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# Molecular diversity of *Sargassum* populations from Persian Gulf Sea coasts

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**A B S T R A C T**

Sargassum is a brown alga in the coasts of warm waters of the world. In Iran this alga found in the coasts of Persian Gulf and Oman Sea. A little information is available from molecular variation of this genus because the genetic variability of this alga. In this study, sixteen populations of five species of Sargassum were analyzed with ISSR bands. These populations were collected from five localities of the Persian Gulf coasts in two seasons (autumn and winter) from 2018 to 2022. We performed clustering and ordination methods for molecular analysis. Also, genetic diversity parameters were measured for all populations. In UPGMA and PCA analyses, the populations of different species separated into distinct clusters. Also, these analyses confirmed the classification of genus to sections. Coefficients of diversity were high for the most populations. The highest amount of diversity was seen in S. swatzii. Furthermore, specific bands were seen in this species.Significant genetic differences and some degree of gene exchange were seen among the studied populations. According to the Mantel test, the genetic diversity of the studied populations is related to geographical distance. In Analysis of molecular variance, 85% of total variation is attributed to among population differences. While 15% of total variation is related to within population variation.

## Introduction

Sargassum C. Agardh (Sargassaceae, Fucales), is a brown alga with different accepted species (Guiry & Guiry, 2013) that distributed in the wide range of warm waters of Iran. Persian Gulf is a Mediterranean sea in west of Asia. This sea is an extension of the Indian Ocean and located between Iran and the Arabian Peninsula. The Persian Gulf has a half dry-half tropical weather. Arvand, Karun, Dialeh, Zab, Jarahi, Mand, Dalaki and Minab are some of the most important rivers that flow into this sea (Mortimer, 1993).

The brown alga Sargassum is a common macro algal genus occurring throughout the coasts of Oman Sea and Persian Gulf (Noormohammadi, et al., 2011; Gharanjik, 2005; Sohrabipour, et al., 2004; Sohrabipour & Rabii, 1999).

These are nutritious and rich source of bioactive compounds such as vitamins, carotenoids, dietary fibers, proteins, and minerals. Also, many active compounds like terpenoids, flavonoids, sterols, sulfated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, pheophytine were isolated from different Sargassum species (Subhash, et al., 2014). These compounds exhibit

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diverse biological activities like analgesic, anti-inflammatory, antioxidant, neuron protective, anti-microbial, anti-tumor, fibrin lytic, immune-modulatory, anti-coagulant, hepatic protective, anti-viral activity etc. (Subhash, et al., 2014).

*Sargassum* with high rate of polymorphism has been studied very little from a view of genetic, reproduction and geographic distribution. DNA-based molecular phylogenetic analyses have shown great promise to resolve taxonomic ambiguity and algal diversity (Leliaert, et al., 2014).

Taxonomic classifications of algae can only be conducted at the genetic level in most cases. These genetic classifications allow for interspecific and intraspecific hypotheses of genetic and phenotype variation. Molecular markers are useful when evaluating variation and estimating inter and intra population diversity in algal species. ISSR markers involve the amplification of DNA segments between two identical microsatellite repeat regions. “ISSRs have high reproducibility due to the use of longer primers (16-25-mers) as compared to RAPD primers (10-mers), which permits to use of high annealing temperature (45-60˚C) leading to higher stringency. This technique requires very small amount of template and is convenient in result recording (Zietkiewicz & al. 1994).

Molecular phylogenetic analyses have been done in this genus by various scientists such as Vos, et al., 1995; Williams, et al., 1990; Zietkiewicz, et al., 1993; Mattio, et al., 2013 etc. So far, few phenetic studies have been done in this genus. As Sargazi & Hashemi-Shahraki, 2022 worked on ISSR study of the genus *Sargassum* from Oman Sea coasts. But, molecular diversity of *Sargassum* species was done in the Oman Sea coasts with ISSR and RAPD markers (Noormohammadi, et al., 2011).

The taxonomy of the genus is very problematic and species identification is complicated by a simple morphology that is often influenced by the conditions of the habitat (Noormohammadi, et al., 2011).

Although recently identification of brown algae, especially *Sargassum* species has attracted attention, in general there is limited information about *Sargassum* populations and the studies performed the identification of species by using morphological characters. And few numerical analyses have been performed to identify the intra and inter population’s variation.

We intend to study genetic variation (among and within *Sargassum* species) in the coasts of the Persian Gulf.

