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Analysis of functional traits in female gametophytic and tetrasporophytic life phases of industrially important red alga *Gracilaria dura* (Rhodophyta: Gracilariacae)

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Abstract

The rhodophyte seaweed genus *Gracilaria* is fast replacing *Gelidium* in global agar trade. Recently, *Gracilaria dura* from Indian waters has emerged as species of choice due to its quality agarose. The triphasic life cycle provides an opportunity for selecting an elite cultivar. Here we report diversity pertaining to functional traits over 3 months (January, February and March) among two distinct life phases, the tetrasporophyte and the female gametophyte. It was evident that functional trait related to growth was distinct in tetrasporophytes than in female gametophytes; while those of survival (antioxidant, proximate composition and pigments) were prominent in female gametophytes. Both the lowest $3.42 \pm 0.38\%$ day^{-1} (female gametophytes) and highest $5.17 \pm 0.21\%$ day^{-1} (terasporophytes) growth rates were in January with 33% higher growth in the latter. Of the survival traits, antioxidant potential ranged from 0.51 ± 0.08 to 1.1 ± 0.03 mg g^{-1} FW ascorbic acid equivalent. Furthermore, female gametophytes collected in March reported 53.60%, 28.40% and 50.40% higher activity than the tetrasporophytes of January, February and March, respectively. The female gametophyte in March showed 23% and 19.40% higher protein content than the tetrasporophytes of January and February, respectively. Sixty-one metabolites were identified of which 29 were common, with sugars as the major portion (77.69% in February to 89.38% in January) and amine derivatives the least (0.30% in January to 0.51% in February). Studies of bio-ecophysiology of this alga are currently being undertaken by our group.

Keywords Female gametophyte · Tetrasporophyte · Rhodophyta · *Gracilaria dura* · Life phases · Metabolite profiling · Proximate composition

Introduction

The Food and Agricultural Organisation of the United Nations started tropical open-water cultivation of *Gracilaria* in Penang, Malaysia and Vedalai and Chinnapalarn, India under its seven-country initiative named Bay of Bengal Program (FAO 1991). The cultivation of *Gracilaria edulis* although abandoned in both villages due to non-availability of mature seed-stock, in-adequate water quality and abundance of grazers had set a tone for experimental farming. Further studies reported a yield of 3.5 to 4 kg fresh weight m^{-1} length of rope using various *Gracilaria* spp. either at experimental or pilot-scale farming (Raju and Thomas 1971; Umamaheswara Rao 1974; Subbaramaiah and Thomas 1990; Oza et al. 1994; Kaladharan et al. 1996). However, cultivation of *Gracilaria* was never been undertaken as a commercial venture and wild harvesting of the resource is still relevant for industrial production. The establishment of the Gulf of Mannar Marine National Park in 1986 strongly affected the industrial production of agar due to stringent conservation measures enforced by Department of Forest, Government of Tamil Nadu (Marirajan et al. 2012). There is thus the opportunity for undertaking commercial *Gracilaria* farming in India.

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The genus *Gracilaria* is the second most diverse seaweed genus comprising around 28 species and 7 varieties in Indian waters (Oza and Zaidi 2001). The studies pertaining to *G. dura* (Meena et al. 2007), *G. edulis* [now *Hydropuntia edulis*], *G. crassa* [now *G. canaliculata*], *G. foliifera*, *G. corticata* (Meena et al. 2008) and *G. debilis* and *G. salicornia* (Mehta et al. 2010) confirmed the availability of superior indigenous feedstock, but industrial exploitation is limited only to *G. edulis* (Ganesan et al. 2019). The prospect of cottage-scale agar production in India has remained unchanged as industrial production is often family-run enterprises, with manufacturing protocols heavily relying on traditional energy-intensive processes and the absence of national standard specifications with respect to gel strength (Mantri et al. 2019). Amidst these shortcomings, *G. dura* is considered a potential species with high commercial prospects. Besides robust growth, its applications such as a plant bio-stimulant, imparting drought tolerance, pigments and high-value agarose obtained from patented technology, have attracted considerable industrial attention (Meena et al. 2014; Baghel et al. 2015; Veeragurunathan et al. 2015a, b; Mantri et al. 2020; Sharma et al. 2019). Furthermore, this species has been reported as the potential source of agar from Mediterranean region (Murano et al. 1992; Marinho-Soriano et al. 2005). The haploid-diploid life cycle in which free-living tetrasporophytes alternate with independent female as well as male gametophytes is not much investigated. The *Polysiphonia*-type or triphasic life cycle consists of identical gametophytes and sporophytes, with physiological (biochemical) and genetic (molecular) differences (Gupta et al. 2011). The phenology of this species in nature is reported with dis-proportionate abundance (Mantri 2010); therefore, it is worthwhile to ascertain differences in life cycle stages related to functional traits of economic importance.

