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Planlet Study of *Kappaphycus Alvarezii* Maumere Variety with Different Colours from Tissue Culture Propagation at Seameo Biotrop Bogor

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Abstract
Tissue culture propagation of *Kappaphycus alvarezii* Maumere variety at SEAMEO BIOTROP Bogor produced planlets exhibiting an unusual colour shift from the original brown (LC) to green (LH) at a colour phenotype ratio of 1:50 within culture bottles. This study aimed to characterise the molecular identity and genetic relationships of these colour-variant planlets using the Internal Transcribed Spacer (ITS) DNA barcoding marker and phylogenetic reconstruction in MEGA X. DNA sequencing using ITS primers revealed that both colour variants (LH and LC) confirmed as *K. alvarezii* based on NCBI BLAST analysis (Query Cover: 92%; Percent Identity: 87.52%; Accession No. JN673973.1). The nucleotide compositions showed T(U): 23.84%, C: 22.48%, A: 25.48%, G: 28.20% (LH) and T(U): 25.28%, C: 26.40%, A: 25.42%, G: 22.89% (LC), with total nucleotide lengths of 734 and 712 bp, respectively. No single nucleotide polymorphism (SNP) was associated with the colour characteristic detected between the two variants. Genetic distance analysis indicated a close relationship between LH and LC (distance: 0.155), while both showed considerable divergence from the reference *K. alvarezii* KC905270.1 (0.626 and 0.629, respectively) and from *Eucheuma isiforme* (0.565 and 0.563). The phylogenetic tree confirmed that LH and LC belong to the same species with a distinct colour phenotype potentially attributable to epigenetic or physiological factors. These findings provide molecular baseline data for the utilisation of tissue-culture-derived *K. alvarezii* Maumere variety in seaweed aquaculture programs in Indonesia.

Keywords: Barcoding; colour phenotype; planlet; seaweed; tissue culture

INTRODUCTION

Kappaphycus alvarezii (Doty) Doty ex P.C. Silva is one of the most economically important red macroalgae in global aquaculture because it is the main raw material for κ-carrageenan production used in the food, pharmaceutical, and cosmetic industries (Satriani et al., 2023). In Indonesia, *K. alvarezii* is a key fisheries commodity, with major cultivation centres in Kalimantan Utara, Sulawesi, Nusa Tenggara, and Maluku (Satriani et al., 2024). The Maumere variety from East Nusa Tenggara has potential for its rapid growth and high carrageenan yield. To meet the increasing demand for high-quality seedlings, tissue culture, or micropropagation has been developed as a promising technique for producing disease-free, genetically uniform seaweed seed stock.

SEAMEO BIOTROP (Southeast Asian Regional Centre for Tropical Biology) Bogor has pioneered tissue culture protocols for *K. alvarezii*, including explant sterilisation, callus induction on Provasoli's Enriched Seawater (PES) medium supplemented with plant growth regulators, somatic embryogenesis, and plantlet development (Sulistiani, 2021; Irawati & Affandi, 2024). During routine maintenance, an intriguing phenotypic change among planlets derived from brown Maumere thalli: approximately 1 in 50 planlets spontaneously changed colour from brown (LC) to green (LH) under culture conditions. Colour variation in *K. alvarezii* is generally

associated with the relative abundance of pigments, including phycoerythrin, chlorophyll, and carotenoids (Thien *et al*., 2021). Still, under in vitro conditions, colour alteration may also result from somaclonal variation, epigenetic modification, or physiological stress rather than stable DNA mutations.

DNA barcoding using the nuclear ribosomal Internal Transcribed Spacer (ITS) region is a widely used approach for species identification and genetic characterisation of macroalgae because it has suitable variability for intra- and interspecific analysis (Bartolo *et al*., 2022). Phylogenetic reconstruction based on ITS sequences can further elucidate the genetic relationships among colour variants and reference taxa, helping to determine whether observed phenotypic differences correspond to distinct genetic lineages. Based on Nurkolis *et* al (2025), the use of RAPD on *K. alvarezii* in Indonesia showed that genetic differences between samples with different morphological colours (green vs brown) were relatively small compared to variations between cultivation sites. This finding indicates that colour differences most likely originate from phenotypic/epigenetic phenomena or environmental influences, rather than major genetic differences, although molecular markers may still indicate general genetic closeness. However, most previous studies on *K. alvarezii* tissue culture have focused on growth performance, pigment content, or seedstock production, without molecular characterisation of spontaneous colour variants that arise in vitro. Therefore, it is still unknown if these colour morphotypes obtained from tissue culture are actually genetic mutations or just physiological or epigenetic reactions.

This study therefore aimed to: (1) confirm the molecular identity of brown (LC) and green (LH) colour-variant planlets of *K. alvarezii* Maumere variety using ITS DNA barcoding; (2) analyse their nucleotide composition and detect any single nucleotide polymorphisms (SNPs) associated with the colour phenotype; (3) estimate genetic distances among LH, LC, and reference sequences; and (4) reconstruct a phylogenetic tree to clarify their evolutionary relationships. The results aim to contribute to the conservation and utilisation of Indonesian *K. alvarezii* germplasm, particularly tissue culture for aquaculture.

