Indian Journal of Geo Marine Sciences
Vol. 53 (04), April 2024, pp. 203-211
DOI: 10.56042/ijms.v53i04.11130

NIScPR
सीएसआईआर–निस्पर

Growth and biochemical changes in response to salinity stress in *Gayralia oxysperma* (Kützing) K.L. Vinogradova ex Scagel *et al*. 1989 (Chlorophyceae)

M C Thakur* & S Lal

Ashok & Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences,
New Vallabh Vidyanagar, Anand, Gujarat – 388 121, India

*[E-mail: mukundcthakur@gmail.com]

Received 14 January 2024; revised 20 April 2024

Gayralia oxysperma (Kützing) K.L. Vinogradova ex Scagel *et al*. 1989, Doty, (Monostromataceae, Ulvales) possesses exceptional nutritional, antioxidant, and medicinal properties, thriving in fluctuating saline environments and demonstrating resilience against salt stress due to its rich composition of nutrients and phytochemicals. This is an estuarine form of seaweed and is subjected to intense salinity regimes, particularly during the monsoon period (June – September). Therefore, the present study was undertaken to investigate the changes in growth and some cellular biochemical constituents of *G. oxysperma* following exposure to selected salinities *viz*. 1.6 ‰, 4.8 ‰, 8 ‰, 16 ‰, and 32 ‰ in the laboratory culture. The growth of fronds increased with increasing salinity and showed an optimum growth of 2.5-fold increase over initial biomass in 1.6 ‰ on day 15. The microscopic observation of fronds from lower salinity showed unevenly distributed elongated cells with low density and large intercellular spaces. In contrast, at a higher range (> 8 ‰), cells were more compact and evenly distributed all over the thallus. The chlorophyll content increased to 2.8-fold in 32 ‰ over the initial salinity of 1.6 ‰. Proline content of fronds cultured in 32 ‰ showed 9.5-fold increase compared to 1.6 ‰ salinity. Similarly, glycinebetaine also increased with salinity (32 ‰) and showed 2.3-fold increases over values obtained for 1.6 ‰. These findings suggest that *G. oxysperma* thalli accumulate a high amount of osmolytes like proline and glycinebetaine to sustain during adverse salinity stress conditions.

[**Keywords**: *Gayralia oxysperma*, Glycinebetaine, Growth measurement, Osmolytes, Proline, Salinity]

Introduction

The genus *Gayralia* Vinogradova 1969 is of considerable commercial importance as an edible alga grows commonly on open coasts, inner bays and estuarine areas in diverse climatic zones worldwide[1]. Several studies indicate that the growth, reproduction, and distribution of seaweed are influenced by factors such as salinity, temperature, light intensity, and photoperiod.

Gayralia oxysperma (Kützing) K.L. Vinogradova ex Scagel *et al*. 1989 is a benthic marine green alga which thrives abundantly on high-to-mid intertidal rocks in the Southwestern Atlantic Ocean[2], with its distribution spanning marine, brackish water and estuarine environments of India and Brazil[3,4]. This genus faces considerable temperature, salinity, and light fluctuations, potentially leading to a decrease in species diversity and a transition in perennial taxa[5,6]. The light, temperature, and salinity conditions affect the net photosynthesis of *Gayralia*. Choi *et al.*[7] reported that *Gayralia* sp. displays high tolerance across a broad spectrum of salinities, ranging from 15 to 45 ‰, showcasing its euryhaline nature. This adaptability is attributed to the species adjustment to fluctuating salinities in coastal waters or estuaries, influenced by factors such as rainwater influx, evaporation, and precipitation[8]. *Gayralia oxysperma* serves as a valuable source of protein, lipids, minerals, and vitamins[9-11], making it highly sought-after in the pharmaceutical, cosmetic, and food sectors. These polysaccharides are renowned for their thickening properties and are widely utilised as food additives[12].

Green algae have attracted considerable attention in research because of their abundance, widespread dispersion, substantial biomass generators, and bioactive compounds. Bioactive substances obtained from the genera *Gayralia* are known to display a variety of biological activities, such as antiviral[13], antioxidant[14], antitumor activity[15] and antivenom[16]. *Gayralia oxysperma* possesses vital nutritional, antioxidant, and medicinal properties, making it suitable for dietary and pharmaceutical purposes. There, the *Gayralia* exist in several estuaries with potential for mariculture globally.

Seaweeds are frequently consumed directly as food in Southeast Asian nations, often incorporated into salads, soups, and various culinary dishes. Leading forefront countries of seaweed utilisation for human consumption are Japan, China, and Korea. In contrast, in India, seaweeds primarily serve as a source for extracting industrially significant phycocolloids like agar, alginate, and carrageenan[17,18].

Estuarine ecosystems with green macroalgal dominance show wide variations in salinity, nutrients, temperature, desiccation and light causing intense environmental stresses for marine flora. Since the salinity of these regions varies markedly, particularly during the monsoon period (June – September), an attempt has been made in the present study to determine salinity tolerance and associated biochemical changes in *G. oxysperma*. Earlier studies have investigated the possible effects of environmental stresses on the structure of benthic communities of intertidal macroalgae, including species distribution, composition, abundance and succession[19-23]. It has been reported that most of the marine algae, during hyper-salinity conditions, maintain constant cell turgor by regulating osmotic potentials through the adjustment of inorganic ions and osmolytes such as trehalose, sucrose, sorbitol, mannitol, glycerol, praline, glycinebetaine and dimethylsulphoniopropionate (DMSP)[19,24,25]. Several studies have shown that proline accumulated in both micro- and macroalgae as a response to hyper-salinity[22,26,27]. It has been well documented that some osmolytes, particularly proline, function as protestants of macromolecules such as proteins and membranes, while glycinebetaine sustains various enzyme activities under different environmental stresses[28,29]. However, the effect of osmolyte on the growth of *Gayralia* spp. is not been reported so far.