The applied aspects pertaining to *Gracilaria* farming in India have so far focused on elucidating effects of growth-promoting substances, tissue culture, protoplast fusion and developing molecular markers (Oza 1971; Kumar et al. 2007; Baghel et al. 2011; Gupta et al. 2011). Experimental or pilot-scale farming of *Gracilaria* has been attempted in Indian waters (reviewed in Ganesan et al. 2017), and the National Fisheries Development Board has recently implemented two missions, related to giving impetus to commercial farming of *Gracilaria* both at the south-east coast (Tamil Nadu) and north-west coast (Gujarat) India, respectively (Mantri et al. 2020). This has necessitated supply of elite germplasm to the commercial growers.

Vegetative clones have been exclusively used for this alga in large-scale operations, thereby providing opportunity for selecting elite cultivars. It may be noted that the selection of cultivar also involves confirming the persistence of characters besides its superiority (Santelices 1992). Nevertheless, reliable screening criteria for choosing desired trait for biomass development are not yet practised in Indian agarophytes (Mantri et al. 2020; Veeragurunathan et al. 2019). The present investigation thus presents initial steps in ascertaining the diversity pertaining to functional traits of potential interest over 3 months in *G. dura* from Indian waters. The detailed characterisation would be helpful for identification, selection and improving the efficiency of germplasm based on different life cycle phases for developing new cultivars.

Materials and methods

Collection and identification of life phases

Individuals of *Gracilaria dura* were collected at low tide from the coast of Veraval (20.910404° N, 70.351273° E), Gujarat, India, in January, February and March 2019. Healthy live fronds were collected and brought to the laboratory immediately under cool conditions. Although occurrence of this alga along the coast of Veraval was reported during December to May (Mantri 2010), well-developed gametophytic and sporophytic plants only appeared from January to March and this study was restricted to these 3 months. Epiphytes and some adhered particles were removed from the collected samples by cleaning with filtered seawater. The type of individual was identified by viewing transverse section under the light microscope. According to the type of reproductive structures (presence of tetrasporangia for tetrasporophyte and cystocarps for female gametophyte), they were segregated.

Experimental design

The cleaned samples collected in each month were divided into two groups, i.e. one was maintained in modified Erdschreiber seawater (ESS) culture medium (Suto 1959) for growth rate experiment. All the cultures were aerated with ambient air under controlled light intensity (55 μmol photons m^{-2} s^{-1}), photoperiod (12:12 h L/D), temperature (25 °C ± 1) and salinity (35 psu). The other group was processed immediately for proximate and metabolic analysis.

Determination of growth

Apical segments of size approx. 2 cm were excised from each group of fronds (tetrasporophyte and female gametophyte). Five of these fragments were then selected by mass of approx. 1 g and placed in 500-mL flasks containing ESS medium (35 psu salinity). All the flasks were subjected to 24 h aeration and the mouth of each flask was closed with a sponge (plugged). The experiment was carried out in triplicates (n = 3) under laboratory conditions as described above. The medium was replenished every alternate day during which fronds were cleaned with a brush to prevent fouling. After 2 weeks of

experimental setup of each month, the fronds were weighed and their daily growth rate was estimated using the formula $\left[\left\{\left(\frac{Wf}{Wi}\right)^{\frac{1}{t}}\right\}-1\right]\times 100$ where, t=time (days), W_i= initial mass and W_f= final mass (Gupta et al. 2011).

Total antioxidant activity estimation

To estimate total antioxidant potential, 100 mg of frozen sample was extracted twice sequentially, initially with ultrapure water and then with 70% methanol of volume 1 mL and both the extracts were then pooled and analysed spectrophotometrically according to Prieto et al. (1999). Briefly, the extract was mixed with 1 mL of reagent solution containing 0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate. Following incubation at 100 °C for 60 min with ascorbic acid as standard, the absorbance was measured at 695 nm.