METHODS

Time and Locations

This study was conducted over 6 months (January-June 2025). Thallus propagation culture *K.alvarezii* Maumere LC and LH produced by SEAMEO Biotrop Bogor. Research Identification of DNA *K. alvarezii* was carried out at the Bioper laboratory of the Aquaculture FPIK UBT and LKIL PCR+1 DKP Kota Tarakan. The 40µL DNA amplicon was sent to GSI (PT. Genetika Science Indonesia) for Sanger sequencing by the 1st Base Laboratory SDN Bhd. Malaysia. A sequence homology analysis was performed by comparing *Eucheumatoid* nucleotides using the BLAST-N (Basic Local Alignment Search Tool for Nucleotide) program in the GenBank database.

Plant Material and Tissue Culture Conditions

The biological material used in this study consisted of planlets of *K. alvarezii* Maumere variety produced through tissue culture at SEAMEO BIOTROP, Bogor, West Java, Indonesia. Broodstock thalli were acclimated for 6 weeks in the laboratory with circulating seawater at 30 ppt salinity and pH 7.5–8.5 before explant preparation. Explants of 0.5–1.0 cm in length were excised from healthy thalli, surface-sterilised in 1% povidone-iodine solution, and rinsed three times with sterile seawater.

Callus induction was carried out on solid PES medium supplemented with 1.25 mL L^{-1} indole acetic acid (IAA), 0.5 mL L^{-1} benzyl amino purine (BAP), and 4.5 g L^{-1} Bacto-agar (Sulistiani & Yani 2019). Seaweed *K. alvarezii* in culture bottles were maintained at 22–23 °C with a light intensity of approximately 1,500 lux and a 12:12 h light–dark photoperiod, and subcultured every 7 days. Planlets classified into two colour morphotypes: brown (LC) and green (LH), with the green morphotype appearing spontaneously at a ratio of around 1:50 within culture bottles.

DNA Extraction and ITS Amplification

Pproximately 50 mg of fresh thallus from one planlet, a brown morphotype (LC), and one planlet green (LH) were used for DNA extraction using a modified cetyltrimethylammonium bromide (CTAB) protocol commonly applied in seaweed tissue culture studies. DNA quality and concentration were determined using NanoDrop spectrophotometry and 1% agarose gel electrophoresis (Satriani *et al*. 2024). The DNA regions of nuclear Internal transcribed spacer ribosomal DNA (ITS) Forward: 5'-TCGTAACAAGGTTTCCGTAGG-3' and Reverse: 5'-TTCCTTCCGCTTATTGATATGC-3' with band DNA size 650-1100 base pairs (Yong *et al*. 2016). Amplified using 25 µL PCR reactions containing 12.5 µL 2× PCR Master Mix, 1 µL of each primer (10 µM), 2 µL template DNA, and 8.5 µL nuclease-free water.

PCR conditions consisted of an initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 7 min. PCR amplicons were analysed by electrophoresis on 1% agarose gels made with 1× TAE Buffer, and dyed with DNA loading dye was used to verify the purity of the extracted DNA before Sanger sequencing.

Sequence Analysis and BLAST Identification

Forward and reverse ITS sequences were edited and assembled using BioEdit v7.2.5 to obtain consensus sequences for each morphotype. BLAST identifies species by searching the NCBI GenBank database. Nucleotide composition (percentages of T(U), C, A, and G) and total sequence length between two colour variants using BioEdit. The ITS primer of *Kappaphycus alvarezii* chloroplast genome, which is accessible in the National Centre for Biotechnology Information (NCBI) database, is used to guarantee specificity and accuracy in targeting the desired regions.

Multiple sequence alignment of LH and LC ITS sequences was used with ClustalW to detect possible SNPs associated with the colour difference. Additional reference sequences of *K. alvarezii* (for example, JN673973.1 (NCBI GenBank 2011), KC905270.1 (NCBI GenBank 2013), *Eucheuma isiforme* MH255768 (NCBI GenBank 2019), were downloaded from GenBank and included in the alignment for subsequent phylogenetic analysis.

Genetic Distance and Phylogenetic Reconstruction

Genetic distances among LH, LC, and reference sequences by the Kimura 2-parameter (K2P) model in MEGA X software (Kumar *et al.* 2018). The Kimura 2-parameter (K2P) model, which is widely recommended for DNA barcoding datasets, accounts for unequal transition and transversion rates in relatively short sequences. Phylogenetic reconstruction performed using the Neighbour-Joining (NJ) method with 1,000 bootstrap replicates in MEGA X, as NJ is a standard, computationally efficient approach for exploring relationships among closely related haplotypes in barcoding studies. The NJ phylogenetic tree replicates to assess clade robustness. *Eucheuma isiforme* was used as an outgroup to root the tree (Roleda *et al.* 2021). The examination to determine the placement of LH and LC within *K. alvarezii* lineages and to evaluate whether the Maumere tissue culture planlets form a distinct genetic group.

