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MOLECULAR DIVERSITY OF SARGASSUM POPULATIONS FROM OMAN SEA COASTS

F. Sargazi & Sh. Hashemi Shahraki

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Sargassum is widely distributed throughout rocky, coastal upwelling areas in the warm water of the Gulf of Oman and the Persian Gulf in Iran. This alga is of great environmental and industrial importance. However, little information is available that documents the genetic variability of the genus. In this study, fifteen populations of six species of *Sargassum* were analyzed with ISSR bands. These populations were collected from four localities of Oman sea coasts in 2 seasons (autumn and winter) from 2017 to 2020. The ISSR bands were treated as binary characters and coded accordingly. Genetic diversity parameters were determined in each population. Grouping of the populations with UPGMA and PCOA analysis separated the studied species into distinct clusters but few individuals of the Zygocarpiae section were spread in clusters. Analysis revealed significant genetic differences among populations and some degree of genetic admixture and gene exchange among the studied populations.

Fatemeh Sargazi (correspondence<fsargazi@science.usb.ac.ir>) & Shahla Hashemi Shahraki, University of Sistan and Baluchestan, Zahedan, Iran.

Keywords*: Sargassum*; Gulf of Oman; genetic variability; ISSR bands; UPGMA; PCOA

تنوع ملکولی جمعیت‌های سارگاسوم از سواحل دریای عمان

فاطمه سرگزی: استادیار گروه زیست شناسی، دانشکده علوم زیستی، دانشگاه سیستان و بلوچستان، زاهدان، ایران

شهلا هاشمی شهرکی: استادیار گروه زیست شناسی، دانشکده علوم زیستی، دانشگاه سیستان و بلوچستان، زاهدان، ایران

سارگاسوم به طور گسترده‌ای در سراسر مناطق صخره‌ای ساحلی در آب‌های گرم خلیج عمان و خلیج فارس در جنوب ایران حضور دارد. این جلبک از اهمیت زیست محیطی و صنعتی زیادی برخوردار است. با این حال اطلاعات کمی از تنوع ژنتیکی گونه‌ها در دسترس است. در این مطالعه، پانزده جمعیت از شش گونه سارگاسوم با باندهای ISSR مورد تجزیه و تحلیل قرار گرفت. این جمعیت‌ها از چهار منطقه از سواحل دریای عمان در دوفصل پاییز و زمستان سال ۱۳۹۷ تا ۱۴۰۰ نمونه‌برداری شد. باندهای به‌دست آمده به عنوان صفات دوحالته در نظر گرفته و کدگذاری شد. پارامترهای تنوع ژنتیکی در هر جمعیت تعیین شد. گروه‌بندی جمعیت‌ها با تجزیه و تحلیل UPGMA و PCOA، گونه‌های مورد مطالعه را به خوشه‌های متمایز جدا کرد اما تعداد کمی از افراد بخش زیگوکارپیا (Zygocarpiae) در خوشه‌ها پخش شدند. تجزیه و تحلیل داده‌ها تفاوت ژنتیکی معنی‌دار در میان جمعیت‌ها و درجه‌ای از اختلاط ژنتیکی و تبادل ژن در میان جمعیت‌های مورد مطالعه را نشان داد.

INTRODUCTION

Sargassum C. Agardh (Sargassaceae, Fucales), is a brown seaweed with 335 currently accepted species (Guiry & Guiry 2013), widely distributed in the warm waters of the Gulf of Oman and the Persian Gulf in Iran. The Gulf of Oman or the Sea of Makran is a gulf that connects the Persian Gulf with the Strait of Hormuz. It borders Iran and Pakistan on the north, Oman on the south, and the United Arab Emirates on the west.

The brown alga *Sargassum*, or gulf weed, belongs to the order Fucales, containing about 150 different species of brown algae which are generally attached to

rocks along temperate coasts or as pelagic (free-floating) algae in the open ocean. *Sargassum* is a common macroalgal genus occurring throughout the world, except in the Polar Regions (Noormohammadi & al. 2011).

