Electronic Journal of Biotechnology 54 (2021) 37–46

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage:

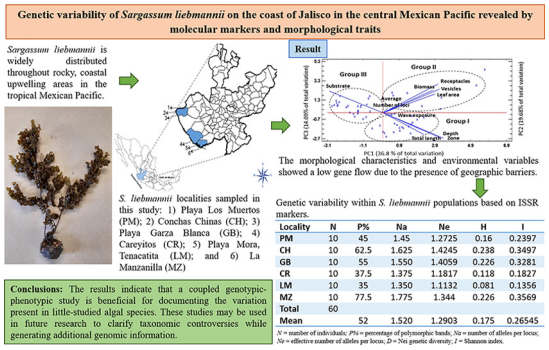
Research Article

Genetic variability of *Sargassum liebmannii* on the coast of Jalisco in the central Mexican Pacific revealed by molecular markers and morphological traits ☆

Hwan Woo Jung-Kim [a], Rosalba Mireya Hernández-Herrera [b], Ildefonso Enciso-Padilla [c], Martha Isabel Torres-Morán [a], Ana Paulina Velasco-Ramírez [a,*]

[a] Departamento de Producción Agrícola, Universidad de Guadalajara, Km. 15.5 Carr. Guadalajara-Nogales, Zapopan, Jalisco, Mexico
[b] Departamento de Botánica y Zoología, Universidad de Guadalajara, Km. 15.5 Carr. Guadalajara-Nogales, Zapopan, Jalisco, Mexico
[c] Departamento de Ecología, Universidad de Guadalajara, Km. 15.5 Carr. Guadalajara-Nogales, Zapopan, Jalisco, Mexico

GRAPHICAL ABSTRACT



Locality	N	P%	Na	Ne	H	I
PM	10	45	1.45	1.2725	0.16	0.2397
CH	10	62.5	1.625	1.4245	0.238	0.3497
GB	10	55	1.550	1.4059	0.226	0.3281
CR	10	37.5	1.375	1.1817	0.118	0.1827
LM	10	35	1.350	1.1132	0.081	0.1356
MZ	10	77.5	1.775	1.344	0.226	0.3569
Total	60					
Mean		52	1.520	1.2903	0.175	0.26545

N = number of individuals; *P%* = percentage of polymorphic bands; *Na* = number of alleles per locus; *Ne* = effective number of alleles per locus; *D* = Nei genetic diversity; *I* = Shannon index.

ARTICLE INFO

Article history:
Received 29 December 2020
Revised 22 April 2021
Accepted 18 August 2021
Available online 24 August 2021

Keywords:
Brown algae
Genetic variability
Inter-population diversity
Inter-simple sequence repeat (ISSR)
Jalisco
Macroalgae
Mexican Pacific
Molecular markers
Morphological traits
Sargassum liebmannii

ABSTRACT

Background: *Sargassum liebmannii* is widely distributed throughout rocky, coastal upwelling areas in the tropical Mexican Pacific. This brown algae is of great environmental and industrial importance. However, no information is available that documents the genetic or phenotypic variability of the species, which is needed to determine how it may react to environmental variation related to climate change. In this study, *S. liebmannii* specimens were collected from the coast of Jalisco, Mexico, and molecular and morphological characterization was conducted. Intraspecific variability was estimated according to the study areas.
Results: The inter-simple sequence repeat (ISSR) markers indicated a polymorphism percentage of 95%. The Shannon index and Nei index showed relatively low values among the populations (0.3569 and 0.081, respectively). On the other hand, the genetic differentiation coefficient indicated inter- and intrapopulation values of 36.69% and 63.31%, respectively. The Jaccard similarity coefficient was used to determine the degree of similarity among individuals by geographical area. The morphological characteristics and environmental variables that were used to correlate phenotypes and genotypes indicated that *S. liebmannii* showed low genetic flow because of the presence of geographical barriers due to substrate that was not optimal for algal development.
Conclusions: The ISSR markers were useful for detecting genetic differences among *S. liebmannii* individuals. The results indicate that a coupled genotypic-phenotypic study is beneficial for documenting the

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso
* Corresponding author.
E-mail address: pauvela73@hotmail.com (A.P. Velasco-Ramírez).

https://doi.org/10.1016/j.ejbt.2021.08.003

variation present in the little-studied algal species. These studies may be used in future research to clarify taxonomic controversies while generating additional genomic information.

How to cite: Jung-Kim HW, Hernández-Herrera RM, Enciso-Padilla I, et al. Genetic variability of *Sargassum liebmannii* on the coast of Jalisco in the central Mexican Pacific revealed by molecular markers and morphological traits. Electron J Biotechnol 2021;54. https://doi.org/10.1016/j.ejbt.2021.08.003

1. Introduction

The macroalgae genus with the highest number of species (539) is *Sargassum*, according to AlgaeBase [1]. Nonetheless, this genus has been the subject of only a few studies in Mexico that have generated information at the genetic level [2]. *Sargassum liebmannii* J. Agardh (Fucales: Phaeophyceae) is a brown benthic seaweed that grows in exposed intertidal or subtidal areas and is generally found between depths of 25–30 m. This macroalga is abundant on rocky coasts where it grows in littoral and sublittoral zones and is often found in warm and well-aerated waters. Along the coast of Jalisco in the tropical Mexican Pacific, *S. liebmannii* develops high-biomass blooms [3] and is used in fertilizer, fodder, and livestock feed and as a source of alginates and potassium salts [4].

The results of genetic diversity studies can be used to help protect species against global climate change, as the stability of a population is often attributed to the degree of genetic variation present. The greater the genetic diversity of a population, the greater are its chances to resist environmental change [5]. *S. liebmannii* presents a high rate of polymorphism, which is common in the marine environment. However, this species has not been evaluated from a genetic point of view, and information that is necessary for an accurate understanding of *S. liebmannii* reproduction and its total geographic distribution is lacking.

In many cases, taxonomic classifications of algae can only be conducted at the genetic level. These genetic classifications allow for interspecific and intraspecific hypotheses of genetic and phenotypic variation to be elucidated, which establish that abiotic factors (i.e., mainly the weather) reflect the degree of migration within a species. Molecular markers are advantageous when evaluating variation and estimating inter- and intrapopulation diversity in algal species. For example, Yao et al. [6] conducted a genetic study of *Sargassum fusiforme* populations on the coast of China using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. Yanhui et al. [7] used simple-sequence repeat (SSR) markers to sequence the entire *Sargassum vachellianum* chloroplast genome and detect highly variable regions. Kubo et al. [8] developed SSR sequences for *Sargassum horneri*. Moreover, Ren et al. [9] used ISSR markers to detect the genetic variation of fixed-culture and floating populations of *S. horneri* along the coast of Zhejiang, China. In addition, Yu et al. [10] used ISSR and SRAP markers to study the population genetics of *S. horneri* to evaluate the relationship between genetic diversity and the geographic distribution of the species.

