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Morphological identification and callus induction of most abundant brown seaweed from the coast of Karachi, Pakistan

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ABSTRACT

BACKGROUND: Marine macro-algae (Phaeophyta) is a plentiful source of structurally diverse bioactive compounds and remains largely unexploited for nutraceutical and pharmaceutical applications. However, species richness and great phenotypic plasticity in the order Fucales make species identification challenging, and complex growth requirements for marine brown algae render the selection of suitable growth media difficult.
OBJECTIVES: This study aimed to identify the most abundantly found brown seaweed at the coast of Karachi and to design a cost-effective growth medium for large-scale macro-algal culturing and production.
METHODS: Brown seaweed was collected from Sandspit Beach, Karachi, and decontaminated by sterilization method. Sargassum species were identified using a traditional morphological approach. Sodium nitrite (NaNO2), Sodium dihydrogen phosphate (NaH2PO4), Ferric Ethylenediaminetetraacetic acid (Fe-EDTA), Manganese (II) chloride (MnCl2), Cobalamin (vitamin B12), Biotin (vitamin B7) and plant growth regulators (PGRs) were selected as essential additives for modifications in Guillard-Ryther F2 media, Murashige-Skoog (MS) media and Walne's media recipes, followed by monitoring of algal growth efficiency on these modified culture media.
RESULTS: The most abundant brown seaweed found on the Karachi coast was identified as Sargassum *fluitans* and it was observed that modified Guillard-Ryther (Guillard F2) media demonstrated higher brown algal growth and less incubation time compared to modified MS media whereas no algal growth was noted on Walne's media.
CONCLUSION: We reported first time the phenotypic characteristics of *S. fluitans* from Pakistan and the modified Guillard F2 media can be used as an algal growth media and useful for industrial-scale applications.

Keywords: Seaweed, Phaeophyta, Phenotype, Callus induction, F2 culture media

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INTRODUCTION

The Karachi coast (100 km) is located on the Arabian Sea (1). and includes numerous beaches and islands. The coastal waters around the Sandspit beach are inhabited by a variety of marine algae. Algae use sunlight to produce their own food and are vital for the aquatic food web and ecosystem and provide habitats and source of food to a variety of marine life. Humans have also using algae for millennia as food supplements and commercial purposes (2). Annually, more than 180,000 tons of dry-weight seaweeds are harvested globally and a turnover of USD 5.7 billion has been estimated (3). Phycocolloids (alginate, agar, agarose, and carrageenan) are prepared from seaweeds, of which alginates are primarily extracted from brown seaweeds (4).

Over 72,500 algal species are found globally, which are broadly divided into macroalgae

(seaweed/sea vegetables) and microalgae (cyanobacteria) (5). Macroalgae are further categorized on the basis of pigment they produce into brown (Phaeophyceae), green (Chlorophyceae), and red (Rhodophyceae) seaweeds. Phaeophyceae are found in salt water only and are most commonly found in the ocean at depth of 50-75 ft (benthic) and/or floating along the coastal area (pelagic) (6). Phaeophyceae is a huge group that senses as "dusky plants" found in temperate or polar seas (7) and some species, such as Ascophyllum nodosum, Kelp, Rockweed (Fucus), and *Sargassum* are commercially important (6). The genus *Sargassum C. Agardh* displays the highest phenotypic plasticity and in the order Fucales, has the highest number of species with at least 570 reported species (2). Currently, the genus *Sargassum* has been divided into four subgenera (*Arthrophycus*, *Bactrophycus*, *Sargassum*, and *Phyllotrichia*) and 12 sections (8). *Sargassum* genus identification goes back to the 19th century and is based on differences in macromorphological features. Several efforts have been made to precisely identify *Sargassum* species based on traditional morphological characteristics such as blade shape, size, length and width, axis development, pneumatocysts (vesicles or bladders), receptacles and holdfast (9). Presence of ambiguous characters or variability in features between individuals of the same species or even within the same individuals or different growth stages, makes species identification notoriously challenging (1). This plasticity may be attributed to climate change or environmental factors. Although, several authors prefer traditional phenotypic approach or in combination with chemical composition (type of metabolite or phycocolloid structure) approach as a taxonomic tool to distinguish species, these values may vary depending on the season specimens are collected. The use of DNA molecular analyses presents an alternative approach to traditional morphological and chemical concepts (8, 10).

