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Research Article

Genetic Diversity and Relationships among Eighteen Algal Accessions Revealed by RAPD and ISSR Markers

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Abstract

Background and Objective: Microalgae represent an important biological resource with significant potential for applications in aquaculture, biotechnology and sustainable bioresource development. However, information regarding the genetic diversity of indigenous Vietnamese microalgal accessions remains limited. This study aimed to assess the genetic diversity and genetic relationships among 18 indigenous microalgal accessions using 15 RAPD and 15 ISSR molecular markers. **Materials and Methods:** Amplified loci were scored as binary data and analyzed using Jaccard similarity coefficients. We inferred genetic relationships through UPGMA clustering and principal coordinate analysis (PCoA). The consistency among marker systems was evaluated using Mantel tests and cophenetic correlation coefficients (CCC). **Results:** RAPD and ISSR markers generated 140 and 188 loci, respectively, with high polymorphism levels of 87.86 and 91.49%. The combined RAPD-ISSR dataset revealed substantial genetic variation among the analyzed accessions, with Jaccard similarity coefficients ranging from 0.263 to 0.938. The UPGMA analysis separated the 18 accessions into three genetic groups, while PCoA produced a congruent distribution pattern. The LC11 and G6 exhibited pronounced genetic divergence from the remaining accessions. Mantel tests revealed strong correlations among RAPD, ISSR and combined datasets ($r = 0.865-0.971$, $p = 0.001$). High CCC values (0.963-0.978) further confirmed the reliability of the inferred genetic relationships. **Conclusion:** The studied microalgal collection possesses substantial genetic diversity and includes highly differentiated genetic lineages. The combined RAPD-ISSR approach provides a reliable framework for genetic characterization and offers valuable information for germplasm conservation, accession selection and future biotechnological exploitation of indigenous microalgae.

Key words: Genetic diversity, ISSR, Marine microalgal germplasm, Microalgae, RAPD

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Microalgae are ecologically and economically important photosynthetic microorganisms with applications in aquaculture, biotechnology and bioproduct development. In Vietnam, however, the genetic diversity of indigenous microalgal resources remains poorly characterized, which may limit their effective conservation and utilization. The Institute of Biology, Vietnam Academy of Science and Technology (VAST), maintains a collection of microalgal accessions whose genetic diversity and relationships have not yet been systematically evaluated. Because morphological identification may not fully resolve phenotypic plasticity and cryptic genetic variation, molecular characterization is needed to assess genetic diversity and clarify relationships among these accessions. Therefore, this study assessed the genetic diversity and genetic relationships among 18 microalgal accessions using RAPD and ISSR markers.

To overcome these limitations, DNA-based molecular markers have become indispensable tools for assessing genetic diversity, identifying cryptic taxa and characterizing population structure in microalgae¹. Using ITS rDNA and *rbcL* markers, significant genetic diversity was detected among *Haematococcus pluvialis* strains. Phylogenetic analysis resolved six distinct lineages, revealing cryptic species diversity and leading to the description of two new species. The ITS marker showed higher discriminatory power than *rbcL* for assessing genetic variation within the genus *Haematococcus*². An investigation into the genetic variation and population differentiation of *Micrasterias rotata* was conducted using sequence data from four nuclear loci (*actin*, *gapC1*, *gapC2* and *oeel*) obtained from 25 isolates. Evaluation of the discriminatory power of these loci revealed that *gapC1* and *actin* were the most effective markers for characterizing population structure within the species³. SSR markers have been successfully applied to assess genetic diversity in microalgae. For example, seventeen polymorphic SSR loci developed for *Chlorella sorokiniana* effectively discriminated among strains and provided valuable tools for population genetic and conservation studies⁴. Although sequence-based markers such as ITS, *rbcL* and SSRs provide high taxonomic resolution, they often require prior sequence information, extensive sequencing efforts, or higher analytical costs. In contrast, RAPD and ISSR markers offer rapid, cost-effective and genome-wide assessments of genetic variation, making them particularly suitable for preliminary characterization of unclassified germplasm collections. Among various molecular marker tools, Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) markers have emerged

as highly effective systems for assessing genetic polymorphism of microalgae⁵. The RAPD is valued for its universal applicability without prior genomic knowledge, while ISSR offers enhanced reproducibility and targets hypervariable regions adjacent to microsatellites. Since these two systems sample different genomic compartments, their synergistic combination provides a more robust and comprehensive assessment of genetic diversity and relationships than any single marker system alone⁶.