Pigment analysis

For analysis of R-phycoerythrin (R-PE) and R-phycocyanin (R-PC), 100 mg of fresh algal tissues was homogenised using liquid nitrogen in mortar and pestle. The homogenate was added to 0.8 mL of 0.1 M phosphate buffer (pH 6.8) and incubated overnight at 4 °C. After incubation, homogenised samples were vortexed and centrifuged at 15,000×g for 10 min at 4 °C. The supernatant was collected and the residue was re-extracted using 0.2 mL phosphate buffer ensuring complete pigment extraction. The absorbance scan was recorded using a dual-beam UV-visible spectrophotometer. For the analysis of chlorophyll-*a* (Chl-*a*), the same process was applied using 90% acetone as solvent. The contents of R-PE and R-PC were calculated according to Sampath-Wiley and Neefus (2007) and the chlorophyll-*a* content was determined according to Jeffrey and Humphrey (1975).

Protein extraction

Individual fronds weighing 100 mg were crushed in liquid nitrogen and subjected to alkaline digestion for protein extraction using 1 mL 1 N sodium hydroxide. The solution was incubated at 80 °C for 1 h for easy protein liberation from the biomass and was then cooled at room temperature and centrifuged. The supernatant was neutralised using 6 N HCl. The protein content of the supernatant was determined by the Lowry method with bovine serum albumin (BSA) as standard (Lowry et al. 1951).

Lipid extraction

Lipid extraction was carried out by the Bligh and Dyer (1959) method. Briefly, 1 g of fresh homogenised algae (crushed using liquid nitrogen) was mixed with chloroform and methanol in the ratio of 1:2. The solution was stirred for 30 min to enhance lipid extraction. Re-extraction was done to ensure complete lipid extraction followed by adding an equal volume of water. By centrifugation, two phases were formed and the lower organic phase was collected. It was then kept in hot air oven overnight to remove the solvent and the total lipid was determined gravimetrically. Then, it was treated with 1% methanolic NaOH at 55 °C for 15 min followed by methanolic HCl at 55 °C for 15 min for methyl ester conversion and analysed for fatty acid composition by GC-MS (QP-2010, Shimadzu, Japan).

Carbohydrate extraction

One hundred-milligram crushed samples were subjected to acid digestion for total carbohydrate extraction. Two hundred fifty microlitres of conc. H_2SO_4 was added to the homogenised tissue and the solution was kept at room temperature for 30 min. This was followed by addition of 3 mL distilled water to the solution and incubation at 121 °C for 1 h. The solution was then vortexed and neutralised with $CaCO_3$. The supernatants were collected after centrifugation at 9500 rpm for 10 min and the carbohydrate content was estimated spectrophotometrically by the phenol and sulphuric acid method at 490 nm. Galactose was used as standard.

Metabolite profiling

Metabolite profiling was performed by gas chromatography coupled with a mass spectrometer (GC-MS QP-2010, Shimadzu, Japan). A total of 100 mg powder crushed tissue were taken and 0.75 mL of methanol (ice cold) and 50 μL of ribitol (0.2 mg mL^{-1}stock) were added. Extraction was carried

Table 1 Analysis of variance (two-way ANOVA) among function traits of female gametophytic and tetrasporophytic plants

Parameter	Variables	df	SS	MS	*F*	*p*
DGR	Month	2	1.21	0.61	2.19	0.1548
	Phase	1	1.82	1.82	6.57	0.0248*
Protein	Month	2	0.05	0.02	3.17	0.0786
	Phase	1	0.19	0.19	25.34	0.0003*
Lipid	Month	2	0.19	0.09	68.92	< 0.0001*
	Phase	1	0.01	0.01	4.66	0.0518
Carbohydrate	Month	2	16.04	8.02	4.49	0.0350*
	Phase	1	11.12	11.12	6.23	0.0282*
R-PE	Month	2	71,414.29	35,707.14	6.70	0.0111*
	Phase	1	75,944.14	75,944.14	14.26	0.0026 *
R-PC	Month	2	38,029.82	19,014.91	8.40	0.0052*
	Phase	1	20,119.08	20,119.08	8.88	0.0115*
Chlorophyll-*a*	Month	2	4649.55	2324.78	11.47	0.0016*
	Phase	1	2686.45	2686.45	13.26	0.0034*