## Materials and Methods

Sixteen populations of *Sargassum* were collected from different localities of Persian Gulf coasts in two seasons (autumn and winter) from 2018 to 2022. These populations belong to 5 species and listed in table 1. These localities were Qeshm (three site), Bandar Abas (one site), and Bandar Lengeh (two sites). Samples from the field were transported fresh to the laboratory, then washed with sterilized seawater and cleaned under a dissecting microscope (Olympus, SZH model) and sorted. Five individuals (talus) were sampled from each population and fixed with silicagel. The voucher specimens are kept in the Herbarium of Sistan and Baluchestan University. The genomic DNA was extracted from 0/3 gr (gram) of talus (leaf-like blades) using CTAB (Cetyl trimethyl ammonium bromide) activated charcoal protocol (Krizman, et al., 2006). The extraction procedure was according to activated charcoal and Polyvinyl Pyrrolidone (PVP) method for binding of polyphenols during extraction and on mild extraction and precipitation conditions. This promoted high-molecular weight DNA isolation without interfering contaminants. The Five ISSR primers were used in this study were UBC 810, 811, 834 and (GA)9T, (GA)9C. In PCR (Polymerase chain reaction) reactions were used 23 µl (microliter) volume containing 18.25 mm (millimeter) $H_2O$; 2.5 mm Tris-HCl buffer at pH 8; 0.35 mm $MgCl_2$; 0.5 mm of each dNTP (deoxy nucleoside triphosphate); 1 µm (micrometer) of a single primer; 20 ng (nanogram) genomic DNA (Deoxyribonucleic acid) and 0.4 mm of Taq DNA polymerase (Bioron, Germany). Amplifications reactions were performed in Techne thermo cycler (Germany) with following steps: an initial denaturation step of 5 min (minute) at 94˚C (centigrade), followed by 35 cycles of pre-amplification (denaturation step of

30 sec (seconds) at 94°C, annealing step of 45 sec at 52°C and extension step two min at 72°C) and a final extension of 10 min at 72° C. Agarose gel electrophoresis and ethidium bromide staining be used for the visualization of PCR products. Band patterns were photographed using a digital camera. Fragment size was estimated by using a 1kb molecular size ladder (Fermentas, Germany).

**Data analyses**

The ISSR bands as binary characters (presence = 1, absence = 0) were coded. The genetic diversity parameters determined in each population included Nei's genetic diversity, Shannon information index (Weising 2005, Freeland & al. 2011), number of effective alleles and percentage of polymorphism. These genetic diversity parameters were performed with GenAlex 6.4 (Peakall & Smouse, 2006).

The UPGMA (UNweighted Pair Group Method Analysis) tree followed by 100 times bootstrapping and and PCA (Principal Coordinate Analysis) after 999 permutations was determined for genetic divergence of the populations. We used both Jaccard and Dice genetic similarity index for these analyses, as they are appropriate for binary data as in ISSR markers. PAST ver. 2.17 (Hammer, et al., 2012) and Darwin ver. 5 (2012) were used for these analyses.

Analysis of molecular variance test to determine genetic differentiation was performed with GenAlex 6.4 and Hickory ver. 1.0 (Holsinger, et al., 2003).

Also The Mantel test was performed to study association between molecular distance and geographical distance for the studied populations (Podani, 2000). The Mantel test was performed with GenAlex 6.4.

**Results**

After electrophoresis and staining of DNA extracted from different populations of this genus, 126 polymorphic and reproducible bands were formed. The highest number of bands (31 bands) was seen in GA(9A). UBC-811 produced the highest number of specific bands. The presence of specific bands was seen in some populations. For example, population of *S. swartzi* from Shibderaz showed highest number of specific bands in UBC-811(600 kb and 1000 kb molecular weight) and UBC-810 (900 kb). *S. swatzii* Zeyton Park and *S. binderi* Shibderaz showed the highest number of total bands (15 bands). Genetic diversity parameters of populations are available in Table 2. The number of effective alleles ranged from 1.150 in Bandar Abas population of *S. cristaefoium* to 1.365 in Shibderaz population of S. swatzii. Shannon index varied from 0.135 Bandar Abas population of *S. cristaefoium* to 0.275 in Shibderaz population of S. swatzii. The highest value of gene diversity was 56.57 that occurred in Shibderaz population of *S. swatzii*.

Grouping of the populations by UPGMA tree can be seen in Figures 1. In general, there are two major clusters. In the first cluster, populations of species of *S.oligocystum* and *S. crassifolium* were placed into separate categories. Populations of these two species are placed in section of *Acanthocarpicae*. Populations of these species are well separated in different subclusters. In the second major cluster were placed populations of *S. cristaefolium*, *S. binderi* and *S. swartzi*. *S. binderi* and *S. swartzi* are placed in a separate sucluster from *S. cristaefolium*. *S. binderi* and *S. swartzi* are in Section of *Binderianeae* and *S. cristaefolium* belongs to section *Sargassum*. Populations were well separated in all species. The results of PCA, as seen in the figure 2; confirm the results of UPGMA method. As can be seen in Figure 2, species and populations of different species of *sargassum* are well located in different parts of the coordinate axis and are well separated.