This study assessed the genetic diversity and genetic relationships among 18 microalgal accessions using a panel of 15 RAPD and 15 ISSR primers. Genetic polymorphism was evaluated through several statistical parameters, including polymorphism information content (PIC) and resolving power (Rp) and genetic relationships were inferred using UPGMA cluster analysis. As the first molecular characterization of this collection, the study provides baseline information for germplasm conservation, accession selection and the future biotechnological utilization of indigenous microalgal resources in Vietnam.

MATERIALS AND METHODS

Study area: This study was conducted at Institute of Biology, Vietnam Academy of Science and Technology (VAST), Vietnam National University of Agriculture (VNUA) and the Institute of Tropical Medicine, Joint Vietnam-Russia Tropical Science and Technology Research Center. The experimental phase, including microalgae cultivation, genomic DNA isolation and molecular marker analysis, was carried out from June 2024 to May 2026.

Microalgal materials: The genetic diversity study utilized a collection of 18 microalgal accessions isolated and maintained by the Institute of Biology (VAST). These accessions are maintained as independent isolates in the VAST microalgal collection. Preliminary morphological observations suggest that the collection contains several morphologically distinct microalgal types. Their accession codes and sampling coordinates are provided in Table 1.

Research procedure

Genomic DNA extraction: Total genomic DNA was isolated with minor modifications⁷. Following isolation and purification, the microalgal accessions were maintained as pure cultures and grown to obtain sufficient biomass. Cells were harvested by centrifugation at 5,000 g for 7 min at 4°C. Cells were homogenized using a mixer mill with extraction buffer and incubated at 65°C for 30 min.

Table 1: Indigenous microalgal accessions collected from the Bai Tu Long marine area and used in the present study

Accession	Sampling coordinates
S2	21°08'48.3"N, 107°39'20.5"E
S7	21°08'49.3"N, 107°39'29.5"E
S16	21°08'55.7"N, 107°39'19.8"E
H1	21°06'16.1"N, 107°34'30.8"E
G4	21°06'20.4"N, 107°34'19.3"E
G5	21°06'20.4"N, 107°34'21.3"E
G6	21°06'20.5"N, 107°34'21.5"E
G9	21°06'20.7"N, 107°34'22.5"E
G12	21°06'21.2"N, 107°34'21.5"E
N7.2	21°06'26.7"N, 107°34'37.8"E
N16	21°06'25.7"N, 107°34'36.0"E
LC14D	21°02'17.7"N, 107°35'15.6"E
LC11	21°02'16.2"N, 107°35'17.1"E
LC3	21°02'14.1"N, 107°35'15.9"E
TN11	21°06'26.1"N, 107°34'36.9"E
TN12	21°06'26.1"N, 107°34'36.7"E
TN2.3	21°06'25.0"N, 107°34'34.8"E
V6	20°59'34.6"N, 107°34'27.3"E

Accession codes (e.g., S2, S7, H1, G4) are internally assigned codes used to identify individual isolates obtained from the collected samples. These codes are not accession numbers obtained from a public repository or database

Purification was achieved through successive extractions with phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1). DNA was precipitated with cold absolute ethanol, washed twice with 70% ethanol and finally resuspended in Tris-EDTA (TE) buffer. DNA concentration and chemical purity (A260/280 and A260/230 ratios) were quantified via spectrophotometry.

PCR amplification and marker screening: Fifteen RAPD primers and fifteen ISSR primers were screened for genetic polymorphism. PCR reactions were performed in a Bio-Rad Thermal Cycler with a total volume of 20 µL. Each reaction mixture contained 25 ng of template DNA, 5 µM of specific primer and 2X My-Taq PCR Master Mix.

The RAPD and ISSR cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 34 cycles of 94°C (30 sec), annealing at indicated temperature on Supplementary Table S1 and Supplementary Table S2 and extension at 72°C (2 min), with a final extension at 72°C for 10 min.

Amplified products were resolved by gel electrophoresis on 1.0% agarose gels stained with ethidium bromide (EtBr) and visualized under a UVP BioDoc-It Imaging System.

Data analysis: Amplified RAPD and ISSR fragments were scored as binary characters, with the presence of a band recorded as "1" and its absence as "0". Separate binary matrices were generated for RAPD and ISSR markers and subsequently combined into a single dataset for integrated analysis.

Marker performance was evaluated by calculating the total number of amplified loci, number of polymorphic loci, percentage of polymorphic loci (PPL), polymorphism information content (PIC) and resolving power (Rp) for each primer⁸, resolving power was estimated following Prevost and Wilkinson⁹.