To address the increasing market demand, seaweed tissue culture (STC) represents a promising emerging field. Selection of fast growing algal species, removing of environmental stressful conditions, adjustment of abiotic factors and addition of growth promoting substances has predicted promising future in algal biotechnology (11). These factors are adjustable in the laboratory under controlled conditions. STC is of two types: direct regeneration and callus induction (indirect regeneration). Callus induction (CI) is a sustainable harvesting method, which yields homogenous cells and disease-free bio-products at a faster rate. Callus is a cluster of undifferentiated and unorganized cells, forms as part of the wound response in plants. CI is a crucial early step and has been found influenced by several abiotic factors including light irradiance, temperature, plant growth regulators PGRs, and gelling conditions (12). Successful STC depends on various critical steps, including surface sterilization, axenic explant preparation, culture conditions and regeneration. These factors vary with selected species and may be influenced by the geographical location or season.

The commonly used light sources for brown seaweeds are white, red, and blue light with intensity 5-200 umol m^{-2} S^{-2} for 12-14 hours and optimum temperature ranges from 15-25 ℃(13). A number of culture media have been proposed appropriate for different seaweed CI including seawater, enriched seawater (ESW), modified ESW, Provasoli enriched seawater (PES) (14), Murashige and Skoog (MS), modified MS, Guillard and Ryther (f/2), modified f/2, ASP 12, ASP-12-NTA, modified ASP-12-NTA with calcium and Walne's media (15). Almost all known PGRs have been detected at levels comparable to those found in higher plants, suggesting their involvement in a variety of complex metabolisms (16). The discrepancy in the response of algae to PGRs may be due to a lack of knowledge about the physiological role of these agents in their growth and cell differentiation (17). However, the effects of PGRs on callus formation in algal explants is disputable, several PGRs had been claimed for CI including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), picloram, kinetin, 1-napthaleneacetic acid (NAA), forchlorfenuron (CPPU), 2,4-dichlorophenoxyacetic acid (2,4-D), uniconazole, and 6-benzylaminopurine (BAP) (18).

The aim of this study was the morphological identification of the most dominant species of *Sargassum* found at the coast of Karachi and to determine the most appropriate algal media for *S. fluitans* callus induction.

MATERIALS AND METHODS:

The sample of healthy, mature brown seaweed (Phaeophyta) was collected as a sub littoral drifting material from Sandspit beach located at 24º 50' 40.8"N, 66º 54' 08.6"E from inter tidal zone in food grade polythene bag during February 2021. The sample was washed with sea water 3 times to remove the epitphytes, debris, and sand. The average weight of the sample was two Kg. Sample from the field was transported fresh to the laboratory and stored at 4-8 ℃. For morphological identification, different parts were fixed with 4% formaldehyde-seawater solution and mounted on herbarium sheets. Brown seaweed was taxonomically identified using traditional morphological characteristics such as algae color, height of talus, leaf shape, leaf margin, leaf apex, leaf width, leaf length, midrib presence/absence, number of vesicles, vesicle apex, vesicle shape, vesicle stalk, receptacle types, and axis development as described previously (1).

Before seaweed tissue culture (STC), the thallus of the *Sargassum fluitans* was washed with sterilized seawater three times to remove the debris and sand. After this the sample was kept in 5% sodium hypochlorite (NaOCl) for 5 minutes, then steeped in a 1.5% KI solution for ten minutes followed by sterilization in 70% ethanol 3 minutes (19). Leaf axenic explants were prepared using previously described method (14).

