, then transferred into Eppendorf tubes, and stored in a freezer at -4°C.

DNA extraction, PCR amplification, and genetic analysis

Molecular DNA analysis using rbc*L* primer pairs: F 5'-AACTCTGTAGTAGAACGNACAAG-3' and R 5'-GCTCTTTCATACATATCTTCC-3' (Tan *et al*., 2017). The rbc*L* gene has a low mutation rate providing an advantage for the study of intraspecies genetic and phylogenetic variation accompanied by high bi-directional sequencing success (Basith, 2015). The extraction method is the CTAB method (Doyle & Doyle, 1990) with a slight modification (adding a 3% PVP). The PCR program was set 94°C for four minutes, 35 cycles at 94°C, 51°C and 72°C with a time one minute each, and the final elongation 72°C for 10 minutes (Ji *et al*., 2012).

Amplification by PCR technique was carried out using two pairs of primers. All samples were amplified at a total reaction of 40 µL with PCR

components, and the program followed standard PCR protocols. PCR products are then sent to the 1st SDN Base Laboratory Bhd. Malaysia for sequencing. Sequence homology analysis was performed by comparing the collection sample sequences with the GenBank database using the BLAST-N program (Basic Local Alignment Search Tool for Nucleotide) (https://blast.ncbi.nlm.nih.gov). Genetic diversity was determined based on phylogeny analysis and haplotype diversity (Zhang *et al*., 2019).

Multiple sequence alignment and phylogenetic tree was constructed using the MEGA X program (Tamura *et al*., 2013). Input data (input file) used to build the phylogenetic tree are nucleotide sequences aligned using the ClustalW program in FASTA format. The method neighbor-joining (NJ) and replication bootstrap 1000 times constructs the phylogenetic tree (Huang, 2018). Specific area tracing was also carried out on the gene sequences according to the primer pairs used through the BioEdit software. The haplotype diversity in three populations of western, central, and eastern Indonesia of seaweed varieties (tissue culture vs local seed) analyzed using DnaSP v6.12.03 software (Rozas *et al*., 2017).

Character of carrageenan

Observation of seaweed carrageenan content was carried out by referring to conventional methods of alkaline precipitation using propanol (Mulyaningrum *et al*., 2009) with a composite sample. Seaweed samples were washed with fresh water to remove salt content and contamination with other impurities. The samples were soaked for two days and then heated in an autoclave at 120°C for 15 minutes using water as a solvent with the seaweed (g) ratio to water (mL). The second sample was cooked at 100°C for 30 minutes until the seaweed was perfectly soft.

The sample is then blended and extracted using hot water with a ratio of 1:30, and then the sample is filtered. The sample was thickened with propanol at a ratio of 1:2.5 to make the solution a gel. The gel formed was then dried at room temperature, which was then weighed to determine the weight of the carrageenan produced. Identification of the chemical composition of carrageenan with the Fourier Transform Infrared (FTIR) spectrum using the Shimadzu FTIR spectrometer was carried out at the Integrated Biofarmaka Laboratory of IPB.

Thallus histological

Observation of seaweed thallus morphology performed on all plantlet culture samples *K. alvarezii* originating from SEAMEO Biotrop Bogor where there is a limited number of thallus dry weight samples for testing carrageenan yield values, as well as several samples that do not meet the minimum number of carrageenan yield tests. The histological analysis used the hematoxylin and eosin (H&E) staining technique with working procedures including dehydration, clarification, embedding block paraffin, and sectioning (Carson & Cappellano, 2015).

RESULTS AND DISCUSSION

Result

Sample of seeds K. alvarezii

All samples in this study amounted to sixteen thallus *K. alvarezii* collected based on information from the location of carrageenan-producing seaweed cultivation with observed sample

Table 1. Information of *K. alvarezii* samples used in this study.