Genetic relationships among microalgal accessions were assessed using Jaccard similarity coefficients, which are considered appropriate for dominant molecular markers because shared absences are not included in similarity calculations. A pairwise similarity matrix was generated for RAPD, ISSR and combined datasets. The most similar and most divergent accession pairs were identified from the combined similarity matrix.

Cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Jaccard genetic distances. The reliability of the resulting dendrograms was evaluated using cophenetic correlation coefficients (CCC), which measure the agreement between the original genetic distance matrix and the corresponding dendrogram¹⁰.

To further visualize genetic relationships among accessions, Principal Coordinate Analysis (PCoA) was conducted based on Jaccard distance matrices¹¹. The consistency among RAPD, ISSR and combined datasets was examined using Mantel tests with 999 permutations¹².

All statistical analyses were performed in R version 4.6.0¹³. Genetic similarity matrices, UPGMA clustering, Mantel tests and principal coordinate analysis (PCoA) were conducted using the packages *vegan*, *ape*, *proxy* and *dendextend* implemented in R.

Table 2: Comparative efficiency of RAPD, ISSR and combined marker systems for assessing genetic diversity among 18 algal accessions

Marker system	Total loci	Polymorphic loci	PPL (%)	Mean PIC*	Mean Rp*
RAPD	140	123	87.86	0.28	3.59
ISSR	188	172	91.49	0.25	4.83
Combined	328	295	89.94	0.27	4.21

PPL: Percentage of polymorphic loci, PIC: Polymorphism information content, Rp: Resolving power and *: Mean PIC and Rp values for the combined dataset were calculated across all RAPD and ISSR primers

RESULTS AND DISCUSSIONS

Marker performance and polymorphism analysis: The efficiency of RAPD and ISSR markers in detecting genetic polymorphism among the 18 microalgal accessions is summarized in Table 2. A total of 140 loci were amplified using 15 RAPD primers, of which 123 loci were polymorphic, corresponding to 87.86% polymorphism. The number of amplified loci per RAPD primer ranged from 5 to 17, with an average of 9.33 loci per primer. The average number of polymorphic loci per primer was 8.20, while the average polymorphism information content (PIC) and resolving power (Rp) were 0.28 and 3.59, respectively (Table 2).

Similarly, the 15 ISSR primers generated 188 loci, including 172 polymorphic loci, resulting in a polymorphism level of 91.49%. The number of amplified loci per primer ranged from 7 to 18, with an average of 12.53 loci per primer. The average number of polymorphic loci per primer was 11.47. The mean PIC and Rp values were 0.25 and 4.83, respectively (Table 2). Compared with RAPD markers, ISSR markers produced a larger number of loci, a higher percentage of polymorphism and greater resolving power, indicating a superior ability to discriminate among microalgal accessions. The combined RAPD and ISSR dataset generated 328 loci, of which 295 were polymorphic, corresponding to an overall polymorphism level of 89.94% (Table 2). Among RAPD primers, OPD01 exhibited the highest PIC value (0.43), whereas OPC04 showed the highest resolving power (Rp = 5.33). For ISSR markers, ISSR14 displayed the highest resolving power (Rp = 9.44), while ISSR5 exhibited the highest PIC value (0.38), indicating that these primers were particularly informative for assessing genetic variation among the studied accessions. Detailed information on primer performance is provided in Supplementary Tables S1 and S2.

The RAPD and ISSR marker systems employed in this study generated high levels of polymorphism (87.86 and 91.49%, respectively), indicating substantial genetic variation among the investigated microalgal accessions. Similar levels of polymorphism have been reported in RAPD- and ISSR-based diversity studies, where polymorphism percentages above 80% are generally considered indicative of extensive genetic variability within germplasm collections¹⁴.

The ISSR markers generated a greater number of loci (188) and exhibited higher polymorphism levels than RAPD markers (140 loci), suggesting a greater capacity to detect genetic variation. This observation agrees with previous studies demonstrating that ISSR markers often reveal higher levels of polymorphism because they target microsatellite-rich regions that are widely distributed throughout the genome⁶. However, ISSR has also been known to have lower effectiveness over RAPD in evaluating the genetic diversity of rice¹⁵ and Indian lettuce (*Lactuca indica* L.)¹⁶.

The average PIC values (0.25-0.28) indicate moderate discriminatory ability of the selected primers and are comparable to values reported in previous RAPD and ISSR studies. Similar PIC values have been reported in RAPD and ISSR studies involving various plant and algal taxa, supporting the suitability of these marker systems for assessing genetic variation among closely related genotypes¹⁴.