Sargassum spp. are potential source of alginate, which is used as food, liquid fertilizer, and animal feed. These are used for the control of heavy metal pollution and to increase crop production. Also, this alga is the source of many metabolites such as alginic acid, alginates, sulfated fucoidans, pigments, oils, sterols, and mannitol (Wong & al. 2004). Although about 40 tons of biomass of *Sargassum* are produced in the south of Iran and these species are also of ecological value, few detailed genetic studies have been performed on populations of *Sargassum* species in the country (Wong & al. 2004).

The results of genetic diversity studies can be used in conservation programs to combat global climate change. The stability of a population is often attributed to the degree of its genetic variation. The greater the genetic diversity of a population, its chances to resist environmental change, the higher (Kilar & al. 1992). *Sargassum* represents a high rate of polymorphism, which is common in the marine environment.

The taxonomy of the genus *Sargassum* is complicated due to many problems of phenotypic variations caused by polymorphisms and polyphenisms as well as genetic differences among populations (Soe-Htun & Yoshida 1986).

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. Molecular markers are advantageous when evaluating variation and estimating inter and intra-population diversity in algal species.

Many scientists have reported the presence of *Sargassum* from the southern coasts of Iran. Borgesen (1939) identified 26 species of brown algae in the Persian Gulf seashore and recently seven *Sargassum* species were identified in this region (Sohrabipour & al. 2004; Sohrabipour & Rabii 1999). Gharanjik (2005) also reported seven *Sargassum* species in the Sistan and Baluchestan sea shores located in the southeast of Iran.

Noormohammadi & al. (2011) studied morphological and molecular variation in three *Sargassum* species located in the southeast of Iran (Oman Sea). Recently, Shams & al. (2013) reported 19 *Sargassum* species in the Persian Gulf and Oman Sea. Furthermore, other molecular markers were investigated in this genus by different scientists including AFLP (Vos & al. 1995), RAPD (Williams & al. 1990), and ISSR (Zietkiewicz & al. 1993).

The nuclear ITS-2 (Internal Transcribed Spacer 2), chloroplastic partial RuBisCO operon, mitochondrial cox3 (Cytochrome c Oxydase subunit 3) and mt23S (mitochondrial ribosomal DNA 23S subunit) regions were PCR amplified using primers listed in Mattio & al. (2013).

A revision of the genus *Sargassum* in South Africa was performed by Mattio & al. (2015). Based on morphological and molecular analyses of the nuclear ITS2, chloroplastic partial RuBisCO operon, and mitochondrial cox3 and 23S. They identified a total of seven taxa including two new records for South Africa.

In this study, the molecular diversity of six species of the genus *Sargassum* has been examined.

MATERIALS AND METHODS

Fifteen populations of six species of *Sargassum* were analyzed in this investigation (Table 1). These populations were collected from four localities of Oman sea coasts in 2 seasons (autumn and winter) from 2017 to 2020. The sampling sites were Tis, Kachu, Tang, and Gwadar. Ten individuals were sampled from each population and three of them who were more complete and healthier samples were examined. Samples from the field were transported fresh to the laboratory, then washed with sterilized seawater and cleaned carefully under a dissecting microscope (Olympus, SZH model), and sorted. Materials for molecular studies were fixed with silica gel. The voucher specimens are deposited in the Herbarium of Sistan and Baluchestan University. The genomic DNA was extracted from thalli using CTAB-activated charcoal protocol (Krizman & al. 2006). The extraction procedure was based on activated charcoal and Polyvinyl Pyrrolidone (PVP) for the binding of polyphenolics during extraction and on mild extraction and precipitation conditions. This promoted high-molecular-weight DNA isolation without interfering with contaminants. The quality of extracted DNA was examined by running on 0.8% agarose gel. Seven ISSR primers were used in this study, namely: UBC 807, 810, 811, 834 designed by the University of British Colombia and (GA)9T, (GA)9A, (GA)9C. PCR reactions were performed in a 23 µl volume containing 18.25 mM H_2o; 2.5 mM Tris- HCl buffer at pH 8; 0.35 mM $MgCl_2$; 0.5 mM of each dNTP (Bioron, Germany); 1 µM of a single primer; 20 ng genomic DNA and 0.4 mM of Taq DNA polymerase (Bioron, Germany). Amplifications reactions were performed in Techne thermocycler (Germany), using the following steps: an initial denaturation step of 5 min at 94°C, followed by 35 cycles of pre-amplification (denaturation step of 30 s at 94°C, annealing step of 45 s at 52°C and extension step 2 min at 72°C) and a final extension of 10 min at

72° C. Agarose gel electrophoresis following ethidium bromide staining is to be used for the visualization of PCR products. Electrophoresis was performed at 45 V cm^{-1} constant wattage. Fragment size was estimated by using a 1kb molecular size ladder (Fermentas, Germany). Band patterns were evaluated manually in a transilluminator, and photographed using a digital camera.