No.	Source of seed *K. alvarezii*	Sample Origin	Quantity
1.	Local Western Indonesia (Java)	Jepara	1
2.	Commercial Tissue Culture	Tambalang (Biotrop) Maumere LC (Biotrop) Maumere LH (Biotrop) Natuna (Biotrop) Kendari (Biotrop)	5
3.	Local Central Indonesia	Sebatik, Nunukan, Tarakan (Amal), Takalar, Bontang	5
4.	Local Eastern Indonesia	Ambon, Tual, Kupang, Sumba (NTT), Papua	5
		Total Sample	16

morphology (Table 2 and Figure 1). Observing the morphology of all samples *K. alvarezii* (Figure 1) is done by measuring the length and diameter of the talus using a digital *dial caliper* and weighing the talus weight using a digital scale. For weights varying from those measuring 0.08-1.43 grams, they are samples of tissue culture thallus, which are available with a wet size of less than 5 grams per sample so that carrageenan yield analysis cannot be continued, so for tissue culture talus, histological slices are observed.

Genetic

From the PCR results, a sample was selected for the DNA sequencing process, which previous UVis observations showed a single band with optimal bands (not too thick for fear that the bands would accumulate or too thin so that the detection of the bases was challenging to identify) to produce the correct sequence of sequenced DNA bases. Good band of sample amplicon DNA (firm, single, and not overlapping) that is selected samples that were PCR amplified with

Table 2. Morphology thallus of seaweed samples *K. alvarezii*.

No	Sample Name	Thallus Length (mm)	Thallus Diameter (mm)			Thallus Weight (g)
			Primary	Secondary	Tertiary	
1.	Sumba	102.8	3.0	2.3	1.2	7
2.	Natuna (Biotrop)	33.8	1.4	0	0	0.46
3.	Tambalang (Biotrop)	35.5	1	0	0	0.27
4.	Kendari (Biotrop)	17.9	0.2	0	0	0.08
5.	Maumere LC ((Biotrop)	40.9	3.1	2.5	0	1.43
6.	Sebatik	143.6	4.4	2.8	1.4	9
7.	Tarakan Coklat	77.2	4.6	3.4	1.9	6
8.	Nunukan Coklat	81.5	5.3	3.9	2.9	6
9.	Bontang	110.0	5.4	3.7	1.6	7
10.	Ambon	156.2	7.1	4.0	1.0	9
11.	Jepara	109.9	5.6	2.1	1.3	7
12.	Kupang	65.7	6.0	3.6	1.8	7
13.	Papua	91.6	5.7	2.3	1.9	7
14.	Takalar	125.8	5.8	2.3	1.4	7
15.	Maumere LH (Biotrop)	49.0	1.7	0.5	0	0.34
16.	Tual	196	6.4	3.3	2.3	10

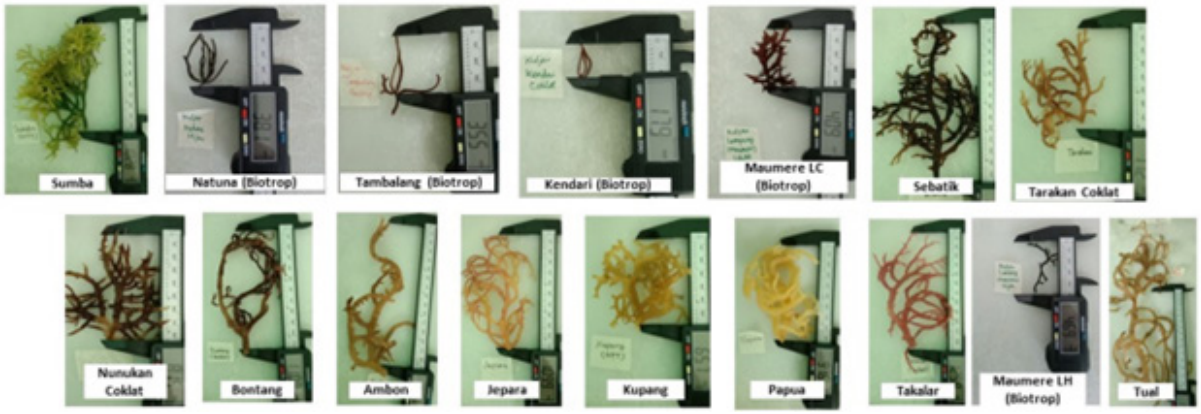

Figure 1. Sixteen of seaweed seeds collection.

rbc*L* primer pairs (Figure 2) and the visualization of the entire band of *K. alvarezii* DNA samples in this study was 800 bp. Subsequent analysis displays the results of the NCBI Blast (Table 3), dendrogram images, the percentage of nucleotides for each sequence, and the genetic distance from the results of the following Mega X software analysis.