Briefly, the blade was cut into two halves (upper-terminal part was labeled as (A) and lower-basal part was labeled as (B). The whole blade with flotation vesicles was labeled as (C). Each leaf axenic explant (A-C) was inoculated onto following three culture media.

1) Modified Guillard-Ryther F2 media: modified by adding 84.5-gram $NaNO_2$, 20-gram Sodium dihydrogen phosphate (NaH_2PO_4), 0.2-gram Cobalamin (vitamin B_{12}), 0.5 gram Manganese (II) chloride ($MnCl_2$) and plant growth regulators (PGRs); 6-benzylaminopurine (BAP) and Indole-3-butyric acid (IBA).

2) Modified Walne's media: modified by adding 2 gram Cobalamin (vitamin B_{12}) and 14 ug biotin (vitamin B_7) and PGRs (BAP and IBA).

3) Modified Murashige-Skoog media: modified by adding 20 gram NaH_2PO_4, 0.02 gram Cobalamin (vitamin B_{12}), 0.5 gram biotin (vitamin B_7), 0.5 gram $MnCl_2$, 0.8 gram Ferric Ethylenediaminetetraacetic acid (Fe-EDTA) and PGRs (BAP and IBA).

All three media were also added with 0.8% Bacto agar (Oxoid, UK). The incubation temperature was 14-22 °C and photoperiod duration was 9:15 (L:D) with cool-white fluorescent lamp at 60 µmol photons m^{-2} s^{-1}. Observations for callus induction were recorded every week up to 03 months.

RESULTS

Morphological identification (*Sargassum fluitans*):

The color of the macroalgae (phaeophycea) was yellow-brown and height of the talus was 20-70 cm. Leaves (blades) were simple, sessile, thin, opaque and arranged in alternate pattern. A total of 20-50 floating vesicles were present on a single mature branch. Receptacles were undifferentiated. This brown alga seems pelagic as holdfast was absent **Figure 1 (A).** Leaf shape was linear-lanceolate with both basal and terminal portions narrow and acute apex. Leaves length was in the range of 1-2 cm and width 0.4-0.8 cm**.** The leaf margins were crenate (rounded toothed) and midrib was absent **Figure 1 (B).** The floating vesicles (air bladders) were stalked, glabrous and round to spherical in shape. The size of the floating vesicle was 0.3-0.6 cm in diameter **Figure 1 (C).** Stem was round, smooth, spineless and glabrous, having the diameter of 0.4 mm **Figure 1 (D).**

Figure 1: This figure represents morphological characteristics of *Sargassum fluitans* that were identified **(A)** Mature branch of *Sargassum fluitans* showing stem, floating vesicles, receptacles and leaf. **(B)** Leaf's shape, length, width, margins, apex and midrib. **(C)** Floating vesicle's shape and size. **(D)** Stem's shape and diameter

Growth of *S. fluitans* on different culture media

Sample A and B from *Sargassum fluitans* showed no callus induction on any three media. Sample C from *S. fluitans* showed significant callus induction on two media *i.e.* modified Guillard-Ryther F2 (Guillard F2) media **(Figure: 2)** and modified Murashige-Skoog media (**Figure: 3)**, whereas no callus induction was noted on modified Walne's media **(Figure: 4)**. *S. fluitans* callus induction on modified Guillard-Ryther F2 media was obvious after six weeks and significant after eight weeks **(Figure: 2).**

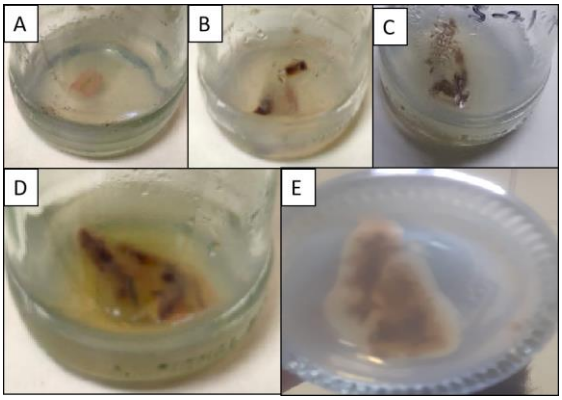

Figure 2: This figure represents callus induction of *Sargassum fluitans* with different time intervals on modified Guillard-Ryther F2 (Guillard F2) media in a screw cap glass jar. (A) Two week (B) Four week (C) Six week (D) Eight week (E) Eight week (jar bottom).