Resolving power (Rp) is an important parameter that reflects the ability of a primer or marker system to discriminate among genotypes. In the present study, ISSR markers exhibited higher mean Rp values than RAPD markers, indicating greater discriminatory capacity for detecting genetic variation among the studied microalgal accessions. Similar findings have been reported in rice, where ISSR markers generated more informative loci and displayed higher resolving power than RAPD markers for assessing genetic relationships among accessions¹⁵. However, the relative performance of RAPD and ISSR markers may vary depending on the species, genome characteristics and primer sets employed. For example, Dinh *et al.*¹⁵ (2022) reported higher resolving power for RAPD than ISSR markers in their analysis, suggesting that marker efficiency is often species- and dataset-dependent rather than being an inherent property of a particular marker system¹⁶.

An important consideration in marker-based diversity studies is whether the number of primers employed provides sufficient genome coverage for reliable estimation of genetic relationships. The 15 RAPD and 15 ISSR primers used in the present study generated a total of 328 loci, including 295 polymorphic loci. This number exceeds those reported in many RAPD/ISSR investigations involving comparable numbers of accessions, where approximately 10-20 primers

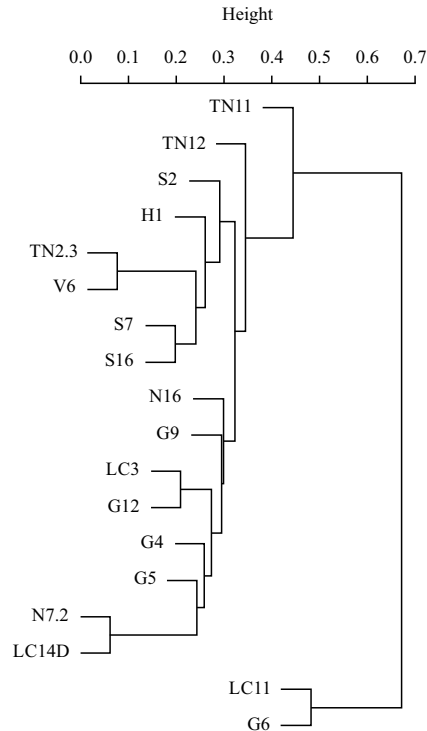


Fig. 1: UPGMA dendrogram showing genetic relationships among 18 indigenous microalgal accessions based on combined RAPD and ISSR markers

The dendrogram was generated using the UPGMA algorithm from the combined RAPD–ISSR dataset. Branch lengths represent genetic distances among accessions

Table 3: Summary statistics of Jaccard similarity coefficients among microalgal accessions based on combined RAPD and ISSR markers

Parameter	Value
Number of accessions	18
Number of pairwise comparisons	153
Minimum similarity coefficient	0.263
Maximum similarity coefficient	0.938
Mean similarity coefficient	0.606
Most genetically similar accession pair	N7.2-LC14D (0.938)
Most genetically divergent accession pair	LC3- G6 (0.263)

per marker system commonly generated between 100 and 250 polymorphic loci¹⁴. The large number of polymorphic loci obtained here suggests that the selected primer set provided extensive genome sampling and sufficient resolution for characterizing genetic diversity among the 18 microalgal accessions.

Overall, the high percentage of polymorphic loci, moderate PIC values, high resolving power and large number of informative loci generated collectively indicate that the selected set of 15 RAPD and 15 ISSR primers was adequate for evaluating genetic diversity and genetic relationships among the studied microalgal accessions.

Genetic similarity among microalgal accessions: Genetic relationships among the 18 microalgal accessions were

evaluated using Jaccard similarity coefficients derived from the combined RAPD and ISSR dataset. The pairwise similarity coefficients ranged from 0.263 to 0.938, indicating substantial genetic variation within the studied collection (Table 3).

The highest genetic similarity (0.938) was observed between accessions N7.2 and LC14D, suggesting a close genetic relationship and a high degree of shared genomic characteristics. In contrast, the lowest similarity coefficient (0.263) was detected between accessions LC3 and G6, indicating pronounced genetic divergence between these two accessions.