In the Analysis of molecular variance, significant genetic difference was observed among the studied species (PhiPT value = 0.94, P = 0.001). In this test 85% of total variation was related to among population differences and 15% was related to within populations. AMOVA-paired revealed significant genetic differences ($P < 0.01$), among the studied populations, which shows in spite of potential across populations gene flow, they still have a mean genetic distinctness that differentiate them from each other. The Mantel test produced significant correlation (P = 0.01) between genetic distance and geographical distance of the studied populations. Then, geographical distance corresponds to the genetic diversity of the studied populations.

**Discussion**

As mentioned in the introduction, many phylogenetic studies have been done on several species of this genus, but there are few studies that have investigated the diversity in this genus. For example, we can mention Sargazi & Hashemi-Shahraki's paper, which investigated genetic diversity on fifteen populations of six species of *Sargassum* with ISSR bands from the coasts of the Oman Sea in 2022. In this study, analysis revealed significant genetic differences among populations and some degree of genetic admixture and gene exchange among the studied populations. Or a molecular analysis of *Sargassum* species in Oman sea coasts was performed with Noormohammadi, et al., 2011. In these studies, the diversity amount among populations was high and the populations are well separated within the clusters. In another study, ITS-2, cox3, and rbcL analysis were performed in the northern China seas. Phylogenetic analyses confirmed *S. shandongense* and *S. qingdaoense*, endemic to the Shandong Peninsula, are shown to be synonymous with *S. vachellianum* (Huang, et al., 2017). In another study, molecular-based identification to *Sargassum* using an ITS2 DNA barcode was done. Fresh algae were gathered from Ekas Bay (EB) and Aan Cape (AC) in Lombok. DNA sequences were examined and traced using the Basic Local Alignment Search Tool (BLAST). DNA sequences were processed in MEGA-X to reconstruct the phylogenetic tree and to estimate the genetic distance. Three species were identified to be *Sargassum cf granuliferum*, *Sargassum polycystum*, and *Sargassum oligocystum* (Sulistiyani, et al., 2022).

There are many other phylogenetic studies about different *Sargassum* species from around the world (Soliman &Tawfik, 2021; Le Cam et al., 2020; Richard et al., 2020 and …).

The studied species were placed in three sections. *S. oligocystum* and *S. crassifolium* belong to the section *Acanthocarpeae*. Also, *S. cristaefolium* belongs to section *Sargassum* and *S. binderi*, *S. aquifolium* and *S. swartzii* belong to section *Binderianeae*. As seen, PCA and UPGMA confirmed this classification.

Furthermore, these analyses were well able to show the relationships between species.

In this study, the characterization of plant materials using molecular characters has been used to assess the genetic diversity of *Sargassum* to determine the divergence among populations. The results of ISSR analysis showed high polymorphism among populations. Nei's genetic diversity, Shannon index showed high values for the most populations.

It is likely that most of the populations in this research are ecotypes due to their high diversity and being separated from each other in analyses. In other words, there are many genotypes that are the same ecotypes in this genus.

The highest amount of diversity was seen *S. swatzii*. Furthermore, specific bands were seen in this species. Analysis indicated the presence of genetic polymorphism in the populations of this species, especially in the in Shibderaz population. The presence of high genetic variation in the species of this genus may show that these species would not be in danger of extinction. This is one of the reasons why this alga covers the coasts of the southern seas of the country.

The results show that the ISSR markers were useful for detecting genetic differences among *Sargassum* species.

**Conclusion**

In this research, the amount of diversity in individuals and populations of sampled Sargassum species was investigated. There is very little research on diversity in this genus. Yet, the genetic diversity data obtained in this study is low, suggesting before unreported introductions in both the Oman Sea and Persian Gulf coasts show that we actually know little about this invasive seaweed. There is an urgent need to conduct more studies in this field.

Further work to examine the genetic diversity of Sargassum species in a wider scope is likely to prove interesting and more conclusive. Extensive phenetic and population genetic frameworks on Sargassum species could help future investigations of under-sampled areas, such as in the Iran.

This research confirmed the existing classifications in the genus. The amount of variation between species and populations was high and S. swatzii had the highest amount of variation. There are many ecotypes in this genus as there are sampled populations. In general, this method was suitable for determining diversity between species and within species. In future research, more diverse techniques such as SCoT can be used, to investigate the diversity of species.

Or you can check what environmental indicators have caused this amount of diversity in this genus.