Despite the environmental and industrial importance of *S. liebmannii* in the central Mexican Pacific [11,12], no information is available on the genetic variability of this species in this region. Thus, the aim of this study was to investigate how the environmental conditions present on the Jalisco coast influence the genetic variability of *S. liebmannii.* This is the first study in the region to generate information that is neither taxonomic nor phylogenetic. The results of this study will allow for problems related to the morphological misidentification of species to be addressed through the determination of the genetic profile of the individuals in this study. Moreover, in the mid-term, the development of new DNA markers will allow for particular population traits to be identified.

2. Materials and methods

2.1. Study area and sample collection

Sixty *Sargassum* specimens were collected by hand from wild populations along the coast of Jalisco in subtidal and intertidal zones by divers at low tide. These specimens were used for molecular and morphological analyses. Specimens were collected in July and August of 2019. A total of six sites were sampled and classified into northern (localities 1, 2, and 3) or southern (localities 4, 5, and 6) zones (Table 1 and Fig. 1). During collection, depth (subtidal or intertidal), wave exposure (exposed or protected), and substrate (rocky or mixed platform) classifications were made for each site. All samples were properly labeled, preserved in cold seawater in an ice chest, and transported to the laboratory for identification and further analysis.

2.2. Preservation of Sargassum liebmannii samples

The collected fragments and whole algae were washed twice with purified water to remove shells and sand and thoroughly rinsed with distilled water. The excess water was then removed. During this process, the absence of epiphytes was verified.

Each collected fragment was labeled (area and location), wrapped in a brown paper, placed inside a white polyethylene bag, and stored in a freezer at −20°C until further analysis.

2.3. Morphological characteristics

Initially, a total of 60 specimens were photographed, and their morphological characteristics were measured using ImageJ v.

Table 1
Study sites of the *Sargassum liebmannii* populations on the coast of Jalisco in the central Mexican Pacific.

Locality	Zone	Depth	Geographic coordinates
1. Playa Los Muertos	Northern	Subtidal	20° 35′ 55″ N–105°14′ 22″ W
2. Playa Conchas Chinas	Northern	Subtidal	20° 37′ 1.2″ N–102° 13′ 48.6″ W
3. Playa Garza Blanca	Northern	Subtidal	19° 35′ 59″ N–105° 09′ 39.17″ W
4. Playa Careyitos	Southern	Intertidal	19° 26′ 07″ N–105° 14′ 22″ W
5. Playa Mora, Tenacatita	Southern	Intertidal	19° 26′ 15″ N–105° 01′ 51″ W
6. Playa La Manzanilla	Southern	Intertidal	19° 16′ 52″ N–104° 47′ 15″ W

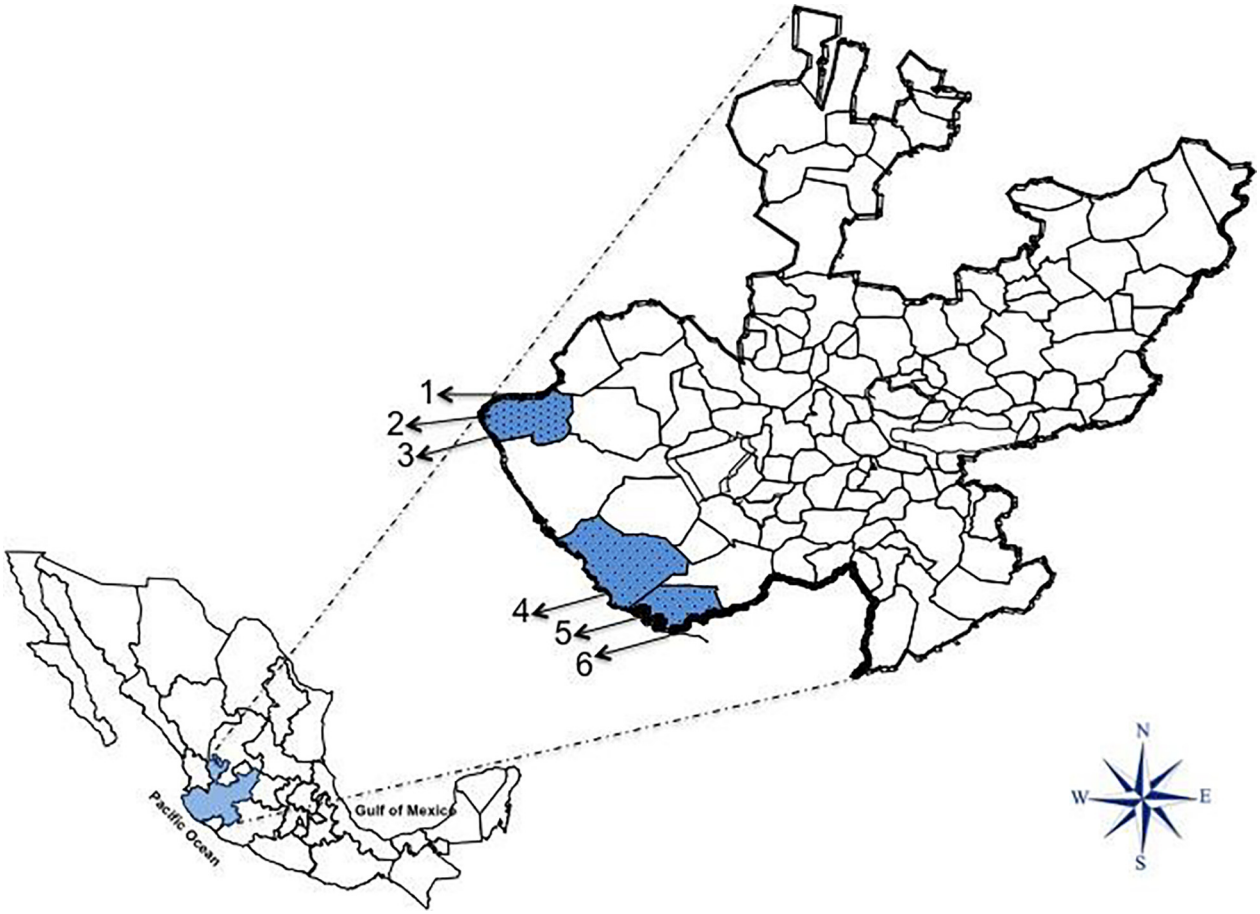


Fig. 1. *Sargassum liebmannii* localities sampled in this study: 1) Playa Los Muertos (PM); 2) Conchas Chinas (CH); 3) Playa Garza Blanca (GB); 4) Careyitos (CR); 5) Playa Mora, Tenacatita (LM); and 6) La Manzanilla (MZ).