The wide range of similarity coefficients observed in the present study demonstrates the effectiveness of RAPD and ISSR markers in detecting genetic variation among microalgal accessions. Moreover, the presence of both highly similar and

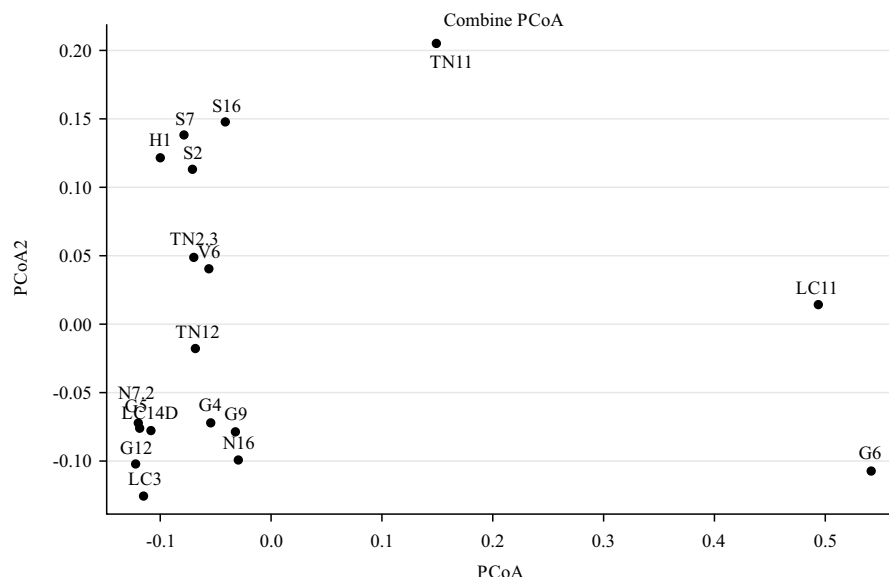


Fig. 2: Principal coordinate analysis (PCoA) of 18 indigenous microalgal accessions based on combined RAPD and ISSR markers
Principal coordinate analysis (PCoA) based on Jaccard similarity coefficients derived from the combined RAPD and ISSR dataset. Each point represents an individual microalgal accession. The ordination illustrates the genetic relationships among accessions and highlights the genetic distinctiveness of LC11 and G6 relative to the main group

highly divergent accession pairs suggests that the analyzed collection contains genetically distinct lineages that may represent valuable resources for future conservation, taxonomic characterization and biotechnological applications.

Genetic relationships and clustering of microalgal accessions: The genetic relationships among the 18 microalgal accessions were further evaluated using UPGMA clustering based on the combined RAPD and ISSR dataset (Fig. 1). The dendrogram revealed a clear genetic structure and separated the accessions into three clusters.

Cluster I comprised the majority of the analyzed accessions, including N7.2, N16, LC14D, LC3, G4, G5, G9, G12, H1, S2, S7, S16, TN11, TN12, TN2.3 and V6. Within this cluster, several closely related subgroups were observed, particularly the pair N7.2–LC14D, which exhibited the highest Jaccard similarity coefficient (0.938). Other closely associated accession pairs included TN2.3–V6 and S7–S16.

Cluster II consisted solely of accession LC11, whereas Cluster III was represented exclusively by accession G6. Both accessions were clearly separated from the remaining genotypes and occupied independent branches in the dendrogram. The isolated positions of LC11 and G6 are consistent with their generally low similarity coefficients relative to the other accessions.

The close association between N7.2 and LC14D reflects their exceptionally high similarity coefficient (0.938), indicating

that these two accessions share a large proportion of amplified loci. Similar clustering of highly related genotypes has frequently been reported in RAPD- and ISSR-based diversity studies¹⁷.

In contrast, LC11 and G6 formed independent clusters, highlighting their pronounced genetic divergence from the remaining accessions. This observation agrees with the pairwise similarity analysis, where G6 displayed consistently low similarity coefficients with most accessions and showed the lowest similarity value with LC3 (0.263). The occurrence of two highly divergent accessions within the collection suggests the presence of distinct genetic lineages that may represent valuable resources for future taxonomic studies, accession improvement programs and biotechnological applications.

To further visualize the genetic relationships among the microalgal accessions, principal coordinate analysis (PCoA) was performed using the combined RAPD and ISSR dataset (Fig. 2). The ordination pattern revealed clear genetic differentiation among the analyzed accessions and was largely consistent with the clustering pattern obtained from the UPGMA dendrogram.

Most accessions formed a compact group located on the left side of the ordination plot, indicating relatively close genetic relationships among them. Within this group, several accessions occupied neighboring positions, reflecting their high genetic similarity as observed in the pairwise comparison matrix.