RESULT AND DISCUSSION

Colour Variant Planlets from Tissue Culture

Tissue culture propagation of *K. alvarezii* Maumere variety at SEAMEO BIOTROP Bogor successfully produced planlets from brown thallus explants. During culture maintenance, a spontaneous colour phenotype from brown (LC) to green (LH) was observed at a ratio of 1:50 within culture bottles (Fig. 1). According to Guillén et al (2022), *K. alvarezii*, chemical regulators IAA and BAP, have been shown to enhance callus formation and shorten induction durations, especially in liquid or enriched-media regimes. This mechanistic insight clarifies how PES (Provasoli's enriched seawater) based or liquid media with growth regulators to induce callus and somatic embryos, followed by regeneration of plantlets in *K. alvarezii* and related taxa, expedite morphogenesis toward thallus-like propagules and enhance embryogenic potential (Tirtawijaya *et al.*, 2022).

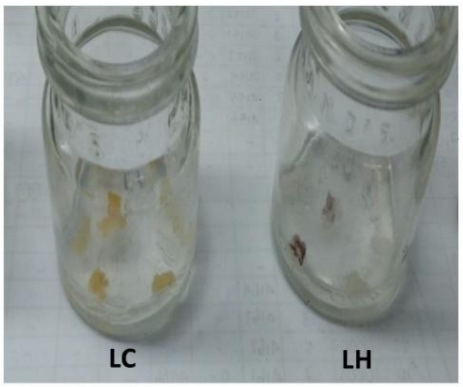

Figure. 1. Brown (LC) and green (LH), with the green morphotype appearing spontaneously at a ratio of around 1:50 within culture bottles.

The green variant planlets (LH) displayed a lighter, distinctly greenish thallus colouration compared to the dark-brown LC planlets, suggesting a possible reduction in phycoerythrin content or a relative increase in chlorophyll expression under in vitro conditions (Fig. 2).

Figure 2. Color-variant thallus planlets of *K. alvarezii* Maumere variety from tissue culture at SEAMEO BIOTROP Bogor: brown morphotype (LC, left) and spontaneous green phenotype (LH, right).

In the Biotrop var. *K. alvarezii* Maumere tissue culture sample after 40 days in PES media, which had initially been a brown thallus (LC), spontaneously changed to green (LH) during the micropropagule process from embryonic callus (Fig.1) at a ratio of 1:50; that is, one green individual collet from a culture bottle containing 50 micropropagules. The green phenotype results were then gathered into a different bottle and kept there until they developed into a plantlet (Figure 2).

ITS Sequencing and NCBI BLAST Identification

PCR testing using ITS primers analysed by UVis-agarose electrophoresis with 100bp DNA ladder markers, the visualisation of all *K. alvarezii* DNA sample bands in this study was 700 bp in size (Figure 3). The findings are consistent with the work by Khan et al. (2025), which found that all DNA samples could be amplified using the ITS primer. ITS DNA barcoding sequencing successfully amplified target regions from both LH and LC samples of *K. alvarezii* Maumere variety Biotrop planlets (Figure 3 and 4).

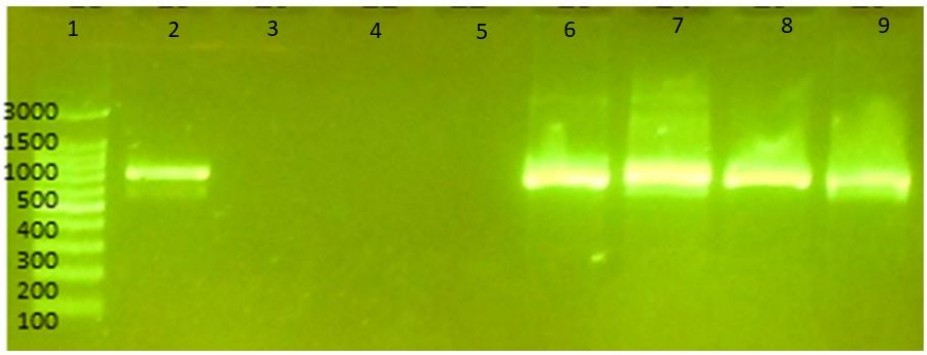

Figure 3. Agarose electrophoresis visualization (1.5% w/v) PCR primer ITS, namely well 1=Marker (100bp DNA Lader Geneaid), 2= positive control, 3= empty, 4&5= negative control (ddH2O), 6&7=