Table 2
Inter-simple sequence repeat (ISSR) primer sequences and polymorphism based on the resulting banding patterns of *Sargassum liebmannii*.

Primer	Sequence (5′–3′)	Amplicons	Polymorphism (%)
7	CTCTCTCTCTCTRTRG	9	100
16	YRG ACA GAC AGA CA	11	100
812	GAGAGGAGAGAGAGAA	8	75
DAT	GAGAGAGAGAGAGARG	6	100
902	GTCTGTGTGTGTAY	6	100
	Global	40	95

1.52a (https://imagej.nih.gov/ij/download.html) [13]. The characteristics were measured according to the methodology of Modelo et al. [14] and were as follows: the total length of the thallus (cm), leaf area of the fronds (cm^2), total number of vesicles, receptacle number, and biomass (fresh weight, g), which was obtained with an electronic balance (Precisa Instrument, Dietikon, Switzerland). Once the morphological characteristics were measured, the seaweeds were used for the molecular analysis.

2.4. DNA extraction and PCR amplification

The molecular analysis was carried out in the Molecular Markers Laboratory of the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) of the Universidad de Guadalajara in Jalisco, Mexico. Each sample was analyzed individually (10 samples per location). The DNA of the algae was isolated according to the CTAB (Cetyl Trimethyl Ammonium Bromide) procedure of Phillips et al. [15]. The samples were then analyzed for purity and concentration by standard electrophoretic and spectrometric methods [16].

Five ISSR primers (7, 16, 812, DAT, and 902) from 15 sequences selected from the University of British Columbia [17] and Ohio State University [18] databases were chosen for amplification (Table 2). Amplification was carried out in 20 μL of 1X PCR buffer containing 2.5 mM $MgCl_2$, 0.8 μM of primer, 0.25 mM dNTPs, 4 ng of DNA, and 0.05 U of Taq DNA polymerase (Promega, Madison, USA). PCR cycling included 3 min at 95°C, followed by 40 cycles of 45 s each at 95°C (DNA denaturalization), 45 s at 52°C (annealing), 1 min 30 s at 72°C (extension), and a final extension at 72°C for 10 min. The amplified fragments were separated by elec-

Table 3
Marker effectiveness. Parameters for the inter-simple sequence repeat (ISSR) markers used with *Sargassum liebmannii*.

Parameters of marker efficiency	ISSR
Number of individuals	60
Total number of bands (*L*)	40
Polymorphic bands (*p*)	38
Total number primer combinations (*T*)	5
Multiplex ratio (MR; *L/T*)	8
Polymorphism percentage (*%p*)	95
Polymorphic information content (*PIC*)	0.61
Average heterozygosity (*Hav*)	0.85
Marker index (*MI*) *Hav* × *MR*	6.8

trophoresis on 2% agarose gels, and ethidium bromide stain was used for visualizing the bands.

2.5. Data analysis

The genetic variability and informativeness of the ISSR markers were tested by polymorphic information content (*PIC*), heterozygosity per locus (*He*), average heterozygosity (*Hav*), the multiplex ratio (*MR*), and the marker index (*MI*) [19]. A binary presence/absence matrix was obtained from the gels of each primer. The bands were considered for subsequent analysis.

POPGENE v. 1.31 [20] was used to evaluate genetic variability from the percentage of polymorphic loci (*P*) with a 99% criterion, the average expected heterozygosity (*H*) assuming a Hardy-Weinberg equilibrium, the Shannon information index of genetic diversity (*I*), total genetic diversity (*HT*), genetic diversity within populations (*HS*), the coefficient of genetic differentiation between different populations (*Gst*), and the Nei genetic distance (*D*) [21].

Variance heterogeneity was tested using Statgraphics (New Jersey, United States) and was found to be not significantly homogeneous. For each of the variables, a mean comparison Tukey test was performed to group statistically equal variables ($P \leq 0.005$). Information on genetic variability, morphological characteristics, and environmental factors was included in a clustering analysis using the unweighted pair-group method with arithmetic average (UPGMA). Analyses were performed using NTSYS v. 2.21 [22]. The results are represented as a dendrogram.

A principal component analysis (PCA) was carried out to evaluate genetic variability and the interdependence of the morphological characteristics (total length of the thallus, leaf area of the fronds, total number of vesicles, receptacle number, and biomass) and environmental variables (locality, depth, wave exposure, and substrate). In addition, a cluster/SIMPROF analysis was performed on the Euclidian distance matrices constructed from square root-transformed descriptor data to identify similar patterns among the variables as well as the characteristics that had the highest descriptive value and that best explained the genetic and morphological variability of the algae.

3. Results

3.1. Molecular marker informativeness

A unique ISSR band profile was obtained for each individual. A total of 40 fragments were amplified. The number of amplicons per primer, the percentage of polymorphism, and the sizes of the amplicons ranged from 6–11, 75–100, and 150–2500 bp, respectively (Table 2). In Table 3, the effectiveness values of the ISSR markers and the average heterozygosity (*Hav*) are shown, with the latter showing a value of 0.85. Considering that the maximum heterozygosity value is 1, the *S. liebmannii* populations appeared to maintain high genetic variability. These results show that ISSR molecular markers can be used to evaluate the genetic variability of *S. liebmannii* individuals.

3.2. Genetic relationships and genetic variability in Sargassum liebmannii populations

The estimations of genetic diversity within the *S. liebmannii* populations (Table 4) indicated that the *P* value (overall average of 52%) was moderately high for each of the six populations analyzed in this study. The lowest *I* value (0.1356) corresponded to Playa Mora, while the highest value (0.3569) corresponded to La Manzanilla. A *D* value of 0.081 was obtained for Playa Mora, while a *D* value of 0.1175 was obtained for Careyitos, and both populations were located in the southern zone. The analysis of genetic diversity using the *I* and *D* indices showed that there was little heterogeneity between populations, indicating that relatively low genetic diversity was present.