The genetic distance of rbc*L* sites (Figure 3) ranged from 0.000-0.005. With the closest kinship *K. alvarezii* **from Ambon** *vs.* **Tual (0.000),** Takalar *vs.* **Tual (0.000), Papua** *vs.* **Tual (0.000),** Kupang *vs.* **Tual (0.000),** Jepara *vs.* **Tual (0.000), Tarakan** *vs.* **Tual (0.000), and farthest Sumba** *vs.* Tual (0.005). Seaweeds with the same or adjacent sequences (nucleotide base sequences)

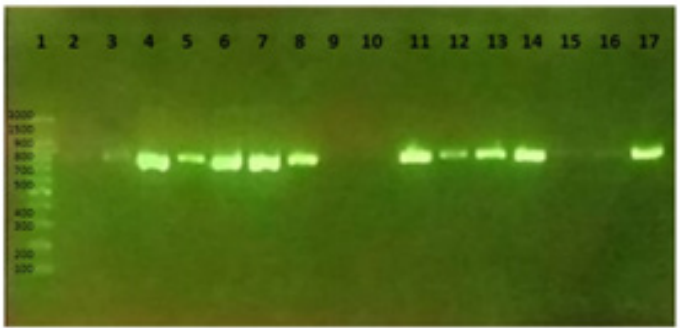

Figure 2. Amplicon of sample *K.alvarezii* analyzed by UVis-electrophoresis agarose with a 100-bp DNA ladder marker (column number 1), negative control ddH_2O (column number 2) and DNA samples (column number 3-17).

Table 3. NCBI nucleotide BLAST results and number of bases in DNA sequencing *K. alvarezii*.

Origin of Samples	Species	rbc*L* sequence			Percentage of rbc*L* (%)				Total Nucleotides
		Query Cover	Percent Ident.	Accession Number	T(U)	C	A	G	rbc*L*
Sumba	*K. alvarezii*	99%	99.46%	KU892652.1	32.20	15.55	30.15	22.10	733
Natuna (Biotrop)	*K. alvarezii*	99%	99.73%	KU892652.1	32.20	15.69	30.42	21.69	733
Tambalang (Biotrop)	*K. alvarezii*	99%	99.87%	KU892652.1	32.06	15.69	30.42	21.83	733
Kendari (Biotrop)	*K. alvarezii*	99%	99.87%	KU892652.1	32.20	15.69	30.29	21.83	733
Maumere LC (Biotrop)	*K. alvarezii*	99%	99.73%	KU892652.1	32.20	15.69	30.42	21.69	733
Sebatik	*K. alvarezii*	99%	99.73%	KU892652.1	32.06	15.69	30.29	21.96	733
Tarakan	*K. alvarezii*	100%	100.00%	KU892652.1	32.06	15.69	30.29	21.96	733
Nunukan	*K. alvarezii*	99%	99.73%	KU892652.1	32.06	15.69	30.29	21.96	733
Bontang	*K. alvarezii*	99%	99.73%	KU892652.1	32.06	15.69	30.15	22.10	733
Ambon	*K. alvarezii*	99%	100.00%	KU892652.1	32.06	15.69	30.29	21.96	733
Jepara	*K. alvarezii*	99%	99.87%	KU892652.1	32.06	15.69	30.29	21.96	733
Kupang	*K. alvarezii*	99%	100.00%	KU892652.1	32.06	15.69	30.29	21.96	733
Papua	*K. alvarezii*	99%	100.00%	KU892652.1	32.06	15.69	30.29	21.96	733
Takalar	*K. alvarezii*	99%	99.87%	KU892652.1	32.15	15.67	30.25	21.93	734
Maumere LH (Biotrop)	*K. alvarezii*	99%	99.87%	KU892652.1	32.20	15.69	30.29	21.83	733
Tual	*K. alvarezii*	99%	100.00%	KU892652.1	32.06	15.69	30.29	21.96	733

will form groups (clusters) in one large group. At the same time, seaweed with different lines will be separated into a separate group. Based on the construction of the rbc*L* phylogenetic tree, it is known that the first group is occupied by *K. alvarezii* from Biotrop var. Maumere LC, Biotrop var. Natuna, Biotrop var. Tambalang, Biotrop var. Maumere LH, Biotrop var. Kendari, Bontang, Nunukan and Sebatik.