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**Table 1- *Sargassum* populations and their localities.**

| List of species | Location | No. Of Station |
|---|---|---|
| *S. binderi* | Qeshm (Shibderaz), Bandar Lengeh (Dolat Park), Bandar Abas | 1 |
| *S. crassifolium* | Bandar Abas, Qeshm (Shibderaz ) and (Zeyton Park) | 2 |
| *S. cristaefolium* | Qeshm (Shibderaz ), Bandar Abas, Bandar Lengeh (Dolat Park) | 3 |
| *S. swartzii* | Qeshm (Shibderaz ) and (Zeyton Park) , Bandar Lengeh (Dolat Park), Bandar Abas | 4 |
| *S. oligocystum* | Qeshm (Shibderaz ), Bandar Abas, Bandar Lengeh (Dolat Park) | 5 |

**Table 2- Genetic diversity parameters in populations of *Sargassum***

| No. population | populations | Ne | I | He | %P |
|---|---|---|---|---|---|
| 1 | *S. crassifolium*, Bandar Abas | 1.225 | 0.255 | 0.115 | 45.38 |
| 2 | *S. crassifolium*, Shibderaz | 1.252 | 0.202 | 0.117 | 48.56 |
| 3 | *S. crassifolium*, Zeyton Park | 1.157 | 0.162 | 0.098 | 39.67 |
| 4 | *S. crassifolium*,Dolat park | 1.155 | 0.152 | 0.094 | 38.67 |
| 5 | *S. cristaefolium*, Bandar Abas | 1.150 | 0.135 | 0.090 | 26.57 |
| 6 | *S. cristaefolium*, Dolat Park | 1.215 | 0.145 | 0.157 | 28.67 |
| 7 | *S. cristaefolium*, Shibderaz | 1.223 | 0.149 | 0.159 | 29.67 |
| 8 | *S. binderi*, Dowlat Park | 1.221 | 0.143 | 0.145 | 28.25 |
| 9 | *S. binderi*, Bandar Abas | 1.235 | 0.165 | 0.169 | 35.86 |
| 10 | *S. binderi*, Shibderaz | 1.240 | 0.185 | 0.189 | 38.86 |
| 11 | *S. swartzi*, Shibderaz | 1.365 | 0.275 | 0.192 | 56.57 |
| 12 | *S. swartzi*, Zeyton Park | 1.335 | 0.262 | 0.160 | 52.28 |
| 13 | *S. swartzi*, Dolat Park | 1.338 | 0.272 | 0.173 | 53. 28 |
| 14 | *S. swartzi*, Bandar Abas | 1.312 | 0.231 | 0.152 | 50.17 |
| 15 | *S. oligocystum*, Dolat Park | 1.332 | 0.258 | 0.162 | 51. 28 |
| 16 | *S. oligocystum*, Shibderaz | 1.335 | 0.262 | 0.160 | 52.28 |
| 17 | *S. oligocystum*, Bandar Abas | 1.341 | 0.265 | 0.190 | 55.17 |

Ne: No. of effective alleles; I: Shannon index;

He: Gene diversity; P%: percentage of polymorphism.

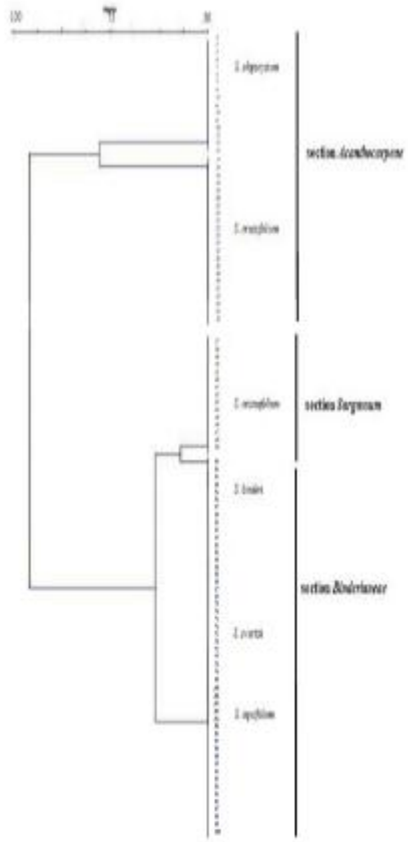

Figure 1. UPGMA tree of populations of the genus Sargassum.

1-15: individuals of *S. oligocystum*, 16-35: individuals of *S. crassifolium*, 36-50: individuals of *S. cristaefolium*, 51-65: individuals of *S. binderi*, 66-80: individuals of S. swartzi.

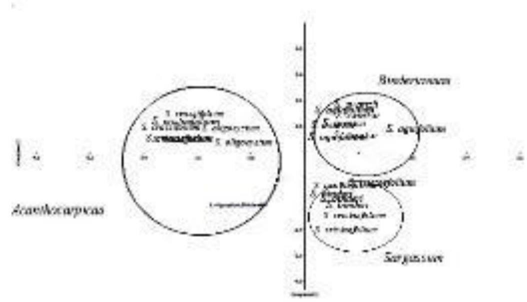

Figure 2. PCA analysis of populations of the genus *Sargassum*.