The mean *HT* and *HS* values of the six study sites were low (0.276 and 0.1747, respectively; Table 5). This level of genetic diversity generated a very high *Gst* value of 0.3669, and the genetic diversity among populations and within populations was 36.7% and 63.31%, respectively. The gene flow number (*Nm*) was low (0.826) among the different *S. liebmannii* populations, indicating that the migration rate per generation among populations was low.

According to the Nei genetic distance matrix (Table 6), relatively homologous values (0.95–0.98) were found within the southern populations, whereas low values (<0.93) were found within the northern populations. The estimate of genetic differentiation between different populations for *S. liebmannii* ranged from 0.0232–0.2421, which clearly indicated a separation between the southern and northern populations. The degree of genetic difference was remarkable in the northern zone (*D* values of 0.7850–0.9771) compared to that of the southern zone (*D* values of 0.1387–0.2421). These *D* values indicated that moderate and high levels of genetic distance were present in the northern and southern zones, respectively [23]. The individuals originating from La Manzanilla beach were genetically connected to the populations of Careyitos (*D* = 0.0232).

3.3. Morphological variation

The morphological characteristics of *S. liebmannii* individuals were analyzed. Significant differences ($P \leq 0.05$) in the total length of the thallus (cm), leaf area of the fronds (cm^2), total number of vesicles, receptacle number, and fresh weight (g) were observed between study zones. In the northern zone, especially in Playa Los Muertos, *S. liembannnii* individuals presented the greatest thallus lengths, foliar areas, fresh weights, and vesicle and receptacle numbers compared to those from the southern zone (Table 7 and Fig. 2).

Table 4
Genetic variability within *Sargassum liebmannii* populations based on inter-simple sequence repeat (ISSR) markers.

Locality	*N*	*P%*	*Na*	*Ne*	*H*	*I*
Playa Los Muertos	10	45	1.45	1.2725	0.16	0.2397
Playa Conchas Chinas	10	62.5	1.625	1.4245	0.238	0.3497
Playa Garza Blanca	10	55	1.550	1.4059	0.226	0.3281
Playa Careyitos	10	37.5	1.375	1.1817	0.118	0.1827
Playa Mora, Tenacatita	10	35	1.350	1.1132	0.081	0.1356
Playa La Manzanilla	10	77.5	1.775	1.344	0.226	0.3569
Total	60					
Mean		52	1.520	1.2903	0.175	0.26545

N = number of individuals; *P%* = percentage of polymorphic bands; *Na* = number of alleles per locus; *Ne* = effective number of alleles per locus; *D* = Nei genetic diversity; *I* = Shannon index.

Table 5
Nei analysis of gene differentiation among *Sargassum liebmannii* populations.

	N	H_T	H_S	*Gst*	*Nm*
S. liebmannii	60	0.276	0.1747	0.3669	0.8626

N = number of individuals; H_T = total genetic diversity; *Hs* = genetic diversity within the population; *Gst* = coefficient of genetic differentiation between different populations; *Nm* = gene flow number.

Table 6
Genetic identities and distances of the six *Sargassum liebmannii* populations based on inter-simple sequence repeat (ISSR) data. Abbreviations: Playa Los Muertos (PM); Playa Conchas Chinas (CH); Playa Garza Blanca (GB); Playa Careyitos (CR); Playa Mora, Tenacatita (LM); and Playa La Manzanilla (MZ).

Locality	MZ	LM	CR	CH	GB	PM
Playa La Manzanilla	––	0.9605	0.9771	0.8488	0.8234	0.8705
Playa Los Muertos	0.0403	––	0.9499	0.8273	0.7875	0.8680
Playa Careyitos	0.0232	0.0514	––	0.8280	0.7850	0.8540
Playa Conchas Chinas	0.1639	0.1896	0.1888	––	0.9163	0.7942
Playa Garza Blanca	0.1943	0.2389	0.2421	0.0874	––	0.8567
Playa Mora, Tenacatita	0.1387	0.1416	0.1579	0.2304	0.1547	––

Nei genetic identity values (above diagonal) and genetic distance values (below diagonal).

Table 7
Morphological characteristics evaluated in *Sargassum liebmannii* individuals from different localities. Abbreviations: Playa Los Muertos (PM); Playa Conchas Chinas (CH); Playa Garza Blanca (GB); Playa Careyitos (CR); Playa Mora, Tenacatita (LM); and Playa La Manzanilla (MZ).

Locality	Total length of the thallus (cm)	Leaf area of the fronds (cm^2)	Vesicle number	Receptacle number	Weight (g)
CH	39.38 ± 5.4^{a}	85.31 ± 36.1^{bd}	17.6 ± 9.76^{abc}	52.6 ± 38.48^{abc}	3.7 ± 2.6^{bc}
GB	33.34 ± 8.1^{ab}	64.64 ± 44.4^{bd}	3.9 ± 4.2^{bc}	30.6 ± 17.6^{bc}	4.3 ± 2.7^{abc}
PM	33.84 ± 17.2^{ab}	164.35 ± 69.8^{a}	41.3 ± 35.3^{a}	101.9 ± 59.7^{a}	10.0 ± 9.4^{a}
CR	21.52 ± 5.5^{b}	42.36 ± 6.0^{cd}	6.5 ± 6.2^{bc}	24.0 ± 11.8^{c}	2.6 ± 1.5^{bc}
LM	28.52 ± 4.9^{ab}	114.45 ± 30.4^{ab}	15.2 ± 14.7^{bc}	78.4 ± 39.6^{ab}	4.8 ± 2.4^{abc}
MZ	30.29 ± 6.3^{ab}	85.97 ± 22.6^{bd}	2.3 ± 3.5^{c}	31.1 ± 27.7^{bc}	2.4 ± 1.0^{c}

Values are mean ± standard deviation (n = 10). Different letters (a–d) within each column indicate significant differences according to the post-hoc Tukey mean comparison test ($P \leq 0.05$).

3.4. Dendrogram classification of S. liebmannii individuals based on genetic variability, morphological characteristics, and environmental factors

The grouping analysis of the morphological descriptors (total length of the thallus, leaf area of the fronds, vesicle number, receptacle number, and biomass), genetic variability (average number of loci detected), and environmental variables (locality, depth, wave exposure, and substrate) allowed for three groups to be identified with similar morphological characteristics that were associated with environmental conditions (Fig. 3).