* Significant difference

out at 70 °C for 15 min followed by centrifugation for 5 min at 10,000 rpm. To the supernatant, 750 μL water and 325 μL chloroform (ice cold) were added. The mixture was then vortexed and centrifuged for 10 min at 5000 rpm. The polar phase (water/methanol) was separated from the non-polar fraction into a new tube. Five hundred microlitre of polar fraction was dried in speed vacuum concentrator. For derivatisation, the dried pellet was reconstituted in 60 μL of 20 mg mL^{-1} solution of methoxyamine hydrochloride in pyridine and shaken at 37 °C for 120 min. Afterward, 130 μL of N-methyl-trimethylsilyl trifluoroacetamide (MSTFA) was added and shaken at 37 °C for 30 min (Lisec et al. 2015). It was then analysed with the thermal program of 80 °C as initial temperature with 2 min hold, from 10 °C increment to 315 °C with hold for 1 min and final temperature 250 °C with total run time of 40 min. The compounds were identified on their m/z ratio with the help of NIST library. The metabolites were quantified by calculating the relative response ratio of the peak area of individual metabolites to that of internal standard adonitol.

Statistical analysis

In all experiments, a minimum of three biological replicates were taken and all the data are expressed as mean ± standard deviation. Data analysis was carried out by two-way analysis of variance, i.e. phase (tetrasporophyte and female gametophyte) and month of collection (January, February and March) with a significance difference level of $p < 0.05$ (Table 1). The functional traits were further analysed using Tukey's post hoc test with the help of software Infostat (Di Rienzo et al. 2018).

Results

The collections acclimatised well to the culture conditions employed during the study period. DGR for tetrasporophytes over the collection months, i.e. January, February and March was statistically similar and the same was seen for female gametophytes. However, in between the life phases, initial assemblage, i.e. January collection showed 33% higher growth rate for tetrasporophytes than female gametophytes of same collection ($F = 6.57$, $p = 0.024$) but in subsequent collections, growth rate was similar for both phases (Fig. 1a).

The antioxidant potential ranged from 0.51 ± 0.08 to 1.1 ± 0.03 mg ascorbic acid (AA) equivalent g^{-1} FW. In the female gametophytes, a steady increase was observed in the activity over the subsequent months (from January to March) whereas for tetrasporophytes, February collection showed more activity than January, which again declined in March. Besides, March-collected female gametophytes showed 53.60, 28.40 and 50.40% higher antioxidant potential than the tetrasporophytes of January, February and March, respectively ($F = 65.85$, $p < 0.0001$) (Fig. 1b).

Protein content for all tetrasporophyte collections was statistically similar over the months and the same trend was observed in female gametophytes. However, among the life phases, female gametophytes collected during March showed 23, 19.40 and 19.66% higher protein content than the tetrasporophytes of January, February and March, respectively ($F = 25.34$, $p = 0.0003$). For the carbohydrate content within the phases, February-collected female gametophytes showed 37.27% higher carbohydrate content than the tetrasporophytes of January ($F = 6.23$, $p = 0.028$). Nevertheless, the lipid content showed decrease over the subsequent collection month considering both life forms. The highest was in January followed by February and March. The maximum value of 0.44% ± 0.06 FW was found in female gametophytes of January while the lowest of 0.18% ± 0.02 FW was observed in tetrasporophytes of March (Fig. 2a–c). Further, the fatty acid composition showed more polyunsaturated fatty acids (PUFA) than saturated fatty acids (SFA) with 3-fold difference. Percentile composition matched in collections of all the months and between the phases, but actual concentration varied. Comparatively, female gametophytes showed higher concentration for SFA (28% in January; 36.75% in February and 62.47% in March), monounsaturated fatty acids (MUFA) (20.25% in January; 44.15% in February and 33.53% in