Table 4: Mantel test correlations and cophenetic correlation coefficients (CCC) for RAPD, ISSR and combined datasets

Analysis	Comparison	Value	p-value
Mantel test	RAPD vs ISSR	$r = 0.865$	<0.001
Mantel test	RAPD vs Combined	$r = 0.959$	<0.001
Mantel test	ISSR vs Combined	$r = 0.971$	<0.001
Cophenetic correlation coefficients	RAPD	CCC = 0.963	-
Cophenetic correlation coefficients	ISSR	CCC = 0.970	-
Cophenetic correlation coefficients	Combined	CCC = 0.978	-

In contrast, accessions LC11 and G6 were clearly separated from the main cluster along the first principal coordinate (PCoA1). G6 occupied the most distant position in the ordination space, whereas LC11 was also distinctly isolated from the majority of accessions. Accession TN11 showed an intermediate but unique position and was separated from the main cluster along the second principal coordinate (PCoA2). The close proximity between N7.2 and LC14D observed in the dendrogram was also evident in the PCoA plot, where these accessions clustered tightly together. Overall, the ordination analysis confirmed the existence of substantial genetic variation within the collection and highlighted the presence of several genetically distinct lineages. The distinct position of TN11 along the second principal coordinate suggests that this accession is genetically differentiated from the remaining accessions. The compact grouping of most accessions in the central region of the ordination plot indicates the existence of a relatively shared genetic background across a large proportion of the collection. Nevertheless, the wide dispersion observed among several accessions demonstrates that substantial genetic variability is still maintained within the germplasm. Similar findings have been reported in studies utilizing combined RAPD and ISSR markers, where the integration of multiple marker systems improved the resolution of population structure and genetic relationships¹⁷.

Taken together, the PCoA results reinforce the conclusions derived from the similarity matrix and UPGMA analysis, confirming that the Vietnamese microalgal collection contains both closely related accessions and highly divergent genetic lineages. Such diversity represents a valuable resource for future taxonomic identification, germplasm conservation and accession selection for biotechnological applications.

Congruence among RAPD, ISSR and combined marker systems: The reliability and consistency of the genetic relationship analyses were assessed using Mantel tests and cophenetic correlation coefficients (CCC) (Table 4). Mantel

tests revealed strong and highly significant correlations among the genetic distance matrices generated from RAPD, ISSR and combined marker datasets. The correlation coefficient between RAPD and ISSR datasets was 0.865 ($p = 0.001$), indicating a high degree of agreement between the two independent marker systems. Even stronger correlations were observed between RAPD and the combined dataset ($r = 0.959, p = 0.001$) and between ISSR and the combined dataset ($r = 0.971, p = 0.001$).

The UPGMA dendrograms generated from all datasets exhibited excellent goodness-of-fit to the original genetic distance matrices. Cophenetic correlation coefficients were 0.963 for RAPD, 0.970 for ISSR and 0.978 for the combined dataset. The highest CCC value obtained from the combined RAPD-ISSR dataset suggests that integrating both marker systems provided the most accurate representation of the genetic relationships among the studied microalgal accessions.

Taken together, the high Mantel correlation coefficients and CCC values indicate that RAPD and ISSR markers generated highly congruent patterns of genetic variation and that the resulting dendrograms accurately reflected the underlying genetic distance structure. These findings confirm the robustness and reliability of the clustering and ordination analyses presented in this study.

Previous studies have reported contrasting levels of agreement between RAPD and ISSR markers. For example, Nguyen *et al.*¹⁸ analyzed 14 Vietnamese ramie accessions using 17 RAPD and 17 ISSR primers and found significant correlations between individual marker systems and the pooled dataset, supporting the use of combined markers for genetic diversity assessment. In contrast, Dinh *et al.*¹⁵ reported that RAPD and ISSR datasets generated from eight brown planthopper-resistant rice varieties were not significantly correlated, indicating that the two marker systems captured different components of genetic variation¹⁵.

The high level of polymorphism detected by both RAPD and ISSR markers, together with the broad range of Jaccard similarity coefficients (0.263-0.938), indicates that the studied

microalgal collection harbors large genetic diversity. Such diversity is particularly important for germplasm conservation and accession selection because genetically diverse collections provide a broader reservoir of adaptive traits and metabolic potential. Similar observations have been reported in microalgal and plant germplasm studies, where high molecular polymorphism was associated with increased opportunities for selecting elite genotypes for biotechnology, aquaculture and breeding applications^{6,19}.