Combined data analysis revealed two groups delimited by geographical area (southern and northern zones), and individuals were grouped according to their populations of origin. The first group (I) was composed of individuals distributed among three localities in the southern zone: La Manzanilla, Careyitos, and Playa Mora. The ISSR markers detected a similarity of 0.33–1.00 among individuals, according to the Jaccard similarity coefficient. The analysis revealed two groups delimited by geographical area (southern and northern zones) in the same way as the morphological data, which grouped individuals to their populations of origin. Individuals belonging to La Manzanilla, Careyitos, and Playa Mora were grouped in the southern zone, while individuals from Playa Garza Blanca, Playa Los Muertos, and Conchas Chinas were grouped in the northern zone. The results of the analysis showed that the highest similarity (0.95) was among individuals from the Playa Mora population, while the lowest similarity (0.63) was found among individuals from Playa Garza Blanca and Playa Los Muertos.

The first group (I) of *S. liebmannii* individuals showed the lowest number of receptacles (average of 24) and biomass (average of 2.5 g). Individuals from this group grew in sites with similar environmental conditions (e.g., intertidal areas not exposed to waves). In this group, an average number of 25.37 loci was also detected.

The second group (II) of *S. liebmannii* individuals was composed of two subgroups, and individuals were distributed in the northern zone among the localities of Playa Garza Blanca, Conchas Chinas, and Playa Muertos (PM). In subgroup 1, individuals showed an average of 40.12 loci and similar characteristics with regard to thallus length (which was the largest in the study; average of 39.38 cm), number of receptacles (average of 78.4), leaf area (average of 114.45 cm^2), and number of vesicles (which was also the largest in the study; average of 17.6). Individuals from subgroup 1 were also distributed in areas exposed to wave action in the subtidal zone. Subgroup 2; was composed of individuals distributed in Playa Muertos (PM). These individuals were collected in the subtidal zone and had morphological characteristics that separated them from those of the other groups with regard to leaf area, (average of 164.34 cm^2), number of vesicles (41.3), number of receptacles (101.9), and biomass (10.1 g), which were the greatest in the study, and an average of 29.51 loci.

A biplot analysis was used to confirm the relationships that were identified in the analysis of variance (ANOVA) among genetic variability, morphological characteristics, and environmental variables. The PCA indicated that three factors explained 70.6% of the total variance. Factor 1 (PC1) explained 36.8% of the variance and was positively correlated with depth, wave exposure, and the total length of the thallus. Factor 2 (PC2) explained 19.68% of the variance and was positively correlated with leaf area, receptacle number, vesicle number, biomass, and the average number of loci. Factor 3 (PC3) explained 14.09% of the variance and was positively correlated with substrate. By plotting data according to PC1, PC2, and PC3, three clusters were identified that showed a clear separa-

Fig. 2. Variations in morphological and structural characteristics (fronds, vesicles, and receptacles) of *Sargassum liebmannii* individuals from different localities in the northern zone [a) Conchas Chinas, b) Playa Garza Blanca, and c) Playa Los Muertos] and southern zone [d) Playa Careyitos; e) Playa Mora, Tenacatita; and f) Playa La Manzanilla].

tion among *S. liebmannii* individuals based on genetic variability, morphological characteristics, and environmental variables (Table 8 and Fig. 4).

The first group was composed of *S. liebmannii* individuals distributed among the La Manzanilla, Careyitos, and Playa Mora sites from the southern zone. These individuals were distributed on sandy rocky platforms not exposed to direct wave action in the intertidal zone. The second group was composed of *S. liebmannii* individuals that presented high total thallus lengths and that were distributed in the subtidal zones of the Garza Blanca, Conchas Chinas, and Los Muertos sites from the northern zone. A higher average number of loci (40.12) was detected in this group compared to that of the first group.

Interestingly, a significant and beneficial effect on morphological characteristics was observed in the third group, which included *S. liebmannii* individuals from Los Muertos in the northern zone. This third seaweed group displayed the highest growth values and reproductive indicators (i.e., leaf area, biomass, number of vesicles, and number of receptacles), which suggests that favorable environmental conditions were present in this site (Fig. 4).

4. Discussion

This is the first study on the genetic variability of *S. liebmannii* populations in the central Mexican Pacific. The Mexican Pacific is