Data analysis

The ISSR bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). Genetic diversity parameters were determined in each population. These parameters were Nei's genetic diversity (He), Shannon information index (I) (Weising 2005, Freeland & al. 2011), number of effective alleles, and percentage of polymorphism. The genetic divergence of the studied populations was checked by the UPGMA tree followed by 100 times bootstrapping, principal coordinate analysis (PCoA) after 999 permutations. PAST ver. 2.17 (Hammer & al. 2012) and DARwin ver. 5 (2012) were used for these analyses.

Genetic differentiation of the studied species and populations was studied by AMOVA (Analysis of molecular variance) test (with 1000 permutations) as performed in GenAlex 6.4 (Peakall & Smouse 2006) and Hickory ver. 1.0 (Holsinger & al. 2003).

The Mantel test (Podani 2000) was performed to study the association between molecular distance and geographical distance of the studied populations.

Table 1. Collection data of the examined *Sargassum* species and populations in this study.

species	Latitude	Longitude	Location	No. of Station
S. angustifolium, S. glaucescence, S. swartzii, S. tenerrimum	25º 16' 57"	60º 38' 52"	Tis	1
S. bacciferum, S. baccularia	25º 14' 32"	60º 50' 51"	Kachu	2
S. glaucescence, S. tenerrimum, S. angustifolium	25º 21' 52"	59º 54' 50"	Tang	3
S. glaucescence, S. swartzii, S. tenerrimum, S. bacciferum, S. baccularia, S. angustifolium	25º 10' 35"	61º 30' 48"	Gwadar	4

Table 2. Genetic diversity parameters measured in populations of *Sargassum*. Abbreviations: Ne: No. of effective alleles; I: Shannon index; He: Gene diversity; P%: percentage of polymorphism.

No. population	populations	Ne	I	He	%P
1	*S. swatzii.* Tis	1.324	0.255	0.167	48.38
2	*S. swatzii.* Gwadar	1.242	0.202	0.137	35.56
3	*S. angustifolium.*Tis	1.167	0.162	0.098	27.67
4	*S. angustifolium.*Tang	1.157	0.142	0.094	26.67
5	*S. angustifolium.*Gwadar	1.155	0.140	0.090	26.57
6	*S. glaucescence.*Tis	1.225	0.245	0.157	45.55
7	*S. glaucescence.*Tang	1.220	0.242	0.155	45.25
8	*S. glaucescence.*Gwadar	1.210	0.225	0.145	44.25
9	*S. tenerrimum.*Tis	1.245	0.215	0.139	35.86
10	*S. tenerrimum.*Tang	1.240	0.205	0.129	33.86
11	*S. tenerrimum.*Gwadar	1.321	0.260	0.150	47.28
12	*S. bacciferum.*Tis	1.332	0.271	0.165	48.28
13	*S. bacciferum.*Gwadar	1.348	0.252	0.173	54. 28
14	*S. baccularia* .Tis	1.342	0.265	0.155	54.17
15	*S. baccularia* .Gwadar	1.351	0.272	0.175	55.17

RESULTS

156 polymorphic and reproducible bands were formed from the seven primers used in this experiment. GA(9C) produced the highest number of bands (34 bands). Highest number of specific bands was produced with UBC-810. Some of the populations showed presence of specific bands. For example, *S. baccularia* showed highest number of specific bands in UBC-810 (1000 kb and 1100 kb molecular weight) and UBC-811(1000 kb). Similarly, *S. swartzii* had single specific bands with 900 kb molecular weight produced by primer GA (9T). The highest number of total bands was observed in *S. swatzii* Tis2 and *S. angustifolium* Gwadar1, with 24 bands.