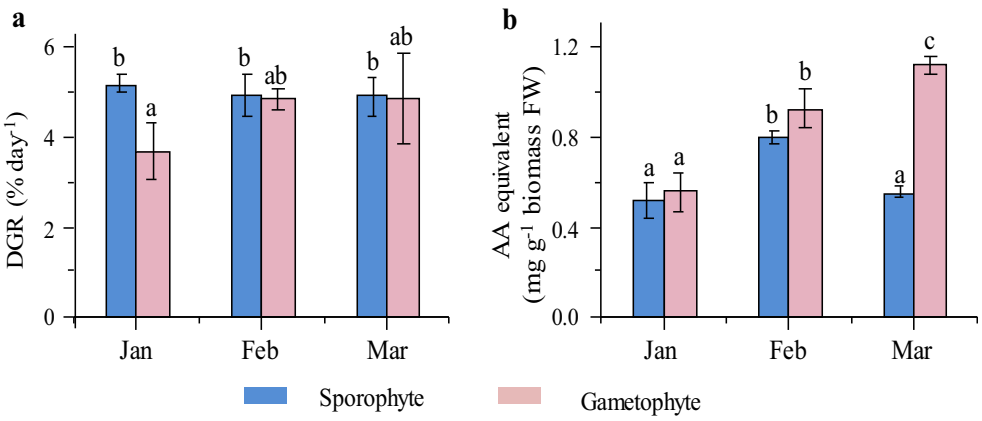

Fig. 1 **a** Daily growth rate (DGR) over a period of 15 days from acclimatised field material to the laboratory condition of 25 °C temperature, 55 μmol photons $m^{-2}s^{-1}$ of light intensity, 12 h light period and 35 psu salinity. **b** Total antioxidant activity equivalent to ascorbic acid (AA) from the field-collected biomass. Different letters above bars depict significant difference between phases (ANOVA, $p < 0.05$). Data shown are the mean ± SD ($n = 3$)

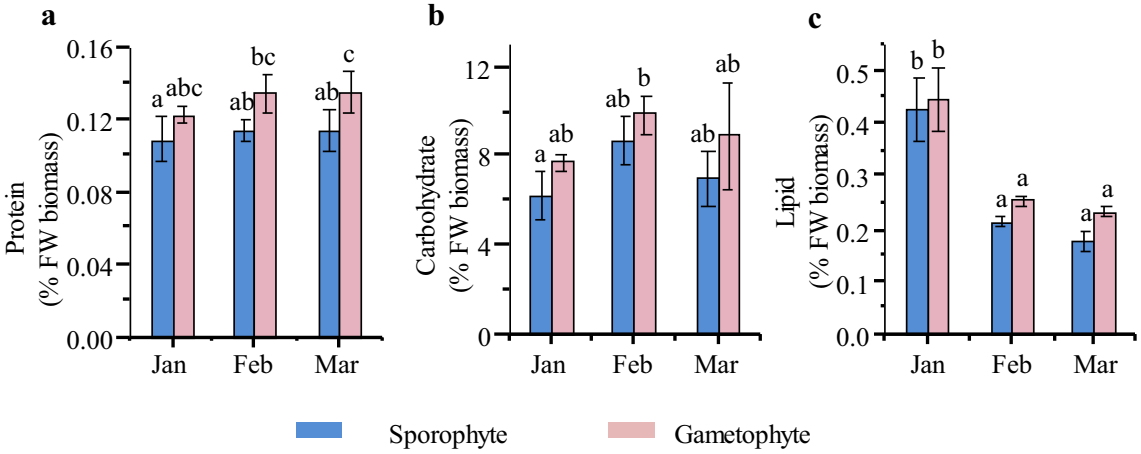

Fig. 2 Total protein (**a**), carbohydrate (**b**), lipids (**c**) content in both life phases of *G. dura* collected from the field. Different letters above bars depict significant difference between phases (ANOVA, $p < 0.05$). Data shown are the mean ± SD ($n = 3$)

March) and PUFA (28.67% in January; 45.48% in February and 62.73% in March).

Figure 3a–c depicts the pigment contents. The Chl-*a* content of tetrasporophytes was higher in February compared with the January collection, whereas the March samples showed intermediate values. For the Chl-*a* content of the female gametophytes, it was statistically similar over the months. Comparing among the phases, March and February female gametophytes had 63.32% and 65.07%, respectively, higher chlorophyll content than the January-collected tetrasporophytes ($F = 13.26$, $p = 0.003$). The R-PC content of female gametophytes showed an increasing trend over the months, but it was statistically similar for tetrasporophytes. The March-collected female gametophytes had a 57.80% higher R-PC content than the January terasporophytes ($F = 8.88$, $p = 0.011$). Similarly, March-collected female gametophytes had a 65.10% higher R-PE content than January tetrasporophytes ($F = 14.26$, $p = 0.002$).