amplicon LC, and 8&9 = amplicon LH

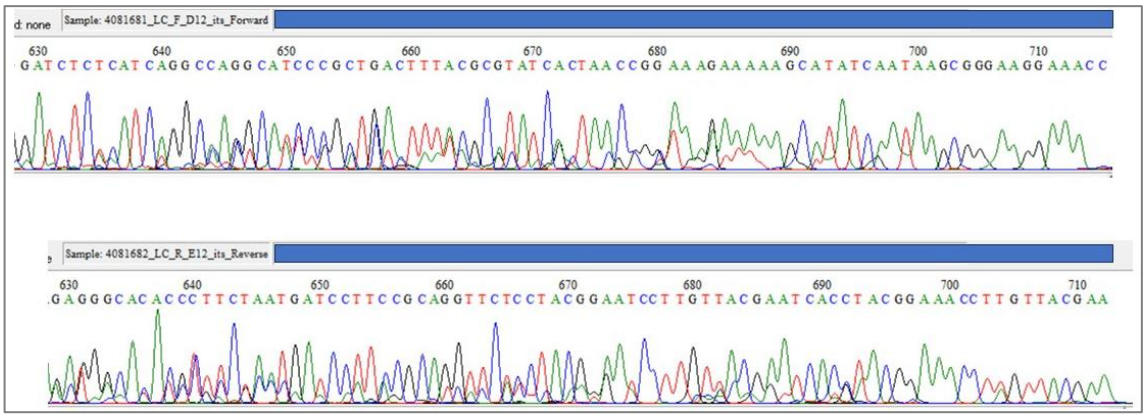

Figure 4. Sequencing chromatogram of LC DNA *K. alvarezii* Maumere amplified by ITS (up: forward, and bottom: reverse) primers

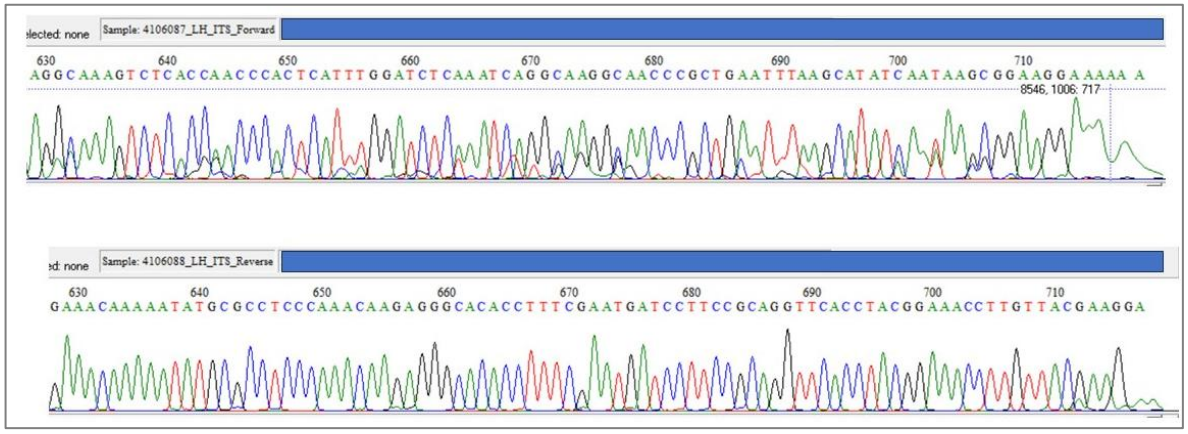

Figure 5. Sequencing chromatogram of LH DNA *K. alvarezii* Maumere amplified by ITS (up: forward, and bottom: reverse) primers

The literature documents broad eucheumatoid studies, providing a rich comparator framework for interpreting ITS based genotypes in relation to known cultivar lineages (Zuccarello & Paul, 2019). Genotype to phenotype association: stratify germplasm for MAS using ITS genotypes, then examine correlations with desirable characteristics (such as carrageenan yield, quality, and growth rate) in multi-site experiments. When single-locus data are unclear or impacted by intragenomic variations, the multi-locus strategy lowers the risk of misclassification and increases confidence in lineage assignment (Tan *et al.* 2021).

NCBI BLAST analysis confirmed that both colour morphotypes belong to the species *Kappaphycus alvarezii* (Table 1). The LH variant yielded a sequence of 734 bp in total length, while the LC variant produced a 712 bp sequence. Both variants showed identical query coverage (92%) and per cent identity (87.52%) when matched to the reference accession JN673973.1 in GenBank. The difference in ITS length between LH (734 bp) and LC (712 bp) reflects small insertions or deletions in parts of the ITS sequence that do not code for proteins and are known to vary within the same species, rather than major genetic changes.

Table 1. Nucleotide percentage composition (a) and NCBI BLAST identification (b) of *K. alvarezii* Maumere variety planlet DNA samples using ITS primers (ITS primer; LH = green morphotype; LC = brown morphotype)

(a) Nucleotide composition

Sample	T(U) %	C %	A %	G %	Total (bp)
Biotrop var. Maumere (LH)	23.84	22.48	25.48	28.2	734
Biotrop var. Maumere (LC)	25.28	26.4	25.42	22.89	712
Average	24.55	24.41	25.45	25.59	723

(b) NCBI BLAST identification

Sample	Species	Query Cover	% Ident.	Accession No.
Biotrop var. Maumere (LH)	*K. alvarezii*	92%	87.52%	JN673973.1
Biotrop var. Maumere (LC)	*K. alvarezii*	92%	87.52%	JN673973.1

Nucleotide Composition and SNP Analysis

The nucleotide composition of the LH variant showed a relatively higher proportion of G (28.20%) and A (25.48%) compared to the LC variant, which exhibited higher T(U) (25.28%) and C (26.40%) content (Table 1). The average nucleotide composition across both samples was: T(U) 24.55%, C 24.41%, A 25.45%, and G 25.59%. Despite observable differences in nucleotide proportions between LH and LC, multiple sequence alignment analysis revealed no distinct SNPs specifically associated with the colour phenotype. Our result suggests that the colour shift from brown to green is unlikely to be caused by single-point mutations in the ITS region under the conditions tested.