Among the analyzed accessions, LC11 and G6 consistently exhibited pronounced genetic divergence from the remaining accessions. Both accessions occupied isolated positions in the UPGMA dendrogram and PCoA plot and showed relatively low similarity coefficients with most other accessions. The repeated detection of these accessions as outliers across independent analytical approaches suggests that they may represent distinct genetic lineages within the collection. Such highly differentiated genotypes are of particular interest because they may contain unique alleles, adaptive traits or metabolic characteristics not present in more closely related accessions. Therefore, LC11 and G6 represent valuable candidates for future taxonomic characterization using DNA barcoding markers (18S rRNA, ITS and rbcL) and for screening programs aimed at identifying accessions with promising biotechnological potential.

The effectiveness of combining RAPD and ISSR markers can be attributed to the complementary characteristics of the two marker systems. RAPD markers employ short arbitrary primers and low-stringency amplification conditions, enabling the detection of polymorphisms across multiple genomic regions without prior sequence information²⁰. As a result, RAPD markers often provide broad genome coverage and are useful for detecting overall patterns of genetic variation. In contrast, ISSR markers target regions flanked by microsatellite sequences using longer primers and higher annealing temperatures, generally resulting in improved reproducibility and greater amplification specificity²¹. Owing to the high variability frequently associated with microsatellite-adjacent regions, ISSR markers have been widely used for assessing genetic differentiation among closely related genotypes and populations²². The flexibility of ISSR in mapping microalgal lineages has been successfully applied in identifying the geographical boundaries of the genus *Haematococcus*⁵ and *Chlorella* sp.¹⁹.

The complementary nature of RAPD and ISSR markers was reflected in the present study. When the two datasets were combined, a total of 295 polymorphic loci were detected, providing a broader representation of genome-wide variation than either marker system alone. Furthermore, Mantel tests revealed strong and highly significant correlations between the combined dataset and the individual marker systems ($r = 0.959-0.971$, $p < 0.001$), indicating a high degree of concordance in the genetic relationships inferred from independent marker sources. These results suggest that integrating RAPD and ISSR markers provides a robust and reliable framework for characterizing genetic diversity and genetic relationships among indigenous microalgal accessions.

Although the accessions were collected from locations distributed within the Bai Tu Long marine area, several genetically related accessions tended to cluster together, whereas others exhibited pronounced genetic divergence. For example, LC11 and G6 were consistently separated from the main group in both UPGMA and PCoA analyses. These observations suggest that geographic distribution alone may not fully explain the genetic relationships observed among the accessions. Similar patterns have been reported in marine microalgae, where genetic differentiation may arise through a combination of historical, ecological and evolutionary processes rather than geographic distance alone^{23,24}.

The occurrence of highly divergent accessions within a relatively restricted sampling area may reflect the presence of distinct genetic lineages that have persisted within local environments. Previous studies have suggested that environmental heterogeneity, localized dispersal, historical isolation and the persistence of dormant propagules may contribute to the maintenance of genetic diversity in microalgal populations^{25,26}. Although the present study did not directly evaluate these factors, the marked genetic separation of LC11 and G6 indicates that further investigation using DNA barcoding markers and ecological characterization would be valuable for clarifying their taxonomic status and evolutionary relationships.

The high cophenetic correlation coefficient obtained from the combined RAPD-ISSR dataset (CCC = 0.978) further demonstrates that integrating multiple marker systems provides a robust representation of genetic relationships among the studied accessions. Similar conclusions have been reported in previous studies where combined RAPD and ISSR datasets improved genome coverage and enhanced the resolution of genetic diversity analyses compared with individual marker systems alone^{6,22}.

Overall, the results demonstrate that the indigenous microalgal collection maintained by VAST contains substantial genetic diversity and several genetically distinct lineages. This diversity represents an important genetic resource for future taxonomic studies, germplasm conservation programs and the identification of accessions with potential applications in aquaculture, biotechnology and bioresource development.

CONCLUSION

This study provides the first molecular assessment of genetic variation among eighteen indigenous marine microalgal accessions collected from Bai Tu Long Bay, Vietnam. Both RAPD and ISSR markers revealed substantial levels of polymorphism and generated congruent patterns of genetic relationships. The combined marker dataset offered the highest discriminatory capacity and produced a robust representation of accession relationships, as evidenced by high cophenetic correlation values and significant Mantel correlations. The pronounced divergence observed in several accessions, particularly LC11 and G6, highlights the presence of valuable genetic resources within the collection. These findings establish a baseline framework for future taxonomic characterization, germplasm management and the exploration of indigenous microalgae for biotechnological applications.