Genetic diversity parameters of populations are provided in Table 2. The number of effective alleles ranged from 1.155 in Gwadar population of *S. angustifolium* to 1.351 in Gwadar population of *S. baccularia*. Shannon index varied from 0.140 Gwadar population of *S. angustifolium* to 0.272 in Gwadar population of *S. baccularia*. The highest value of gene diversity occurred in the Gwadar population of *S. baccularia* was 55.17.

Grouping of the populations based on Nei's genetic distance by the UPGMA tree is visible in Fig.1. Two major clusters were formed. In the first main cluster, populations of species of *S. baccularia*, *S. swartzii*, and *S. bacciferum* were placed into separate categories and well separated. Populations of *S. baccularia* are placed in the section *Polycystae*. Also, populations of *S. swartzii* and *S. bacciferum* are included in sections *Binaderiana* and *Sargassum*, respectively. The populations and individuals of these species are also well separated in the first major cluster.

The second major cluster is comprised of populations of *S. tenerrimum*, *S. glaucescens*, and *S. angustifolium* populations, and each of these species is placed in a separate subcluster. *Sargassum angustifolium*, *S. tenerrimum*, and *S. glaucescens* are in section *Zygocarpiae*. Some individuals of populations showed similarities and did not separate well in the second major cluster. In PCoA analysis (Fig. 2), species and their populations in section *Zygocarpiae* are well separated from the other sections and are located farther away from them. This analysis was well able to show the relationships among species. As can be seen in Fig. 2, species and populations of different species of *sargassum* are well located in different parts of the coordinate axis and are well separated.

AMOVA test produced significant genetic differences among the studied species (PhiPT value = 0.92, P = 0.001). The test also revealed that 82% of the total variation is attributed to among-population differences and 18% due to within-population variation. Therefore, these results revealed the genetic distinctness of the studied species. The Mantel test was not significant and geographical distance did not correspond to the genetic diversity of the studied populations (R^2 = 0.03, P = 0.20).

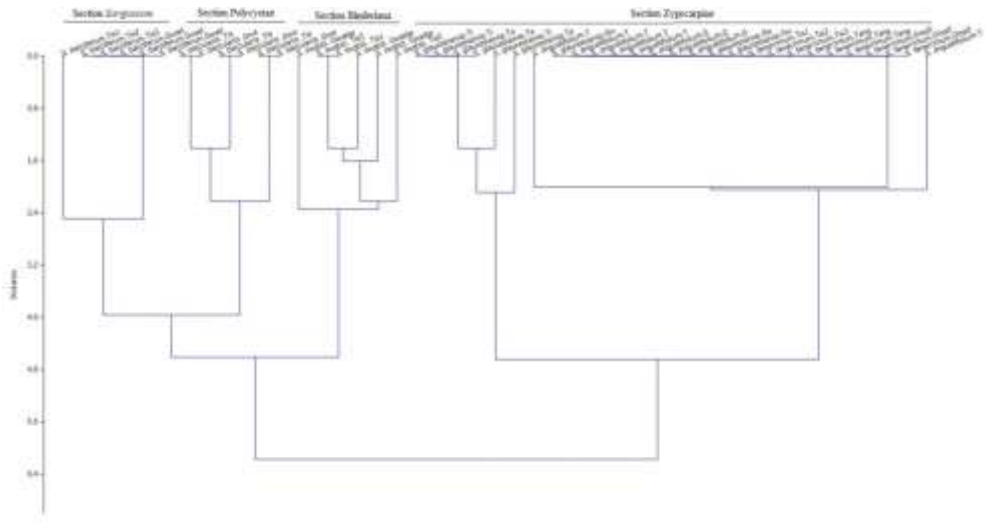

Fig. 1. UPGMA tree of populations of *Sargassum* showing two major clusters and related subclustered.

DISCUSSION

Noormohammadi & al. (2011), studied three species of *Sargassum* including *S. tenerrimum*, *S. glaucescens* and *S. ilicifolium*, that are widely distributed the Oman Sea coasts, ISSR marker. In this study, results of ISSR analysis showed high polymorphism. Twelve ISSR markers including single and combined primers showed high polymorphism (>94%). Nei's genetic diversity, Shannon index showed high values between populations.