Genetic Distance and Phylogenetic Analysis

The Kimura 2-parameter (K2P) genetic distance matrix revealed the closest relationship between the LH (green) and LC (brown) colour variants of Biotrop Maumere, with a distance of 0.155 (Table 2). Both variants showed considerable divergence from the reference *K. alvarezii* KC905270.1 (LH: 0.626; LC: 0.629). The outgroup *Eucheuma isiforme* showed the greatest divergence from all *K. alvarezii* samples (distances: 0.504–0.565).

Table 2. Genetic distance matrix (Kimura 2-parameter) of *K. alvarezii* Maumere variety planlets (LH and LC) and reference sequences based on the ITS marker

Primer ITS	E. isiforme	K. alvarezii LH	K. alvarezii LC	K. alvarezii KC905270.1
E. isiforme	-	-	-	-
K. alvarezii LH	0.565	-	-	-
K. alvarezii LC	0.563	0.155	-	-
K. alvarezii KC905270.1	0.504	0.626	0.629	-

The Neighbour-Joining phylogenetic tree (Fig. 6) reconstructed from ITS sequences showed that the LH and LC samples formed a distinct clade, separate from the reference *K. alvarezii* KC905270.1. This topology indicates that the Biotrop Maumere variety represents a genetically discrete lineage within *K. alvarezii*, consistent with the geographic isolation and selection history of this Indonesian variety. The clustering of LH and LC within the same clade, with *E. isiforme* at the base, supports their conspecific status.

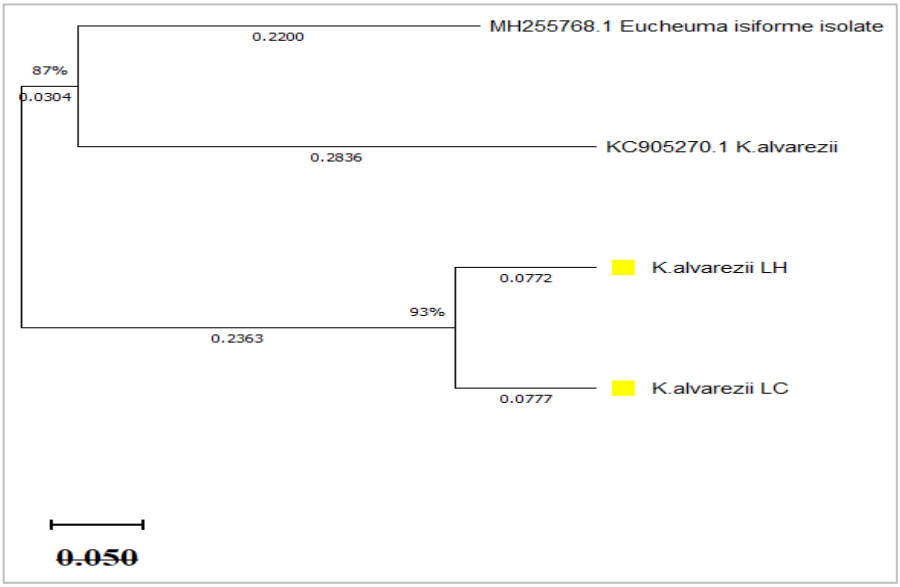

Figure. 6. *Neighbour-Joining phylogenetic tree of K. alvarezii Maumere variety planlets (LH and LC) reconstructed with MEGA X based on ITS sequences.*

DISCUSSION

A future aquaculture sector built on a variety of high-productivity, stress-tolerant strains will be supported by macroalgal germplasm banking and domestication programs. Culture propagation of *K. alvarezi*i will guarantee a steady supply of biomass, disease resistance, and climate change adaptation, supporting sustainable feeds and ecosystem services in marine farming systems (Wade *et al*. 2020).

The efficiency of ITS as a molecular identifier for species identification in this commercially significant red alga was the successful amplification and sequencing of the ITS region from *K. alvarezii* Maumere variety planlets. DNA samples from Biotrop var. Maumere (LC) and Biotrop var. Maumere (LH) had total nucleotide counts of 712 and 734, respectively. On the ITS site, the sequences were highly similar to *K. alvarezii* JN673973.1 (92%). Two cluster groups found by phylogenetic tree reconstruction (Figure 6): cluster 1, which contains *K. alvarezii* (accession code: KC905270.1) and *E. isiforme* (accession code: MH255768), and cluster 2, which contains *K. alvarezii* from Biotrop var. Maumere LH and LC with bootstrap percentages>90%. A percentage of identity, or sequence similarity with the database, and query cover, or the percentage of sequence alignment results that match the data per species in the database, were also obtained using the nucleotide BLAST analysis (ncbi.gov) of the samples sequenced in this investigation. In line with earlier studies showing genetic heterogeneity among *K. alvarezii* populations, the comparatively moderate identity value (87.52%) may represent natural intraspecific variation within this species across various geographic origins and cultivation histories (Cabrera et al., 2019; Valderrama et al., 2021).