Still, in the first group *K. alvarezii* **from Ambon, Tual,** Takalar, Papua, Kupang, Jepara, and Tarakan form separate branches of the phylogenetic tree but are still in the first significant group. The second large group is occupied only by *K. alvarezii* **from Sumba. The grouping of phylogenetic trees in one group for** Biotrop origins in the same branch (clustered) illustrates the closeness of their kinship. The more sequences that are the same, the higher the similarity value, the more the phylogenetic tree's components will be closer together (Figure 3).

The AMOVA (analysis of molecular variance) based on 16 sample collections (Table 4) were grouped into four different groups of seedling acquisition, namely tissue culture samples from SEAMEO Biotrop-Bogor (var.Natuna, var. Maumere LH, var.Maumere LC, var.Tambalang, and var.Kendari), seeds from Central Indonesia cultivation centres (Bontang, Nunukan, Sebatik, Tarakan, and Takalar) and seed samples from Eastern Indonesia cultivator networks (Sumba, Kupang, Ambon, Tual, and Papua). The F_{ST} = 0.32, meaning there is little structuring. From the results of the haplotype network analysis from sixteen samples from Indonesia (Figure 4B), a total of 7 haplotypes were found. Haplotype 1 is the most common because it is owned by seven other samples: Tual, Ambon, Takalar, Papua, Kupang, Jepara, and Tarakan. There are only three unique haplotypes (unique/private haplotype), meaning haplotype is only owned by one sample. The three unique haplotypes are haplotype 2, which

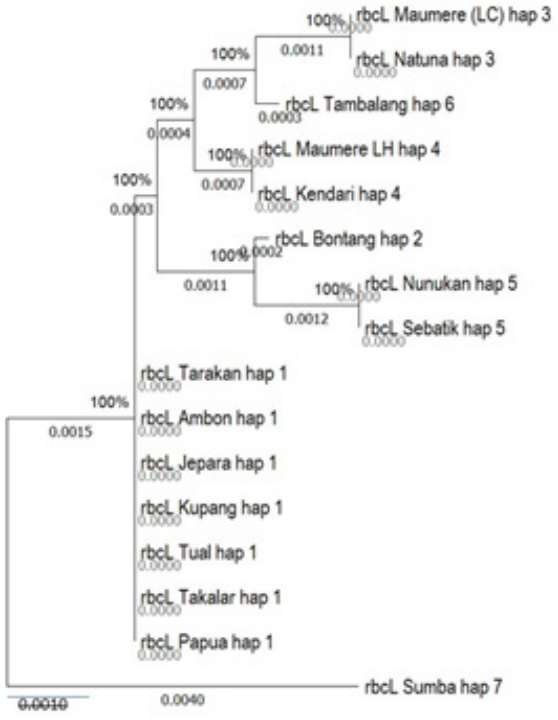

Figure 3. Reconstructed phylogenetic diagram of Mega X on DNA sample sequencing *K. alvarezii* **with** rbc*L* primers.

Table 4. AMOVA using DNA sequencing data *K. alvarezii* rbc*L* site.

Analysis of variation	d.f.	Percentage of variation (%)	F_{ST}	Significance
Among population	3	32.14	0.321	P<0.05
Within population	12	67.86		
Total		100.00		

Note: d.f. = degree of freedom.

is only held by Bontang, haplotype 6, owned by Tambalang; and haplotype 7, which belongs to Sumba. The circle size shows the number of samples that have haplotype (Figure 4A).

Carrageenan

Test results of FTIR analysis of the characterization of carrageenan aerogel microparticles using a frequency range of 250-4750 cm and a resolution of 0.04/cm produced a characteristic peak of carrageenan (kappa) present in all the research samples tested. Carrageenan yields from samples of 11 different locations (Table 5) for functional groups using FTIR showed the presence of sulfate esters, glycosidic bonds, 3.6 anhydrogalactose groups and galactose-4-sulfate groups in the carrageenan yields tested. No galactose-2-sulfate groups were found at all (wavelength 825-830 cm), which is an additional feature iota and lambda, carrageenan, and galactose-6-sulfate groups (wavelength 810-820 cm) which is an additional feature lambda carrageenan (Diharmi, 2016). Thus, it was concluded that all carrageenan yields produced by extraction techniques in this study were *kappa* types. The carrageenan yield value