SIGNIFICANCE STATEMENT

This study provides the first comprehensive assessment of genetic diversity among 18 indigenous microalgal accessions from Vietnam using combined RAPD and ISSR markers. The results revealed substantial genetic variation and demonstrated strong concordance between the two marker systems. These findings provide a valuable molecular foundation for microalgal germplasm conservation, accession identification and the selection of genetically diverse materials for future biotechnological and aquaculture applications.

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SUPPLEMENTARY DATA

Supplementary Table S1: Characteristics and polymorphism parameters of the 15 RAPD primers used for genetic diversity analysis of 18 indigenous microalgal accessions

No.	Primer	Nucleotide sequence (5'-3')	Annealing temperature (°C)	Total loci (loci)	Polymorphic (%)	Polymorphism amplified	Total bands	PIC	Resolving power (Rp)
1	OPA03	AGTCAGCCAC	34	17	13	76.47	221	0.19	4.11
2	OPB04	GGACTGGAGT	34	10	10	100	84	0.27	3.78
3	OPB06	TGCTCTGCCC	34	11	8	72.73	135	0.19	2.78
4	OPC01	TTCGAGCCAG	34	11	11	100	97	0.3	4.78
5	OPC02	GTGAGGCGTC	34	11	9	81.82	163	0.18	2.33
6	OPC03	GGGGGTCTTT	34	5	5	100	39	0.26	1.89
7	OPC04	CCGCATCTAC	34	10	10	100	106	0.35	5.33
8	OPC05	GATGACCGCC	34	10	6	60	155	0.16	2.11
9	OPD01	ACCGCGAAGG	34	6	6	100	58	0.43	4.22
10	OPD02	GGACCCAACC	34	12	10	83.33	120	0.24	4.22
11	OPN01	CTCACGTTGG	32	5	5	100	40	0.34	2.44
12	OPN02	ACCAGGGGCA	34	10	9	90	121	0.3	4.33
13	OPO02	ACGTAGCGTC	32	7	6	85.71	75	0.3	3.22
14	OPS08	TTCAGGGTGG	32	9	9	100	69	0.34	4.56
15	OPS10	ACCGTTCAG	32	6	6	100	50	0.37	3.78
Total				140	123	87.86	1533	-	-
Mean				9.33	8.2		102.2	0.28	3.59

Supplementary Table S2: Characteristics and polymorphism parameters of the 15 ISSR primers used for genetic diversity analysis of 18 indigenous microalgal accessions

No.	Primer	Nucleotide sequence (5'-3')	Annealing temperature (°C)	Total loci (loci)	Polymorphic (%)	Polymorphism amplified	Total bands	PIC	Resolving power (Rp)
1	ISSR1	CAGCAGCAGCAGCAG	51.6	11	5	45.45	175	0.06	0.78
2	ISSR3	AGAGAGAGAGAGAGTC	53.9	14	13	92.86	140	0.21	3.78
3	ISSR4	ACACACACACACACT	50.0	7	7	100	85	0.34	3.44
4	ISSR5	AGAGAGAGAGAGAGGTG	57.3	14	14	100	152	0.38	8.22
5	ISSR7	CAACAACAACAACA	53.7	14	14	100	107	0.24	4.56
6	ISSR13	CAGCACACACACACACA	57.3	15	15	100	116	0.34	7.56
7	ISSR14	CGTCACACACACACACA	44.0	18	18	100	123	0.36	9.44
8	ISSR3	ACACACACACACCG	42.0	13	12	92.31	154	0.25	4.44
9	UBC809	AGAGAGAGAGAGAGAGG	51.9	13	12	92.31	135	0.11	4.78
10	UBC826	ACACACACACACACACC	51.9	11	10	90.91	140	0.29	4.67
11	UBC835	AGAGAGAGAGAGAGAGYC	53.9	8	6	75	111	0.22	2.78
12	UBC836	AGAGAGAGAGAGAGAGYA	51.9	13	13	100	156	0.24	3.78
13	UBC840	GAGAGAGAGAGAGAGAYT	51.9	7	5	71.43	58	0.22	2.44
14	UBC841	GAGAGAGAGAGAGAGAYC	53.9	13	12	92.31	114	0.22	3.56
15	UBC876	GATAGATAGACAGACA	42.0	17	16	94.12	180	0.32	8.22
Total				188	172	91.49	1946		
Mean				12.53	11.47		129.73	0.25	4.83