In metabolite profiling, we recorded a total of 61 metabolites incorporating sugars, amino acids, amine derivatives, fatty acids, polyols, organic acids and organooxy compounds (Supplementary material Table 1). A total of 29 metabolites were common in all 3 months, and among these, sugars were the major portion (77.69% in February to 89.38% in January) and amine derivatives (0.30% in January to 0.51% in February) were the least (Supplementary material Fig. 1a, 2a). Within phases, 52 metabolites were common (Supplementary material Fig. 1b, 2b). The sugars were highest and amine derivatives were least. Mannobiose was highest of all, i.e. 4-fold higher in January collection in both the phases compared with February and March collection. Together, 22 metabolites were common between phases and collection months. The concentration of each was subjected to log10 transformation for normalisation and a common pattern of decline in concentration among the subsequent collections was observed (Fig. 4).

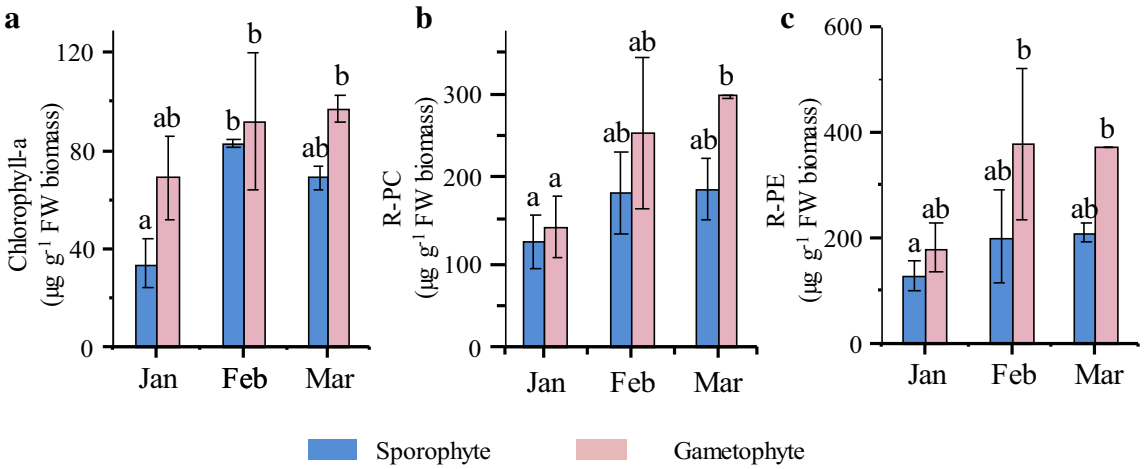

Fig. 3 Chlorophyll-*a* (**a**), R-PC (**b**), R-PE (**c**) content in both life phases of *G. dura* collected from the field. Different letters above bars depict significant difference between phases (ANOVA, $p < 0.05$). Data shown are the mean ± SD ($n = 3$)