We also observed high polymorphism in these species. Genetic diversity parameters that were determined in each population, showed that polymorphism percentage in *S. tenerrimum* is low, a similar result as Noormohammadi & al. (2011). But, their polymorphism percentage in *S. glaucescens* was higher than our study, which can be related to the differences in the studied populations. In both studies, the species of *S. tenerrimum* and *S. glaucescens* were placed in separate clusters.

Lydiane & Claude (2010) studied morphological diversity and DNA phylogenies of species of *Sargassum*. They performed Neighbour-Joining (NJ) analysis based on some Gene Bank sequences. Results highlighted the weak taxonomic value of traditional characters used to classify species, and they mentioned some sections of this genus need to be re-assessed. Classification of this genus by NJ analysis in different sections is in agreement with our study. In this phylogenetic study, re-classification and improvement of treatment of species of the genus are emphasized, but the genetic variation among the populations of species is not discussed. However, in the present study we evaluated genetic variation among and within the species.

Our results showed that the ISSR analysis can well cluster the different populations of *Sargassum* species. Also, PCoA analysis was well able to show the relationships between species and populations. In the classifications of *Sargassum*, three species including *S. angustifolium*, *S. tenerrimum*, and *S. glaucescens* are placed into section *Zygocarpiae*. *Sargassum swartzii* is placed in section *Binaderiana*. *Sargassum baccularia* and *S. bacciferum* are placed in sections *Sargassum* and *Polycystae*, respectively. The UPGMA tree and the PCoA analysis also clearly separated these sections (Figs. 1 & 2).

In ISSR trees, all species were located in separate clusters. Few individuals of populations of section *Zygocarpiae* showed similarities, but individuals of sections *Sargassum*, *Binaderiana* and *Polycystae* were well separated.

Diversity indices in *S. baccularia* were higher than in other species. Furthermore, specific bands were seen in this species. Analysis indicated the presence of genetic polymorphism in the populations of this species, especially in the Gwadar population. The presence of a high genetic variation in this species may indicate that it would not be in danger of extinction because of its genetic content.

The ISSR markers were useful for detecting genetic differences among *Sargassum* species. The results indicate that a genotypic study is beneficial for documenting the 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. However, we suggest larger collections and sampling throughout the entire year in different habitats to be incorporated into future studies.

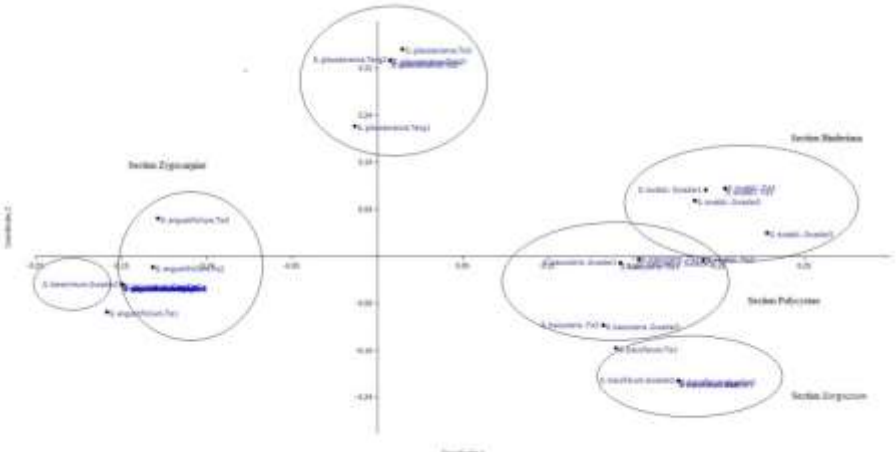

Fig. 2. PCoA analysis of the population of *Sargassum*, showing the grouping of species in different sections.

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REFERENCES

Borgesen, F. 1939: Marine algae from Iranian Gulf. In Danish Scientific Investigation in Iran. Jesen K & Sparck Reds., 42-141 Copenhagen. Einar Munksgaard.

Freeland, J.R. & Kirk, S.D.P. 2011: Mol. Eco. Wiley-Blackwell, UK, pp. 449.

Gharanjik, B.M. 2005: Determination of biomass and expansion of Algae and preparation of Persian Golf and Oman Sea Algae Atlas. Published in: Research Institute of Fisheries of Iran.

Guiry, M.D. & Guiry, G.M. 2013: World-wide electronic publication, National University of Ireland, Galway.