One significant discovery is the lack of SNPs in the ITS region that correspond with the colour difference between LH and LC variants. It implies that alterations in this specific chromosomal region are not the cause of the green colouration in LH planlets. The relative amount of phycoerythrin, which gives red algae like *K. alvarezii* its reddish-brown color, phycocyanin, and chlorophyll a are the main factors that govern colour (Thien et al., 2021). The transcriptional regulation of genes encoding phycobiliprotein subunits may be changed under artificial light conditions in tissue culture, especially when light wavelength composition and intensity differ from natural sunlight. This could result in decreased phycoerythrin synthesis and a relative predominance of chlorophyll, giving rise to the green phenotype. Transcriptomic findings showing that light wavelength strongly controls the expression of pigment production genes in *K. alvarezii* provide credence to this theory (Thien et al., 2021).

Even though the LH and LC variants are the closest pair in the matrix, their genetic distance of 0.155 is non-negligible and could point to minor genomic divergence brought on by somaclonal variation during in vitro development. In plant tissue culture, somaclonal variation is a well-known phenomenon that can result from de novo mutations brought on by the culture microenvironment or from pre-existing genetic or epigenetic heterogeneity in donor tissues (Duta-Cornescu et al., 2023). To fully define the genetic basis of the observed colour variation, future research using more variable markers such as microsatellites, simple sequence repeats (SSRs), or whole-genome SNP profiling would be required.

The Maumere variety is genetically different from the reference sequence submitted in GenBank, as evidenced by the significant genetic difference between the Biotrop Maumere variants (both LH and LC) and the reference accession KC905270.1 (0.626–0.629). This discovery emphasizes how crucial it is to preserve and describe locally adapted Indonesian *K. alvarezii* cultivars as distinct genetic resources. Trait-specific genetic characteristics, such as adaptability to local hydrodynamic, temperature, and salinity conditions, may be present in the Maumere variety but are not reflected in reference sequences that have been deposited yet.

Theoretically, the findings extend the interpretation of colour variation in tissue-culture-derived Kappaphycus alvarezii by showing that an observable green-brown phenotype is not necessarily associated with ITS-level SNPs. This supports a model in which colour morphotypes may arise from physiological regulation, pigment-expression dynamics, or epigenetic responses to in vitro conditions rather than species-level divergence. The close LH-LC genetic relationship,

combined with their separation from reference accessions, refines existing assumptions about K. alvarezii diversity by suggesting that local Maumere germplasm may carry lineage-specific molecular characteristics while maintaining phenotypic plasticity under tissue culture conditions.

Pedagogically, the study can be used in science education, biotechnology training, and aquaculture extension as an authentic case for teaching DNA barcoding, sequence alignment, BLAST interpretation, phylogenetic reasoning, and evidence-based discussion of genotype-phenotype relationships. Using real chromatograms, distance matrices, and phylogenetic trees may improve student engagement, scientific data literacy, and confidence in interpreting molecular evidence in applied biology. At the policy level, the findings indicate the need for stronger seed-quality control, standardized molecular documentation of tissue-culture-derived K. alvarezii planlets, and germplasm banking policies that protect locally adapted Indonesian seaweed varieties while supporting sustainable aquaculture, biosecurity, and traceable seed distribution.

The novelty of this study lies in its molecular examination of spontaneous colour-variant planlets of the Maumere variety generated through tissue culture, particularly the green morphotype that appeared at a low frequency within culture bottles. Unlike previous work that mainly emphasized growth, cultivation performance, pigment content, or general molecular identification, this study links ITS-based DNA barcoding, BLAST identification, nucleotide composition, genetic distance, and phylogenetic reconstruction to the interpretation of colour variation in tissue-culture-derived K. alvarezii. Its main contribution is the provision of baseline molecular evidence that LH and LC planlets are conspecific, that the observed colour shift is not explained by detectable ITS SNPs, and that Indonesian Maumere germplasm deserves further characterization as a potentially distinct local genetic resource for aquaculture and science education.

This study has several limitations that may influence the interpretation of the findings. First, the analysis relied on a single nuclear marker (ITS) and a limited number of representative colour morphotypes, which restricts the capacity to detect fine-scale genetic variation and to generalize the results across broader K. alvarezii populations. Second, the study did not include direct measurements of pigment concentration, photosynthetic performance, transcriptomic expression, epigenetic regulation, or field-based growth and carrageenan-yield performance; therefore, the physiological mechanism underlying the green phenotype remains inferential. Future research should involve larger and replicated samples from different culture batches and cultivation sites, integrate multi-locus markers such as COI, rbcL, cox2-3 spacer, SSRs, or whole-genome SNP profiling, and combine molecular analysis with pigment biochemistry, controlled light and nutrient experiments, transcriptomic or epigenomic profiling, and open-water cultivation trials. These directions would strengthen causal interpretation, clarify genotype-phenotype relationships, and determine whether the LH variant has practical value for commercial seed production and sustainable seaweed aquaculture.

The presence of color-variant planlets in culture bottles at a 1:50 ratio calls for consideration in seed production quality control from the standpoint of aquaculture management. The green (LH) variant's photosynthetic efficiency, carrageenan content, growth performance, and environmental adaptability under open-water cultivation circumstances need to be assessed, even though it is confirmed to be *K. alvarezii* and exhibits no harmful SNPs in the ITS region. This differentiation is crucial since constant carrageenan yield and thallus quality are what determine the economic worth of *K. alvarezii* farming (Rimmer *et al.* 2021).