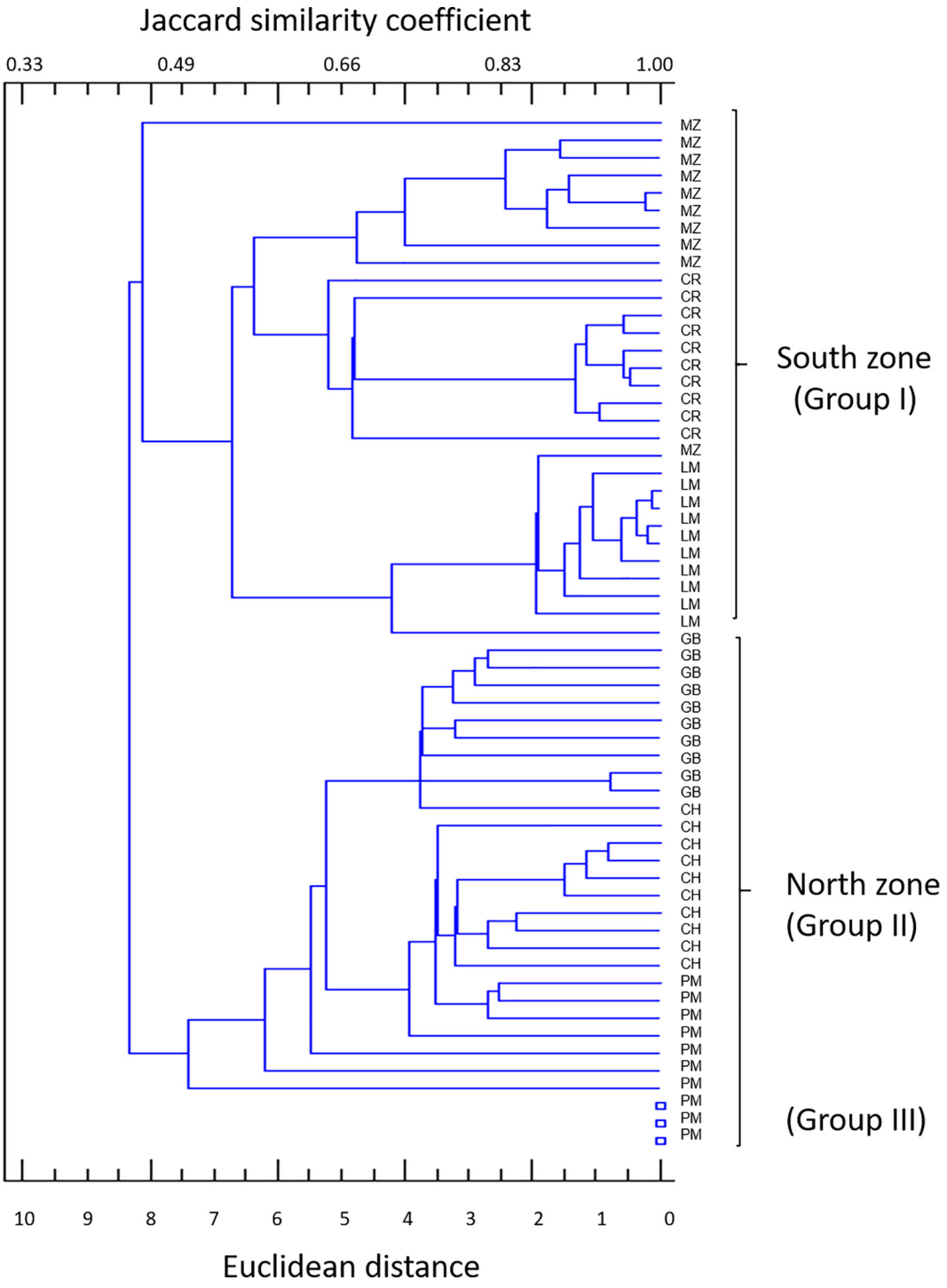


Fig. 3. Dendrogram based on the genetic variability, morphological characteristics, and environmental factors of the *Sargassum liebmannii* populations of the coast of Jalisco.

an area that is highly dynamic given that the California Current, which flows toward the equator, and the Mexican Coastal Current, which flows poleward and to the east, meet in this region. This region is also located in a tropical-subtropical transition zone with a very shallow oxygen minimum zone [24]. The study area is characterized by a salinity gradient of approximately 34.6–34.9 g kg^{-1} and pH values that range between 7.5 and 8.4. The beaches in the northern zone are mainly characterized by being protected by coastal rocks and dunes, whereas the beaches in the southern zone are characterized by current channels and coral reefs [25]. Moreover, in this region, *Sargassum* species are important due to their great industrial potential.

The results of this study are based on 40 loci from 60 individuals, which meets the requirements for an acceptable sample size for the genetic analysis of populations [21,26]. The total number of loci detected with the ISSR markers in this study was considered reliable for genetic analysis [27]. As mentioned by Ren et al. [9], ISSR markers have proven to be efficient and appropriate for detecting high levels of polymorphism in *S. horneri*. The results obtained in this study further support the conclusion that ISSR

Table 8
Loading values of the genetic variability, morphological characteristics, and environmental variables identified by the principal component analysis (PCA) for *Sargassum liebmannii* populations of the coast of Jalisco.

	PC1	PC2	PC3
Locality	0.395886	−0.377459	−0.235462
Depth	0.38006	−0.379441	−0.261611
Wave exposure	0.0820039	−0.0220485	0.569568
Substrate	−0.352579	0.321597	−0.104241
Total length	0.267668	−0.32366	0.365018
Leaf area	0.340174	0.279108	0.337603
Vesicles	0.378169	0.358222	−0.0906936
Receptacles	0.360282	0.42638	0.0594901
Biomass	0.326542	0.334514	−0.231279
Average number loci	0.0313399	0.0704311	−0.477886

markers are useful for detecting genetic variability among *Sargassum* species [28,29].

The level of polymorphism found in this study was 95% (Table 3), which is similar to the results reported by Yu et al. [10] in *S. horneri* and Yao et al. [6] in *S. fusiforme*. The *Hav* value of 0.85 suggests the presence of high genetic variability, indicating that this species is not in danger of extinction. Intra- and interpopulation variability was also found in *S. liebmannii*. The group associations observed in the dendrogram are quite clear because these groupings were based on the geographical area to which each population belonged, which is similar to that reported by Yu et al. [10] and Yao et al. [6].

The *Gst* and *Nm* values suggest that gene drift acts independently in each population, which was also observable in the geographic separation of the groups that were formed in the PCA. Between the southern and northern regions of the Jalisco coast, a large extension of sandy beaches may be found that ranges from Punta Perula in the southern zone to Cabo Corrientes in the northern zone. This confirms the presence of a spatial barrier between the populations and agrees with the results obtained by Kalvas and Kautsky [30].

The analysis of morphological markers is the basis of variability studies. Nonetheless, it is important for morphological marker information to be correlated with the information generated by molecular markers, as much of the morphological variation, despite being under genetic control, depends on environmental stimuli and changes with the maturity stage of the individuals in question [31]. The morphological analysis indicated considerable phenotypic variability among *S. liebmannii* populations along an environmental gradient. Specifically, the *S. liebmannii* populations were grouped according to the degree of habitat similarity, considering spatial interactions (geographic scale) and the results of the genetic analyses.

Overall, the *S. liebmannii* populations in this study showed characteristics that were related to ecological factors and to specific geographic locations. The morphological division became evident in the grouping analysis (Fig. 3), in which the individuals of six populations were divided into three groups according to habitat type (group I, intertidal environment exposed to waves; group II, subtidal zone protected from waves; and group III, subtidal). A similar study was carried out with *S. polyceratium* on Martinique Island [32] and showed that morphological differences were correlated with two environmental factors: water depth and wave exposure. Engelen et al. [33] described an increase in the biomass of algae in wave-exposed subtidal populations, as was found with the Playa Los Muertos population in this study. In some cases, macroalgae assemblages are small and hard because they inhabit intertidal areas with high wave exposure [34], such as in Careyitos.

Other studies of various species of brown macroalgae, such as those that have evaluated abiotic stress, have found that morphological variability is due to differences in coastal environments. For example, Fowler-Walker et al. [35] showed severe morphological changes in *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum* due to wave exposure that were reflected in low frond size and the number of vesicles. In contrast, in coastal regions of the United Kingdom, Fowler-Walker et al. [35] found six species of brown macroalgae that showed increased frond thickness associated with wave exposure. These kinds of notable changes constitute an ecological strategy that seaweed species may employ to mitigate mechanical disturbances or desiccation stress, which are the main factors that drive population fragmentation [36]. In addition, Kalvas and Kautsky [37] stated that populations of *Fucus vesiculosus* were disturbed by waves, which altered the morphological characteristics of the seaweeds. In particular, biomass, thallus lengths, and receptacle frequencies were found to be low, suggesting that these variations were the main factors constituting morphological barriers among populations in nearby and adjacent geographic regions.