Hammer, O., Harper, D.A.T & Ryan, P.D. 2012: PAST: Paleontological Statistics software package for education and data analysis. Paleo. Electro. 4: 9.

Holsinger, K.E. & Lewis, P.O. 2003: Hickory: a package for analysis of population genetic data v1·0.University of Connecticut, Storrs*:* Distributed by the authors*;* Version 1.0.4 available for download at http://darwin.eeb.uconn.edu/ hickory/hickory.html.

Kilar, J.A., Hanisak, M.D. & Yoshida T. 1992: On the expression of phenotypic variability: why is Sargassum so taxonomically difficult? I.A. Abbott (Ed.), Taxonomy of Economic Seaweeds with reference to Pacific and Western Atlantic species, pp. 95-117.

Krizman, M., Baricevic, D. & Prosek, M. 2006: Fast quantitative determination of volatile constituents in fennel by headspace gas chromatography. Anal. Chim. Acta. 557: 267–271.

Mattio, L., Zubia, M., Loveday, B., Crochelet, E., Duong, N., Payri, C.E., Bhagooli, R. & Bolton, J.J. 2013: *Sargassum* (Fucales, Phaeophyceae) in Mauritius and Reunion, western Indian Ocean: taxonomic revision and biogeography using hydrodynamic dispersal models. Phycol. 52: 578–594.

Mattio, L., Anderson, R.J. & Bolton, J.J. 2015: A revision of the genus Sargassum (Fucales, Phaeophyceae) in South Africa South African. Jour. Bot. 98: 95–107.

Noormohammadi, Z. Ghasemzadeh, Baraki S. & Sheidai M. 2011: Preliminarily report on molecular diversity of *Sargassum* species in Oman Sea by using ISSR and RAPD markers. -Act. Bio. Szeg. 55(1): 19-26.

Noormohammadi, Z., Ghasemzadeh Baraki, S., Sheidai, M., Rafiee, F. & Gharanjik, B. M. 2011: Morphological diversity of Sargassum species of Iran. Gen. Cons. 10 (39): 1-22.

Peakall, R. & Smouse, P. E. 2006: GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Eco. Notes. 6: 288–295.

Podani, J. 2000: Introduction to exploration multivariate biological data. Backhuys publisher, Leiden, pp. 407.

Shams, M., Afsharzadeh, S., Balali, Gh & De Clerck, O. 2013: Revision *Sargassum* species (Fucales, Phaeophyceae) from Persian Gulf and Oman Sea (Iran) based on morphological and phylogenetical analyses. 18th National Symposium on Applied Biological Sciences, on February, Ghent University, Ghent, Belgium.

Soe-Htun, U. & Yoshida, T. 1986: Studies on morphological variations in *Sargassum cristaefolium* C. Agardh (Phaeophyta, Fucales). -Jap. J. Phycol. 34 (4): 275-281.

Sohrabipour, J., Nejadsatari, T., Assadi, M. & Rabii, R. 2004: The marine algae of the southern coast of Iran, Persian Gulf, Lengeh area. -Iran. J. Bot. 10 (2): 83-93.

Sohrabipour, J. & Rabii, R. 1999: A list of marine Alexandria University for his kind help and reviewing the algae of seashores of the Persian Gulf and Oman manuscript. Sea in the Hormozgan Province. Iran. J. Bot. 8: 131-162.

Vos, P., Hogers, R., Bleeker, M., Reijians, M., Lee, T., Hornes, M., Frijters, A., Pot, J., Pleman, J., Kuiper, M. & Zabeau, M. 1995: AFLP, a new technique for DNA fingerprinting. Nucleic Acid Res. 23: 4407-4414.

Weising, K., Nybom, H., Wolff, K. & Kahl, G. 2005: DNA Fingerprinting in Plants. Princi ples, Methods and Applications. Taylor and Francis.

William, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. -Nucleic Acid Res 18: 6531-6535.

Wong, C.L., Gan, S. & Phang, S. M. 2004: Morphological and molecular characterization and differentiation of *Sargassum baccularia* and *S. polycystum* (Phaeophyta). -Jour. App. Phyco. 16: 439-445.

Zietkiewicz, E., Rafalski, A. & Labuda, D. 1993: Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. -Genomics 20: 176-183.