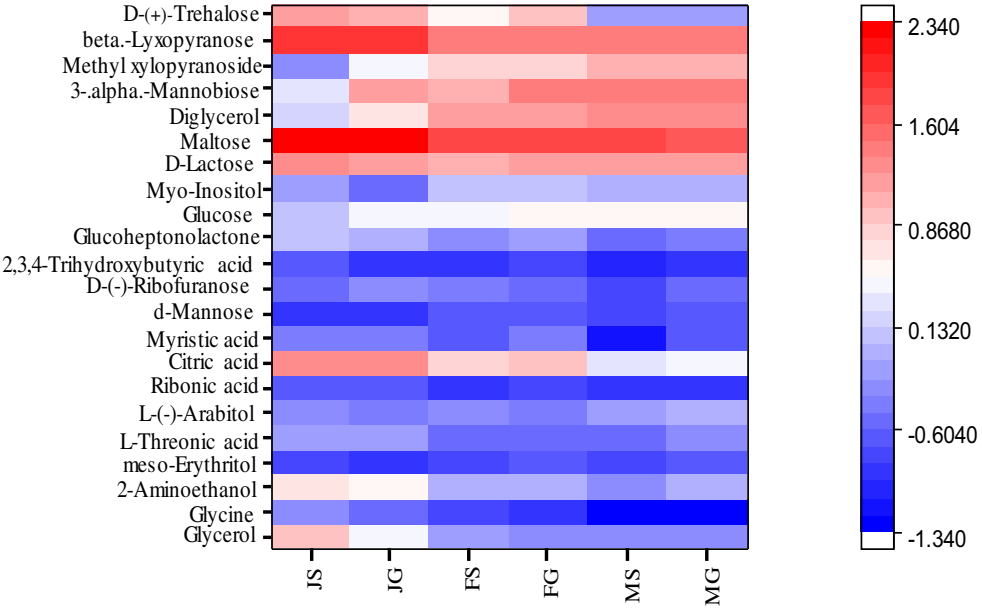

Fig. 4 Heatmap of *G. dura* phases collected from the field, metabolite concentration (μg per 100 mg FW biomass) subjected to $\log_{10}$ transformation

Discussion

Gracilaria, being a prominent feedstock for industrial agar production, is cultivated extensively replacing *Gelidium* and substituting the wild harvest (Porse and Rudolph 2017). This is primarily due to harvest ban from Mediterranean countries coupled with decline in wild populations due to overharvesting. Despite availability of several local agrophytes, *G. dura* has gained prominence due to its high agarose content (Meena et al. 2007). The cultivation practices have been standardised and large-scale farming has been successfully implemented (Mantri et al. 2020). Genetic improvement studies through breeding, mutation and chromosome manipulations remain inconclusive in *Gracilaria* (Van der Meer and Bird 1977, Patwary 1987; Zhang and Fei 1990; Patwary and Van der Meer 1992). It is evident from the studies that different cytological processes involved in reproduction (Scrosati and DeWreede 1999) and niche partitioning (Destombe et al. 1989) are responsible for competitive abundance of life cycle stages at given point of time. Thus, strain selection with improved functional trait from wild population is essential for developing and selecting elite germplasm. With this leitmotif, we have studied different functional traits pertaining to biochemical and growth characters in tetrasporophyte and female gametophyte phases during different time intervals.

The results indicated that female gametophytes differ from tetrasporophytes considerably over the collection months among functional traits studied. The investigations on effect of culture conditions on growth have been well illustrated the but influence of life cycle stage on growth is seldom studied (DeBusk and Ryther 1984; Raikar et al. 2001). The growth rate with respect to life cycle stages has been documented in *G. lemaneiformis* (Zheng and Gao 2009), *G. tenuistipitata* [now *Agarophyton tenustipitatum*] (Skriptsova and Nabivailo 2009), *G. arcuata*, *G. incurvata*, *G. textorii*, *G. lichenoides* [now *Hydropuntia edulis*] and *G. foliifera* (Raikar et al. 2001). In the present study, DGR was higher in tetrasporophytes initially in January but was then similar for both phases in March. It may be noted that high growth in tetrasporophytes of *G. caudata* [now *Crassiphycus caudatus*] has been reported (Araújo et al. 2014) together with the report by Barufi et al. (2010) where higher growth rate was observed in tetrasporophytes compared with female gametophytse for *G. tenuistipitata* in the first week and almost similar in following weeks for both the phases. Furthermore, the differences with respect to growth rates initially can be related to the different nutritional needs, which are advantageous for the survival of the species in heterogeneous environments (Ursi and Plastino 2001), but the inconsistency in growth is also essentially linked to environmental conditions. These variations favour specific life phase under wild conditions to ascertain the maintenance of taxa for a given point of time. But this approach may be successfully exploited for selecting specific life stage for specific farming site that would act as most conducive environment (Araújo et al. 2014).

It is considered that the antioxidant activity of seaweeds is caused not only by phenolic compounds but also by other water-soluble compounds, i.e. phycobiliprotein (Kuda et al. 2006). The sequential extraction performed in this study with both water and methanol was helpful in documenting the higher values than in individual solvent extraction. It may be further noted that significantly high values were observed in gametophytic phase of March collection. This dominance can be explained by the fact that by this time, the alga reaches its reproductive maturity. This period is characterised by high seawater temperature, longer daylength coupled with elevated light intensity which leads to ceased growth and elevated

reproductive activities. The pre-dominance of tetrasporophytes over gametophytes in *G. dura* has been documented (Mantri 2010). The diploid nature of tetrasporophyte was responsible for higher viability in this phase in *G. verrucosa* [now *Gracilariopsis longissima*] (Destombe et al. 1989) and *G. domingensis* (Guimarães et al. 1999). The higher antioxidant response by female gametophytes (March samples) suggests that this life phase struggles to maintain its viability. Therefore, one might need to preferably select sporophytic life phase during summer month for farming.