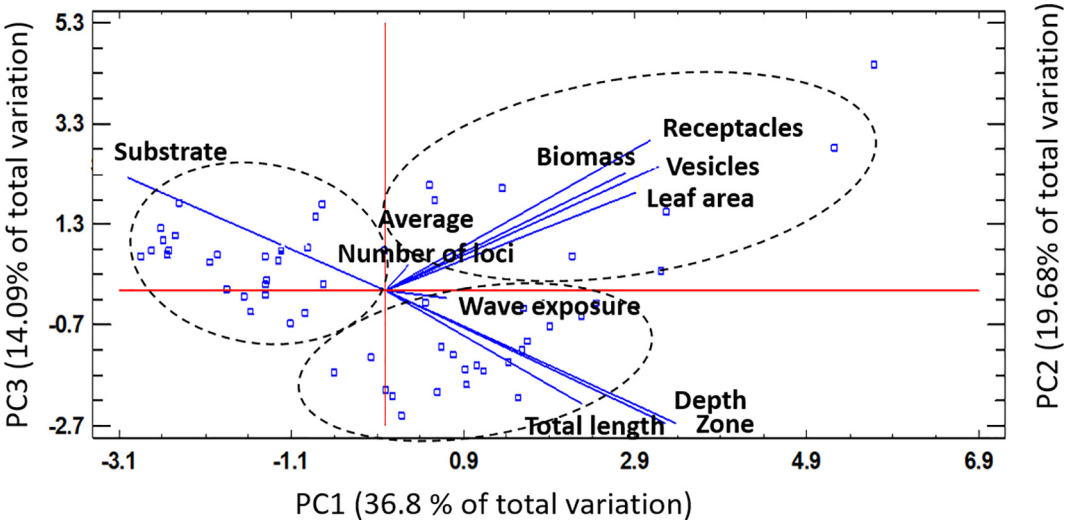


Fig. 4. Principal component analysis (PCA) of the genetic variability, morphological characteristics, and environmental factors of the *Sargassum liebmannii* populations of the coast of Jalisco.

De Paula [38] analyzed the intraspecific morphological variation of *Sargassum cymosum* based on indeterminate barriers of geographical and ecological isolation and found that hybridization, which is a process in which a new species is generated from the crossing of two different species, was at work. Likewise, Fowler-Walker et al. [35] performed a reciprocal transplantation experiment with *Ecklonia radiata* and found that individuals that were transplanted from protected to exposed habitats suddenly changed their morphology, although algae transplanted from exposed to protected sites also altered their morphology albeit slowly. The potential for these effects should not be ruled out from this study. However, these effects may be evaluated in future studies that include larger collections and sampling throughout the entire year in different habitats.

5. Conclusions

A molecular profile was achieved with ISSR markers for all individuals included in this study. This molecular characterization generated information on *S. liebmannii* genotypes on the Jalisco coast. The results of the morphological analysis allowed for three groups with similar morphological characteristics to be identified, which were mainly influenced by the environmental variables of water depth and wave exposure, in addition to their associations with geographical areas. The results presented in this study will contribute valuable information on the *S. liebmannii* genome that may form the basis of future research and may aid in the development of new DNA markers that may specifically characterize populations.

Financial support

This study was supported by the National Council for Science and Technology (CONACyT) [grant number 926742, awarded to Jung-Kim]. This study was financed through the PRO-SNI fund (2019) of the University of Guadalajara.