The callus induction of *Sargassum fluitans* was comparatively slow on modified Murashige-Skoog Media and was noted after eight weeks **(Figure: 03)**. The rate of *S. fluitans* callus induction was significantly lower 4:3 on modified Murashige-

Skoog media compared to modified Guillard-Ryther F2 media.

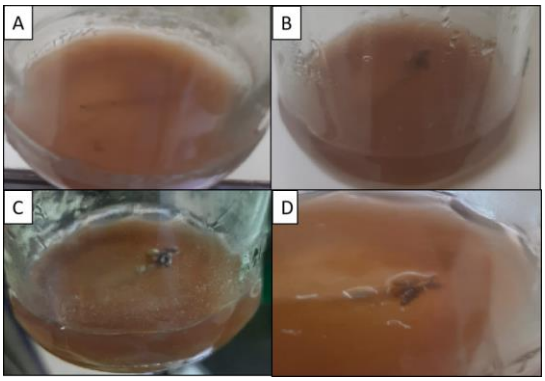

Figure 3: This figure represents callus induction of *Sargassum fluitans* with different time intervals on modified Murashige-Skoog media in a screw cap glass jar. (A) Two week (B) Four week (C) Six week (D) Eight week

There was no callus induction noted on modified Walne's media upto eight week **(Figure: 04).**

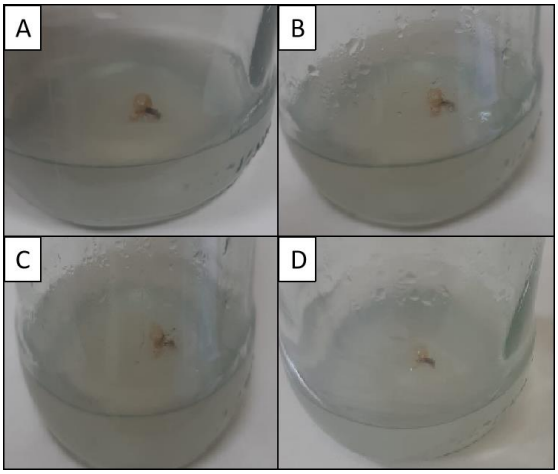

Figure 4: This figure represents callus induction of *Sargassum fluitans* with different time intervals on modified Walne's media in a screw cap glass jar. (A) Two week (B) Four week (C) Six week (D) Eight week

DISCUSSION:

Sargasso seaweeds have grown in the Sargasso sea for millennia and recently the blooming of massive quantities of two holopelagic species *Sargassum fluitans* and *S. natans* have been noticed in the Arabian sea coast. The first taxonomical record from northern Arabian sea dates back to 1934 which enlist several brown algae (20). A comprehensive taxonomic study of brown algae on the coast of Karachi reported 14 species; however, the morphological characters of only *Sargassum tenerrimum* were described (21). Another survey from the coast of Makran enlisted 61 genera and 99 species, of which they reported 07 species of *Sargassum* however, they did not describe the morphological features (22). A recent study from the coastal waters of Sindh reported 36 species, of which 16 were Phaeophyceae, 12 Rhodophyta, and 08 Chlorophyta and again morphological characteristics were missing (23). Another recent study at the Karachi coast listed 17 species and they reported most of the self-contained underwater breathing apparatus (SCUBA) diving sites were dominated by *Sargassum* communities (24). To our knowledge, though several studies from Pakistan report a wide variety of brown seaweeds (25), information describing detailed morphological characteristics is lacking. In this study, we report first time the morphological features of *Sargassum fluitans* based on previously described traditional characters.