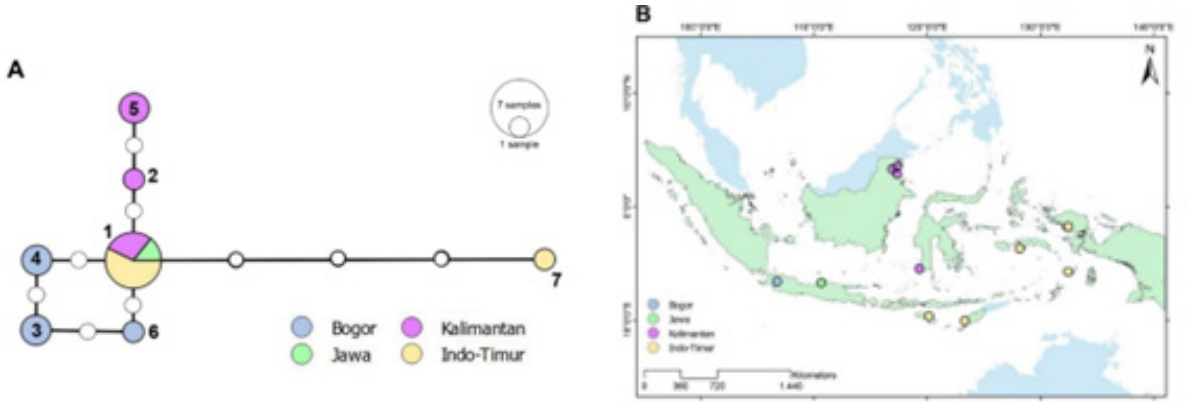

Figure 4. **Haplotype networking (A) and map of the location of the sample origins** *K. alvarezii* **(B).**
Note: (A). Haplotype network using method *Minimum Spanning Network* (Bandelt *et al*., 1999) on PopART software. Haplotype 1 (Tual, Ambon, Takalar, Papua, Kupang, Jepara, Tarakan); haplotype 2 (Bontang); haplotype 3 (Biotrop var. Maumere LC, Biotrop var. Natuna); haplotype 4 (Biotrop var. Maumere LH, Biotrop var. Kendari); haplotype 5 (Nunukan, Sebatik); haplostype 6 (Biotrop var. Tambalang); haplotype 7 (Sumba), white circle shows the number of mutations. (B). Map of the location of the origin of the sample based on four groupings, namely Biotrop-Bogor, Java (Jepara), Central Indonesia (Kalimantan and Takalar), and Eastern Indonesia (Sumba, Kupang, Ambon, Tual, and Papua). Map processed from ArcMap 10.3.

Table 5. Analysis of seaweed carrageenan yield *K. alvarezii*.

Origin of samples	Average yield of carrageenan (%)	Average water content of carrageenan (%)	Wavelength (cm)			
			Galactose 4 sulfate	3.6 anhydrogalactose	Glycosidic bond	Ester sulfate
Nunukan	50.30	13.37	885.28	-	1059.93	1385.84
Sebatik	50.86	11.99	846.29	924.88	1066.14	1259.55
Takalar	27.00	7.91	846.45	926.20	-	1259.13
Tarakan	45.20	12.31	845.43	-	1064.27	-
Kupang	35.00	6.09	846.84	925.19	1067.67	1259.22
Sumba	48.31	16.02	884.59	-	1061.41	-
Bontang	23.00	6.35	846.48	-	-	1258.96
Ambon	28.00	12.32	848.21	926.34	1068.43	1255.65
Papua	38.63	12.12	846.64	914.71	1067.00	-
Jepara	49.80	11.20	*	*	*	*
Tual	29.00	11.37	*	*	*	*

Note: (-) does not have this type of wave crest; (*) not FTIR tested.

required by the industry is ≥20%, where the carrageenan yield percentage value in this study (23%-50.86%) exceeds the industry requirements and is generally higher than the carrageenan yield percentage value reported by Simatupang *et al*. (2021).