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Guiry MD. AlgaeBase. In: Guiry MD, Guiry GM. 2021. World-wide electronic publication, National University of Ireland, Galway. Available at: https://www.algaebase.org/search/genus/detail/?tc=accept&genus_id=77&-session=abv4:AC1F06400a3c12AB03pk8B0937D0 [Accessed: 18 April ,2021].
[2] Díaz-Larrea J, Pedroche FF, Sentíes A. Impacto de los estudios moleculares en la ficología comparada mexicana: El caso de las macroalgas marinas. Cymbella 2016;2(1):1–15.
[3] Tapia-Martinez J, Hernández-Cruz K, Franco-Colín M, et al. Safety evaluation and antiobesogenic effect of *Sargassum liebmannii* J. Agardh (Fucales: Phaeophyceae) in rodents. J Appl Phycol 2019;31:2597–607. https://doi.org/10.1007/s10811-019-1752-y.
[4] Hernández-Herrera RM, Santacruz-Ruvalcaba F, Ruiz-López MA, et al. Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). J Appl Phycol 2014;26:619–28. https://doi.org/10.1007/s10811-013-0078-4.
[5] Kilar JA, Hanisak MD, Yoshida T. On the expression of phenotypic variability: why is Sargassum so taxonomically difficult? In: Abbott IA, editor. Taxonomy of Economic Seaweeds with reference to Pacific and Western Atlantic species. p. 95–117.
[6] Yao J, Shuai L, Li S, et al. Genetic analysis of selected *Sargassum fusiforme* (Harvey) Setchell (Sargassaceae, Phaeophyta) strains with RAPD and ISSR markers. J Ocean Limnol 2019;37:783–9. https://doi.org/10.1007/s00343-019-8140-y.
[7] Bi Y, Li J, Zhou Z. Complete sequence of chloroplast genome from *Sargassum vachellianum* (Sargassaceae, Phaeophyceae): Genome structure and comparative analisis. Aquacult Fish 2019;2:157–64. https://doi.org/10.1016/j.aaf.2017.06.006.
[8] Kubo N, Douke A, Nishigaki T, et al. Development and characterization of simple sequence repeat markers for genetic analyses of *Sargassum horneri* (Sargassaceae, Phaeophyta) populations in Kyoto, Japan. J Appl Phycol 2017;29:1729–33. https://doi.org/10.1007/s10811-016-1041-y.
[9] Ren JR, Yang R, He Y, et al. Genetic variation of *Sargassum horneri* populations detected by inter-simple sequence repeats. Genet Mol Res 2015;14:619–25. https://doi.org/10.4238/2015.January.30.3.
[10] Yu S, Chong Z, Zhao F, et al. Population genetics of *Sargassum horneri* (Fucales, Phaeophyta) in China revealed by ISSR and SRAP markers. Chin J Oceanol Limnol 2013;31:609–16. https://doi.org/10.1007/s00343-013-2064-8.
[11] Rodríguez-Montesinos YE, Arvizu-Higuera DL, Hernández-Carmona G. Seasonal variation on size and chemical constituents of *Sargassum sinicola* Setchell et Gardner from Bahía de la Paz, Baja California Sur, Mexico. Phycol Res 2013;56:33–8. https://doi.org/10.1111/j.1440-1835.2008. 00482.x.
[12] Marín A, Casas-Valdez M, Carrillo S, et al. The marine algae *Sargassum* spp. (Sargassaceae) as feed for sheep in tropical and subtropical regions. Rev Biol Trop. 2009;57(4):1271–8. https://doi.org/10.15517/rbt.v57i4.5464.
[13] Rasband, WS. ImageJ, US National Institutes of Health, Bethesda, Maryland, USA. Available at: https://imagej.nih.gov/ij/, 1997-2018 [Accessed 23 October 2019].
[14] Modelo RB, Umezaki I, Liao LM. Morphology of *Sargassum corderoi* (Phaeophyta, Fucales), a New Seaweed Species from Central Philippines. Algae 1998;13:79–83.
[15] Phillips N, Smith CM, Morden CW. An effective DNA extraction protocol for brown algae. Phycol Res 2001;49:97–102.
[16] Velasco-Ramírez AP, Torres-Morán MI, Molina-Moret S, et al. Efficiency of RAPD, ISSR, AFLP and ISTR markers for the detection of polymorphisms and genetic relationships in camote de cerro (*Dioscorea* spp.). Electron J Biotechnol 2014;17:65–71. https://doi.org/10.1016/j.ejbt.2014.01.002.
[17] University of British Columbia. 2015. Ubc Issr Primer Set Canada https://es.scribd.com/doc/155984113/Ubc-Issr-Primer-Set. Accessed: 12 September 2019.
[18] University State the Ohio. 2000. ISSR Protocols. https://studylib.net/doc/7559279/protocols-for-issr-and-pcr. [Accessed: 12 September 2019].
[19] Roldán-Ruiz I, Dendauw J, Van Bockstaele E, et al. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium spp.*). Mol Breed 2000;6:125–34. https://doi.org/10.1023/A:1009680614564.
[20] Yeh FC, Yang RC, Boyle TBJ, et al. POPGENE, the user-friendly shareware for population genetic analysis. Edmonton, Alberta, Canada: Molecular Biology and Biotechnology Center, University of Alberta; 1997.
[21] Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 1979;76(10):5269–73. https://doi.org/10.1073/pnas.76.10.5269. PMid: 291943.
[22] Rohlf FJ. NTSYSpc: numeral taxonomy system. Ver. 2.21. Exeter Software. New York: Stauket; 2009.
[23] Wright S. Evolution and the genetics of populations, Vol. 4: Variability within and among natural populations. University of Chicago Press, Chicago. 1978:1-573.
[24] Cepeda-Morales J, Gaxiola-Castro G, Beier E, et al. The mechanisms involved in defining the northern boundary of the shallow oxygen minimum zone in the eastern tropical Pacific Ocean off Mexico. Deep-Sea Res 2013;76:1–12. https://doi.org/10.1016/j.dsr.2013.02.004.
[25] Enciso-Padilla I, Ríos-Jara E, Esqueda-González MC. Inventario y diversidad taxonómica de las macroalgas de la costa de Jalisco, México. Revista Bio Ciencias 2019;6:. https://doi.org/10.15741/revbio.06.e656e656.
[26] Brown ADH, Weir BS. Measuring genetic variability in plant populations. In: Tankesley SD, Orton TJ (eds), Isozymes in plant genetics and breeding, Part A. 1983:219-239. https://doi.org/10.1016/B978-0-444-42226-2.50016-5.
[27] Ng WL, Tan SG. Inter-simple sequence repeat (ISSR) markers: are we doing it right. ASM Sci J 2015;9(1):30–9.
[28] Zhao G, Zhang Z, Sun H, et al. Isolation of Ty1-copia-like retrotransposon sequences from the apple genome by chromosome walking based on modified Site Finding polymerase chain reaction. Acta Biochim Biophys Sin 2007;39:675–83. https://doi.org/10.1111/j.1745-7270.2007.00328.x.
[29] Noormohammadi Z, Baraki SG, Sheidai M. Preliminarily report on molecular diversity of Sargassum species in Oman Sea by using ISSR and RAPD markers. Acta Biol Szeged. 2011;55(1):19-26. Available at: http://abs.bibl.u-szeged.hu/index.php/abs/article/view/2707 [Accessed: 23 October, 2020].
[30] Kalvas A, Kautsky L. Morphological variation in *Fucus vesiculosus* populations along temperature and salinity gradients in Iceland. J Mar Biol Assoc UK 1998;78:985–1001. https://doi.org/10.1017/S0025315400044921.
[31] Sánchez GJJ, Goodman MM, Stuber CW. Racial diversity of maize in Brazil and adjacent areas. Maydica 2007;52:13–30.
[32] Thabard M, Gros O, Hellio C, et al. *Sargassum polyceratium* (Phaeophyceae, Fucaceae) surface molecule activity towards fouling organisms and embryonic development of benthic species. Bot Mar 2011;54:147–57. https://doi.org/10.1515/bot.2011.014.

[33] Engelen AH, Åberg P, Olsen JL, et al. Effects of wave exposure and depth on biomass, density and fertility of the fucoid seaweed *Sargassum polyceratium* (Phaeophyta, Sargassaceae). Eur J Phycol 2005;40:149–58. https://doi.org/10.1080/09670260500109210.

[34] Hurd CL. Water motion, marine macroalgal physiology, and production. J Phycol 2000;36:453–72. https://doi.org/10.1046/j.1529-8817.2000.99139.x.

[35] Fowler-Walker MJ, Wernberg T, Connell SD. Differences in kelp morphology between wave sheltered and exposed localities: morphologically plastic or fixed traits? Mar Biol 2006;148:755–67. https://doi.org/10.1007/s00227-005-0125-z.

[36] Cappelatti L, Mauffrey AR, Griffin JN. Applying continuous functional traits to large brown macroalgae: variation across tidal emersion and wave exposure gradients. Mar Biol 2019;166:145. https://doi.org/10.1007/s00227-019-3574-5.

[37] Kalvas A, Kautsky L. Geographical variation in *Fucus vesiculosus* morphology in the Baltic and North Seas. Eur J Phycol 1993;28:85–91. https://doi.org/10.1080/09670269300650141.

[38] De Paula EJ, de Oliveira F° EC. Wave exposure and ecotypical differentiation in *Sargassum cymosum (Phaeophyta—Fucales)*. Phycologia. 1982;21:145-53. https://doi.org/10.2216/i0031-8884-21-2-145.1.