Sargassum species identification is challenging as morphology displays great phenotypic plasticity which is further augmented by polymorphism and a large number of species in the order Fucales (26). Several attempts have been made to precisely list the differences in traditional macro-morphological characters (8, 27). A relatively detailed study from Oman described the morphological diversity of six *Sargassum* species using 23 characters (26). Another study from Mexico corroborated two approaches (morphological and chemical) (1). Another study thoroughly described the traditional characteristics of the four *Sargassum* subgenera and further they highlighted the taxonomical ambiguities and proposed the advent of DNA phylogenies using markers for nucleus, chloroplast and mitochondria (8). Another study from India reported 52 species of *Sargassum* and described the vegetative morphology of *Sargassum vulgare* based on thallus, holdfast, blades and stipes (28). In this study, we identified our brown seaweed as *Sargassum fluitans* based on morphological features of thallus, leaves, bladder and presence or absence of holdfast. We did not use receptacles for identification as it has been reported that pelagic *Sargassum* species reproduce by vegetative fragmentation and lack sexual reproduction (29, 30). Recent approaches however prefer to employ molecular markers in combination with morphology to enquire the evolutionary relationships and differences among genera, sub-genera and species of Sargassaceae (2, 31).

Seaweed tissue culture (STC) is relatively an evolving biotechnology discipline compared to those of terrestrial plants tissue culture (PTC) (29, 30). PTC is currently enjoying the advent of methods for genetic manipulation whereas STC is limited to micro-propagation and callus induction practices (32). Growing knowledge of the fundamental aspects of callus formation, abiotic factors, role of carbon sources and PGRs has resulted isn the callus induction and plant regeneration in > 40 seaweeds (13, 14). In this study, the induction of pigmented callus was

observed from whole leaf axenic explants of *Sargassum fluitan*s grown in modified Guillard-Ryther F2 media and modified Murashige-Skoog media. Furthermore, we report that brown seaweed growth rate on different culture media is not same because we used three culture medias and callus induction was observed on modified Guillard-Ryther F2 after 45 days and on modified Murashige-Skoog media after 60 days and no callus induction was observed on Walne's media. With these findings, we report that modified Guillard-Ryther F2 media seems more optimum and favourable compared to Murashige-Skoog media for callus induction and micro-propagation of *S. fluitans* and can be used in future for further research.

Callus induction rate varies with species and irradiance. Of the irradiances investigated, 30 μmol photons $m^{-2}s^{-1}$ produced calluses in as many as 40% of the explants and the explants cultured at 5 and 70 μmol photons $m^{-2}s^{-1}$ did not produce any callus induction in all the algal species studied. In this study, the optimum callus growth was observed at 60 μmol photons m^{-2} s^{-1} which is in agreement with the previous studies. Many studies indicate temperatures ranging from 8-26 °C is suitable to induce callus in seaweeds. Furthermore, the callus was optimally induced at temperatures 10-20 °C and temperature higher than 26 °C caused a decrease in callus induction and even mortality of the explants. In this study, we report the optimal callus induction at 14-22 °C which is in the harmony with the previous studies. PGRs can be applied at specific growth stages to influence plant development. Synthetic as well as natural PGRs have been utilized for seaweed callus induction, In comparison to other abiotic variables, the effect of PGRs on callus induction in seaweed has only recently been intensively studied. A previous study indicate that calluses were only induced in media containing kinetin or UNIconazole and other PGRs were not as effective (18). Our results are in contrast to previous study and we report that two PGRs, 6-benzylaminopurine (BAP) and Indole-3-butyric acid (IBA) has significant effect on brown seaweed callus induction.