Histological

Some of the samples that were owned were still small plantlets from tissue culture from the SEAMEO-Biotrop culture laboratory, which could not be analyzed for carrageenan yield because the number was so small that histology was performed in the form of a cross-section of the following thallus (Figure 5).

Discussion

The morphology of sixteen samples of *K. alvarezii* (Table 1) was observed, and the histological of planlets K. *alvarezii* analyzed comparison in the form of cultivated thallus samples from local seed *K. alvarezii* var Sumba. In measuring the weight of samples originating from local seed seaweed cultivation centers, they have a wet weight ranging from 7-10 grams (Table 2) to meet the minimum requirements for the number of extraction samples (>5 grams) to continue the analysis of carrageenan yield. Nucleotide BLAST analysis (ncbi.gov) of the samples sequenced (Table 3) in this study also yielded identity percentages, namely sequence similarity with the database and cover queries in the form of percentage sequence alignment results that matched the per-species data in the database. All lines obtained are similar to *K. alvarezii* (99-100%). The phylogenetic tree shows that the results of the BLAST analysis match the characteristics of the branches formed by the phylogenetic tree (Figure 3).

According to Madduppa *et al*. (2020) phylogenetic trees can provide information on population classification based on their evolutionary relationships. The line on the haplotype indicates sample proximity. Here it meant the entire sample of the thallus *K. alvarezii* of tissue cultures originating from SEAMEO Biotrop Bogor tend to be closer to one another and are more clumped or not blended with samples from Java (Jepara), Central Indonesia (Kalimantan and Takalar), and Eastern Indonesia. The white circle is the number of mutations (Figure 4).

Haplotype, the one from Sumba, is so far apart from the others because, based on the sequence, between the Sumba sample sequences and the others, there are three different mutations (due to substitution). From the analysis results of haplotype, there is little influence on the locality of the sample locality by analysis haplotype because some samples are grouped according to location, for example, Biotrop-Bogor and Kalimantan, although some others are also mixed. AMOVA results in higher genetic variation within the population than between populations, indicating that it is genetically more heterogeneous. Heterogeneity in the AMOVA population of rbc*L* sites because each sample has a different haplotype in the population.

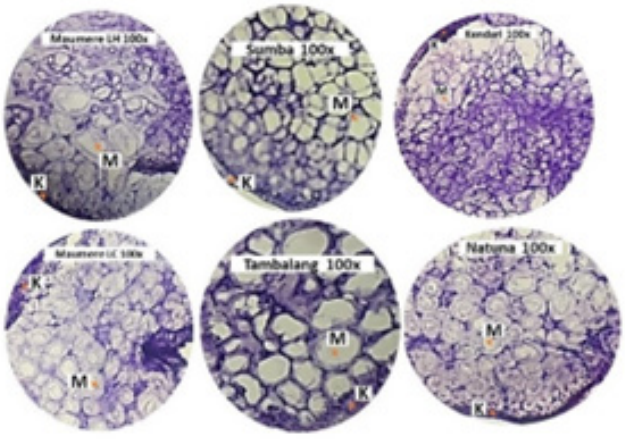

Figure 5. Cross section of seaweed thallus from SEAMEO-Biotrop plantlets and cultivated seaweed thallus at 100× magnification, M=medulla with round parenchymatous cells, K=cortex. From top left to bottom right, samples from Maumere LH (Biotrop), Sumba, Kendari (Biotrop), Maumere LC (Biotrop), Tambalang (Biotrop), and Natuna (Biotrop).

The F_{ST} is a value that indicates whether a population is genetically isolated or not in terms of gene flow (*gene flow*). It can also be used to determine whether there is population structuring/ subdivision. The *F*ST is 0–1, the closer it is to 1, the more isolated the populations are from one another or in the sense that there is no gene flow rbc*L* site; these results are supported by scores *F*ST, which is low on *cox* 2-3 spacer 0.014 (Satriani *et al*., 2023) shows no geographic population structuring or subdivision but differs on rbc*L*, shows F_{ST} = 0.32, meaning there was little structuring. However, it is not classified as strong because it is still below 0.50. The difference in rbc*L* is likely because this gene is included a coding *gene,* which is generally more varied, so it is often used as barcoding (Leliaert *et al*., 2014).