Advances in DNA sequencing and genotyping will enable detailed profiling of genetic diversity in algal germplasm banks, guiding selection of elite lines, maintaining “heirloom” wild diversity, and linking genotype to desirable phenotypes such as growth, stress tolerance, and biochemical composition for aquaculture applications. In order to provide robust, high-yielding algal seedlings while protecting biodiversity, adhering to access-and-benefit regulations, and promoting long-term food security and industrial macroalgae production, future elite-seed

programs will incorporate genetic profiling, germplasm banking, and coordinated international sharing of wild and cultivated strains.

CONCLUSIONS

This study aimed to molecularly characterize brown (LC) and green (LH) colour-variant planlets of *Kappaphycus alvarezii* Maumere variety derived from tissue culture propagation at SEAMEO BIOTROP Bogor using ITS DNA barcoding, nucleotide composition analysis, SNP detection, genetic distance estimation, and phylogenetic reconstruction. The findings confirmed that both LH and LC planlets belong to *K. alvarezii*, indicating that the spontaneous colour variation observed during in vitro propagation did not correspond to species-level genetic differentiation. The absence of SNPs specifically associated with the colour phenotype suggests that the green colouration is unlikely to be driven by point mutations in the ITS region, but may instead reflect physiological adjustment, epigenetic regulation, or pigment-related responses under tissue culture conditions. The relatively close genetic distance between LH and LC further supports their conspecific status, while their divergence from reference sequences highlights the genetic distinctiveness of the Maumere variety as a valuable Indonesian seaweed germplasm resource.

Theoretically, this study contributes to the refinement of molecular interpretation in seaweed tissue culture by demonstrating that visible phenotypic variation does not necessarily indicate stable genetic mutation within commonly used barcoding regions. Methodologically, it reinforces the utility of ITS-based barcoding as an initial tool for species confirmation and genetic relationship assessment, while also revealing its limitations for explaining fine-scale phenotypic variation. Practically, the findings provide a molecular baseline for seedstock quality control, germplasm conservation, and future selection of tissue-culture-derived *K. alvarezii* variants for sustainable aquaculture. However, this study was limited by the use of a single nuclear marker, a restricted number of colour morphotypes, and the absence of pigment profiling, transcriptomic analysis, and open-water growth performance evaluation. Future research should integrate multi-locus genotyping, high-resolution SNP panels, epigenetic analysis, pigment quantification, and field-based agronomic trials to clarify the mechanisms underlying colour variation and assess its implications for carrageenan yield, environmental adaptability, and commercial cultivation. In this way, the present study advances molecular baseline knowledge while opening a broader research pathway for improving seaweed germplasm management and sustainable aquaculture development.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCE

Bartolo, A. G., Zammit, G., & Küpper, F. C. (2022). *Ulva* L. biodiversity in the central Mediterranean Sea: Cryptic species and new records. *Cryptogamie, Algologie, 43*(14), 215–225. https://doi.org/10.5252/cryptogamie-algologie2022v43a14

Cabrera, R., Umanzor, S., Díaz-Larrea, J., & Araújo, P. G. (2019). *Kappaphycus alvarezii* (Rhodophyta): New record of an exotic species for the Caribbean coast of Costa Rica. *American Journal of Plant Sciences, 10*(10), 1888–1902. https://doi.org/10.4236/ajps.2019.1010133

Duta-Cornescu, G., Constantin, N., Pojoga, D.-M., Nicuţă, D., & Simon-Gruiţă, A. (2023). Somaclonal variation—Advantage or disadvantage in micropropagation of the medicinal plants. *International Journal of Molecular Sciences, 24*(1), 838. https://doi.org/10.3390/ijms24010838

Guillén, P. O., Motti, P., Mangelinckx, S., De Clerck, O., Bossier, P., & Van den Hende, S. (2022). Valorization

of the chemical diversity of the tropical red seaweeds *Acanthophora* and *Kappaphycus* and their applications in aquaculture: A review. *Frontiers in Marine Science, 9*, 957290. https://doi.org/10.3389/fmars.2022.957290

Irawati, B. A., & Affandi, R. I. (2024). Kultur jaringan rumput laut *Kappaphycus alvarezii* dengan metode embriogenesis somatik. *Ganec Swara, 18*(1), 358–368. https://doi.org/10.35327/gara.v18i1.768

Khan, A. Z., Fernando, A. P., Pamungkas, E. A., Andhira, A., Ishlah, R. N., Resmi, D. D. C., Damayanti, F., & Rahmawati, D. (2025). Effectiveness of DNA barcoding primers in red algae (Rhodophyta) identification. *BIODIVERS-BIOTROP Science Magazine, 4*(1), 1–8. https://doi.org/10.56060/bdv.2025.4.1

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

National Center for Biotechnology Information. (2011). *Kappaphycus alvarezii* accession no. JN673973.1. *GenBank*. Retrieved March 1, 2026, from https://www.ncbi.nlm.nih.gov/nuccore/JN673973.1