CONCLUSION:

This study aimed to identify the most abundantly found brown seaweed at the coast of Karachi and to design the cost-effective growth medium. Brown seaweed was identified as *Sargassum fluitans.* Modified Guillard-Ryther media demonstrated higher brown algal growth compared to other media. Further studies are needed to identify the other *Sargassum* species from the coast of Karachi and we recommend further research to regenerate the phaeophaceae plant from callus.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTION:

All authors contributed equally in the study design, manuscript writing, editing, checking, analysis, interpretation, revision, drafting, final approval, and agreement to be accountable of all aspects of the work.

REFERENCES

1. Rosado-Espinosa LA, Freile-Pelegrín Y, Hernández-Nuñez E, Robledo D. A comparative study of Sargassum species from the Yucatan peninsula coast: morphological and chemical characterisation. Phycologia. 2020;59(3):261-71.
2. Hasan AHH, Al-Bader DA, Peters AF, Küpper FC. Revision of the family Sargassaceae (Fucales, Phaeophyceae) from Kuwait (Arabian/Persian Gulf, NW Indian Ocean) based on analysis of morphology and ITS2 sequences. Ukrainian Botanical Journal. 2023;80(1):3-20.
3. Sharma A, Arya SK. Ecological and environmental services of microalgae. Valorization of Microalgal Biomass and Wastewater Treatment: Elsevier; 2023. p. 261-315.
4. Hernandez-Carmona G, Freile-Pelegrín Y, Hernández-Garibay E. Conventional and alternative technologies for the extraction of algal polysaccharides. Functional ingredients from algae for foods and nutraceuticals: Elsevier; 2013. p. 475-516.
5. Singh S, Kate BN, Banerjee U. Bioactive compounds from cyanobacteria and microalgae: an overview. Critical reviews in biotechnology. 2005;25(3):73-95.
6. Kandale A, Meena A, Rao M, Panda P, Mangal A, Reddy G, et al. Marine algae: an introduction, food value and medicinal uses. Journal of Pharmacy Research. 2011;4(1):219-21.
7. Mori M, Parmar E, Taral P. SEAWEEDS PROCESSING AND ITS APPLICATION IN FOOD AND INDUSTRIES. Innovations in Agriculture, Environment and Health Research for Ecological Restoration. 2019:82.
8. Mattio L, Payri CE. 190 years of Sargassum taxonomy, facing the advent of DNA phylogenies. The Botanical Review. 2011;77:31-70.
9. Nguyen VT. Seaweed diversity in Vietnam, with an emphasis on the brown algal genus Sargassum. 2014.
10. Rousseau F, De Reviers B. Phylogenetic relationships within the Fucales (Phaeophyceae) based on combined partial SSU+ LSU rDNA sequence data. European Journal of phycology. 1999;34(1):53-64.