In general, based on Madduppa *et al*. (2021) haplotype network is used to study genealogy (genealogical lineage) in an organism based on data haplotype (DNA sequences that represent a group of the same sequence). In contrast to the phylogenetic tree, preferring single *nucleotide polymorphism* (SNP) in determining tree shape and due to genetic distance, haplotype does not have an outgroup (tree root). It only involves the organism under study (*ingroup*). The phylogenetic tree shows that the results of the BLAST analysis match the characteristics of the branches formed by the phylogenetic tree. Following sixteen DNA sample collections using rbc*L* primer pairs acquires a high resemblance to *K. alvarezii* (99%), producing the primary metabolites in kappa carrageenan.

The FTIR profile also confirms the central peak indicating the functional group kappa carrageenan in the seaweed samples tested in this study. Thus, the seaweed from Tarakan, from Biotrop *K. alvarezii* var. Natuna (which are reared at the BBPBL Lampung nursery) and seaweed *K. alvarezii* from Kupang, each representing a group (cluster), has the potential to be selected and developed as a nursery candidate for seaweed cultivation. The growth and development of a plant are influenced by the condition of the arrangement of plant cells because it is related to the diffusion process of nutrients, water and minerals needed to expand plant growth (Satriani *et al*., 2022). In figure 5, a cross-section of the seaweed thallus from the SEAMEO-Biotrop plantlet and cultivated seaweed thallus (Sumba) at 100 times magnification shows the arrangement of medulla cells (M) in the form of round parenchymatous cells and cortical cell walls (K).

In medullary cells, *K. alvarezii* has an irregular arrangement, oval to spherical, and there is space between cells, while the cortical cells on the edges look tighter and more regular. Compared with other Rhodophyta, *Gracilaria verrucosa*, according to Othman *et al.* (2018), has a cell arrangement of 2-3 layers of cortex accompanied by transitions of the medulla and cortex in a random sequence. According to Charrier *et al.* (2015), seaweed medulla cells *G.gigas* consists of 5-8 layers of unpigmented, spherical cells with vacuoles that can increase in diameter to 600 µm, and the cortex consists of rounded cells with dense cytoplasm. *K. alvarezii* is stenohaline, which means it has a narrow tolerance for salinity (30-34 ppt) in contrast to *Gracillaria* sp., which is euryhaline (29-30 ppt).

Differences in the density of medulla and cortex cells in macroalgae affect physiology and biochemistry, especially osmotic pressure, which is closely related to the role of cell membranes in transporting nutrients and stimulating seaweed growth (Fadilah *et al*., 2016). In this study, infrared spectroscopy (FTIR) was carried out to determine the type and structure of carrageenan, indicated by the absorption number 1010-1080 cm. The results of the FTIR analysis showed that the identified groups were total sulfate esters at 1210-1260 cm, galactose 4 sulfate 840-850 cm, galactose sulfate 825-839 cm, 3.6-anhydrogalactose 927-928 cm, and 3.6-anhydrogalactose 2 sulfate 800-805 cm (Table 5). The results of the research by Fauzi *et al*. (2020), who also used FTIR to determine gelation conditions in carrageenan shells, showed that the concentration of critical gelation could be indicated by spectrum analysis or the prominent peaks indicated functional groups of carrageenan shells, and carbohydrate absorbance at 600-1270 cm called fingerprint.

All FTIR waves of the samples tested produced galactose 4 sulfate peaks with additional variations each in the form of absorption peaks of sulfate ester waves, glycosidic bonds, 3.6 anhydrogalactose groups which are characteristic kappa carrageenan. The average percentage of carrageenan yield of seaweed samples in this study ranged from 23.00 to 50.86% (Table 5). Pacheco-Quito *et al*. (2020) stated that carrageenan is the main constituent of red algal cell walls representing 30-75% of its dry weight. The cell walls of red algae consist of pectic and cellulose-containing hydrocolloids or polysulphate esters in the form of agar or carrageenan (Knudsen, 2015).

CONCLUSION

Sequencing DNA of sixteen seed sample collections different seaweed varieties in this study have confirmed their high resemblance with *K. alvarezii* (99%), which produces the primary metabolites of kappa carrageenan. Results haplotype network using the rbc*L* marker can make seven haplotypes unique as a cultivar stock candidate for developing a nursery business in seaweed cultivation *K. alvarezii*.

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