The proximate content was evident with significant difference in both life phases during different collections. The total carbohydrate content values observed were similar in range as described in *Gracilaria* spp. as per Hong et al. (2007). The protein content observed in present study was low, but the lipid percentage was similar to the different *Gracilaria* spp. studied previously (Kumar et al. 2011). Considering fatty acid composition, higher PUFA to SFA ratio is important, which was found to be 2.6 that falls within the prescribed nutritional guidelines. The pigment contents followed a similar trend to that of proximate composition. The March- and February-collected female gametophyte showed higher pigment content than January-collected tetrasporophytes (Chl-*a* and R-PE). Considering R-PC, the March female gametophytes showed dominance over the January tetrasporophytes and also gradual increase was observed over the months with the intermediate values in February collection. Guillemin et al. (2014) also demonstrated higher pigment content for vegetative stage of female gametophytes in *G. chilensis* [now *Agarophyton chilense*]. It may be also noted that the pigments were in a positive correlation with the growth only in the case of female gametophyte. Nevertheless, in the case of tetrasporophyte, growth was not co-related with pigments. Araújo et al. (2014) on the contrary reported that higher pigment content (Chl-*a*; carotenoinds, R-PE, R-PC and APC) in tetrasporophytes contributed to higher viability of this phase when compared with female gametophyte in *G. caudata*.

Gracilaria is a rich source of sulphated polysaccharide; thus, in the metabolic profiling, we observed more number and concentration of sugars which included the precursors of sulphated polysaccharides, i.e. xylose, mannobiose, maltose, galactose and glucose. Polyols were also higher in February as well as in March. They are considered the main storage compounds. The analysis also reported a number of antioxidant organooxy compounds such as gluconoheptolactone that is also a precursor to ascorbic acid biosynthesis (Belghit et al. 2017). The increase in the number of metabolic compounds with the collection intervals in the present investigation might be possibly due to the metabolic switching towards phenological abundance of life cycle stages or seasonal acclimation of alga. There are reports describing the differences in agar yield and viscosity among the life phases of *G. bursapastoris* and *G. dura* (Marinho-Soriano et al. 1999; Baghel et al. 2011; Gupta et al. 2011), but to the best of our knowledge, this is the first study describing the subtle differences of proximate content, metabolite profile together with the different life phases of *G. dura*. It was amply clear from the present investigation that functional trait related to growth (DGR) was more pronounced in tetrasporophytes than in female gametophytes, while those of survival (antioxidant, proximate composition and pigments) were prominent in later-collected female gametophytes. These observations were in accordance with results reported in *G. chilensis* on fertility potentials and survival of haploid and diploid life cycle stages and could be associated with differentiation on evolution and maintenance of gametophytes and tetrasporophytes life cycles (Vieira et al. 2018).

Gracilaria has played an important role in Indian agar industry since its inception, but unfortunately, feedstock is being harvested from natural resource. Considering potential demand in pharmaceutical as well as processed food industry, there has been consistent effort for the last 3 to 4 years to promote agarophyte farming through various government and non-government agencies. The hydrocolloid from the farmed biomass has been recently validated by leading agar manufactures and they have envisaged interest to procure the farmed biomass. In conclusion, isomorphic gametophytes and tetrasporophytes collected from the same location harboured unique traits and responded differently based on time of collection. The current practise of selecting seed from natural stock without knowing life cycle stage for farming is not apt for sustainable production. The study clearly demonstrated influence of life cycle–based material in selecting different functional traits of commercial importance, the knowledge which dictates essential features for selecting particular life forms as seed for successful farming as well as management practices. Finally, our results reveal that tetrasporophytes were more competitive in terms of growth while the other traits related to accumulation of proximate compounds, antioxidant potentials and concentration of pigments were superior in female gametophyte considering over different collection months. This provides greater opportunity for selection of cultivar-based functional traits of economic interest in red algal farming such as *G. dura* which is domesticated recently. It may also be noted that detailed bio-ecophysiology of this alga is being undertaken by our group to understand this agarophyte which is highly potential for the industry.

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