11. Ahmad A, Banat F, Alsafar H, Hasan SW. Algae biotechnology for industrial wastewater treatment, bioenergy production, and high-value bioproducts. Science of The Total Environment. 2022;806:150585.
12. Tirtawijaya G, Negara BFSP, Lee J-H, Cho M-G, Kim HK, Choi Y-S, et al. The Influence of Abiotic Factors on the Induction of Seaweed Callus. Journal of Marine Science and Engineering. 2022;10(4):513.
13. Uji T, Nanaumi D, Kawagoe C, Saga N, Miyashita K. Factors influencing the induction of adventitious bud and callus in the brown alga Sargassum horneri (Turner) C. Agardh. Journal of Applied Phycology. 2016;28:2435-43.
14. Kumar GR, Reddy C, Jha B. Callus induction and thallus regeneration from callus of phycocolloid yielding seaweeds from the Indian coast. Journal of Applied Phycology. 2007;19:15-25.
15. Ismail MM, El-Sheekh M. Enhancement of biochemical and nutritional contents of some cultivated seaweeds under laboratory conditions. Journal of dietary supplements. 2018;15(3):318-29.
16. Stirk WA, Gold JD, Novák O, Strnad M, Staden Jv. Changes in endogenous cytokinins during germination and seedling establishment of Tagetes minuta L. Plant Growth Regulation. 2005;47:1-7.
17. Tarakhovskaya E, Maslov YI, Shishova M. Phytohormones in algae. Russian Journal of Plant Physiology. 2007;54:163-70.
18. Muhamad SNS, Ling AP-K, Wong C-L. Effect of plant growth regulators on direct regeneration and callus induction from Sargassum polycystum C. Agardh. Journal of applied phycology. 2018;30:3299-310.
19. Zuo-mei Y, editor Studies on tissue culture of Laminaria japonica and Undaria pinnatifida. Eleventh International Seaweed Symposium: Proceedings of the Eleventh International Seaweed Symposium, held in Qingdao, People's Republic of China, June 19–25, 1983; 1984: Springer.
20. Borgesen F. Some marine algae from the northern part of the Arabian Sea with remarks on their geographical distribution, Kongelige Danske Videnskabernes Selskab. Biologiske Meddelelser. 1934;11.
21. Shaikh W, Shameel M. Taxonomic study of brown algae commonly growing on the coast of Karachi, Pakistan. 1995.
22. Shameel M, Aisha K, Khan S. A preliminary survey of seaweeds from the coast of Makran, Pakistan. 1996.
23. Ali A, Siddiqui PJA, Aisha K. Characterization of macroalgal communities in the coastal waters of Sindh (Pakistan), a region under the influence of reversal monsoons. Regional Studies in Marine Science. 2017;14:84-92.
24. Ali A, Malik S, Zaidi AZ, Ahmad N, Shafique S, Aftab MN, et al. Standing stock of seaweeds in submerged habitats along the karachi coast, pakistan: An alternative source of livelihood for coastal communities. Pak J Bot. 2019;51(5):1819-30.
25. Bashir F, Abbas A, Shaukat SS, Siddiqui MF, Qureshi IA. DISTRIBUTION AND DIVERSITY OF MARINE ALGAE OF SINDH COASTAL AREA: ALPHA, BETA AND GAMMA DIVERSITY. Pak J Bot. 2022;54(6):2377-82.
26. Sargazi F. MORPHOLOGICAL DIVERSITY OF SARGASSUM SPECIES OF OMAN SEA COASTS. The Iranian Journal of Botany. 2021;27(1):62-70.
27. Godínez-Ortega JL, Cuatlán-Cortés JV, López-Bautista JM, van Tussenbroek BI. A Natural history of floating Sargassum species (Sargasso) from Mexico. Natural History and Ecology of Mexico and Central America: IntechOpen London, UK; 2021. p. 35.
28. Kumari B, Sharma V. A comparative study on morphology of plants: Sargassum vulgare Vol. 3(3), pp. xxx-xxx, May 2012
29. Dibner S, Martin L, Thibaut T, Aurelle D, Blanfuné A, Whittaker K, et al. Consistent genetic divergence observed among pelagic Sargassum morphotypes in the western North Atlantic. Marine Ecology. 2022;43(1):e12691.
30. Hanisak MD, Samuel MA, editors. Growth rates in culture of several species of Sargassum from Florida, USA. Twelfth International Seaweed Symposium: Proceedings of the Twelfth International Seaweed Symposium held in Sao Paulo, Brazil, July 27–August 1, 1986; 1987: Springer.
31. Mattio L, Payri CE, Stiger-Pouvreau V. TAXONOMIC REVISION OF SARGASSUM (FUCALES, PHAEOPHYCEAE) FROM FRENCH POLYNESIA BASED ON MORPHOLOGICAL AND MOLECULAR ANALYSES 1. Journal of phycology. 2008;44(6):1541-55.
32. Baweja P, Sahoo D, García-Jiménez P, Robaina RR. Seaweed tissue culture as applied to biotechnology: problems, achievements and prospects. Phycological Research. 2009;57(1):45-58.