National Center for Biotechnology Information. (2013). *Kappaphycus alvarezii* accession no. KC905270.1. *GenBank*. Retrieved March 1, 2026, from https://www.ncbi.nlm.nih.gov/nuccore/559148104

National Center for Biotechnology Information. (2019). *Eucheuma isiforme* accession no. MH255768. *GenBank*. Retrieved March 1, 2026, from https://www.ncbi.nlm.nih.gov/nuccore/MH255768

Nurkolis, F., Aldian, F. M., Alfaray, R. I., Johan, A. R. D., Frediansyah, A., Permatasari, H. K., Taslim, N. A., Tjandrawinata, R. R., Mustika, A., Kim, B., Santini, A., & Miftahussurur, M. (2025). Integrative pharmacoinformatic and experimental validation of Indonesian red algae as novel functional food for anti-gastric cancer agents. *Molecular Nutrition & Food Research, 69*(23), Article e70282. https://doi.org/10.1002/mnfr.70282

Rimmer, M. A., Larson, S., Lapong, I., Purnomo, A. H., Pong-Masak, P. R., Swanepoel, L., & Paul, N. A. (2021). Seaweed aquaculture in Indonesia contributes to social and economic aspects of livelihoods and community wellbeing. *Sustainability, 13*(19), 10946. https://doi.org/10.3390/su131910946

Roleda, M. Y., Aguinaldo, Z.-Z. A., Crisostomo, B. A., Hinaloc, L. A. R., Projimo, V. Z., Dumilag, R. V., & Lluisma, A. O. (2021). Discovery of novel haplotypes from wild populations of *Kappaphycus* (Gigartinales, Rhodophyta) in the Philippines. *Algae, 36*(1), 1–12. https://doi.org/10.4490/algae.2021.36.2.18

Satriani, G. I., Soelistyowati, D. T., Alimuddin, A., Arfah, H., & Effendi, I. (2023). Molecular assessment of *Kappaphycus alvarezii* cultivated in Tarakan based on cox2-3 spacer. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 18*(1), 52–64. https://doi.org/10.15578/squalen.736

Satriani, G. I., Soelistyowati, D. T., Alimuddin, A., Arfah, H., & Effendi, I. (2024). Identification of *Kappaphycus alvarezii* seaweed based on phylogenetic and carrageenan content. *Jurnal Akuakultur Indonesia, 23*(1), 1–11. https://doi.org/10.19027/jai.23.1.1-11

Sulistiani, E. (2021). Overexpression of Gα gene increases growth and hyposaline tolerance in *Kappaphycus alvarezii* transgenic plantlets. *Biotropia, 28*(3), 181–192. https://doi.org/10.11598/btb.2021.28.3.1047

Sulistiani, E., & Yani, S. A. (2019). *Kultur jaringan rumput laut kotoni (Kappaphycus alvarezii)*. SEAMEO BIOTROP.

Tan, P., Poong, S., Tan, J., Brakel, J., Gachon, C. M. M., Brodie, J., Sade, A., & Lim, P. (2022). Assessment of genetic diversity within eucheumatoid cultivars in east Sabah, Malaysia. *Journal of Applied Phycology, 34*(1), 709–717. https://doi.org/10.1007/s10811-021-02608-8

Thien, V. Y., Rodrigues, K. F., Voo, C. L. Y., Wong, C., & Yong, W. T. L. (2021). Comparative transcriptome profiling of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in response to light of different wavelengths and carbon dioxide enrichment. *Plants, 10*(6), 1236. https://doi.org/10.3390/plants10061236

Tirtawijaya, G., Negara, B. F. S. P., Lee, J., Cho, M.-G., Kim, H. K., Choi, Y.-S., Lee, S., & Choi, J. (2022). The influence of abiotic factors on the induction of seaweed callus. *Journal of Marine Science and Engineering, 10*(4), 513. https://doi.org/10.3390/jmse10040513

Valderrama, D., Cai, J., Hishamunda, N., Ridler, N., Neish, I. C., Hurtado, A. Q., Msuya, F. E., Krishnan, M., Narayanakumar, R., Kronen, M., Robledo, D., Gasca-Leyva, E., & Fraga, J. (2015). The economics of *Kappaphycus* seaweed cultivation in developing countries: A comparative analysis of farming systems. *Aquaculture Economics & Management, 19*(2), 251–277. https://doi.org/10.1080/13657305.2015.1024348

Wade, R., Augyte, S., Harden, M., Nuzhdin, S., Yarish, C., & Alberto, F. (2020). Macroalgal germplasm banking for conservation, food security, and industry. *PLOS Biology, 18*(2), e3000641.

https://doi.org/10.1371/journal.pbio.3000641

Yong, W. T. L., Chin, G. J. W. L., & Rodrigues, K. F. (2016). Genetic identification and mass propagation of economically important seaweeds. In *Seaweed biology: Novel insights into ecophysiology, ecology and utilization*. IntechOpen. https://doi.org/10.5772/62802

Zuccarello, G. C., & Paul, N. A. (2019). A beginner's guide to molecular identification of seaweed. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 14*(1), 43–53. https://doi.org/10.15578/squalen.v14i1.384