In India, the genus *Gayralia* is represented by a single species of *G. oxysperma* (Kutzing), which was earlier known by *Monostroma oxyspermum* (Kutzing) Doty. *Gayralia* occurs dominantly in brackish water habitats forming extensive mats in mangrove swamps[7,30]. The thallus of estuarine forms exceeds over 30 cm in size in the peak growth period. On the contrary, the marine form is relatively smaller in size (< 3 cm) than brackish water forms and occur as scanty patches with stunted growth on rocks in the uppermost region of the upper littoral zone of Okha coast (west coast of India) almost throughout the year[31,32]. Both forms have been found to propagate asexually through biflagellate swarmers, which develop directly into monostromatic membranous fronds. There exist several estuaries with potential for mariculture of *Gayralia* in India. Since the salinity of these regions varies markedly, particularly during the monsoon period (June – September), an attempt has been made in the present study to determine the salinity tolerance range and associated biochemical changes in *G. oxysperma*.

The current study investigated growth and changes in the concentration of cellular proline, glycinebetaine, carbohydrates and chlorophylls produced in response to salinity induced stress by *G. oxysperma* grown in varying salinities in laboratory cultures. Studying the growth and biochemical changes of *G. oxysperma* under salt stress is crucial for understanding its remarkable resilience and potential applications. By investigating its response mechanisms, the study can uncover valuable insights into plants salt tolerance strategies, leading to the development of more resilient crops and sustainable agriculture practices. Additionally, this research can contribute to the exploration of novel bioactive compounds and the optimisation of cultivation techniques for this promising seaweed species.

Materials and Methods

Plant material and growth conditions

The thallus of *G. oxysperma* used in the present study was regenerated from protoplasts and maintained as an unialgal species in aerated cultures in the laboratory. Prior to growth studies, selected thalli were cleaned gently with a brush in autoclaved seawater (ASW) and grown in aerated flasks containing 250 ml Provasoli's Enriched Seawater (PES) medium[33] under white cool fluorescent tube lights at 30 μ mol photons $m^{-2}.s^{-1}$ with a 12:12 h light:dark cycle at 22±1 °C for 15 days.

Following pre-growth studies, the cultures of *G. oxysperma* were further acclimatised to different salinities by growing as mentioned above in six different flasks, each containing four pieces of 0.25 cm^2 area. During the acclimatisation period, the medium was replenished at weekly intervals. In order to determine the growth responses of *G. oxysperma* to different salinities, the PES medium was prepared using pre-diluted ASW with deionised water to 100 %, 70 %, 50 %, 25 %, 15 %, and 5 % whose final salinities corresponded to 32 ‰, 16 ‰, 8 ‰, 4.8 ‰, and 1.6 ‰, respectively. The salinity of the culture

medium was gradually reduced from 32 ‰ to lower salinities in the above-mentioned order at weekly intervals in 35 days duration by changing the culture medium with the next lower salinity while retaining the preceding higher salinity concentration each time. The alga *G. oxysperma* experiences a wide range of salinity during the monsoon season, and hence the different salinity levels were maintained throughout the present study.

The segments thus acclimatised at 32 ‰, 16 ‰, 8 ‰, 4.8 ‰ and 1.6 ‰ salinities were chosen for studying growth. Five pieces with 6.25 cm^2 in area were cut from each flask and further cultured in a 500 mL round flat-bottom flask filled with 400 mL of sterile seawater supplemented with PES medium in aerated condition. This setup was maintained for a duration of 15 days at a temperature of 25 °C, illuminated by white cool fluorescent light with an irradiance of 50 μmol photons m^{-2} s^{-1} under a day-night photoperiod of light: dark 12:12 h medium with respective salinities (32 ‰, 16 ‰. 8 ‰, 4.8 ‰ and 1.6 ‰). The growth, in terms of increase in fresh weight, was measured on 3rd, 6th, 9th, 12th, and 15th days from the start of the experiment and expressed in terms of times fold increase with respect to initial biomass. The culture medium was changed every third day and cultures were maintained under conditions described for pre-growth studies. The growth experiment was repeated twice, and the data is presented as mean values over two replicates.

Proline, glycinebetaine, and mannitol are known to function as cytosolic osmolytes, interacting with cellular macromolecules including enzymes, to stabilise their structure and function. Additionally, they serve as an energy source post-stress release and regulate cellular redox status. Therefore, the chlorophyll, total sugar, proline and glycinebetaine analysis were done in the present study. The final biomass obtained after 15 days of growth was used for analysing chlorophyll, total sugar, proline and glycinebetaine contents of fronds grown in different salinities.

The tissue paper blotted algal material was ground to a fine powder in mortar and pestle using liquid nitrogen and stored in vials at -40 °C for carrying out above-mentioned biochemical analyses.

Chlorophyll estimation

The chlorophyll content of each sample was estimated using the standard method[34]. To the 100 mg powdered sample, added 10 ml of 80 % acetone and incubated at room temperature for 30 min. Following the incubation, the contents were centrifuged at 12000 rpm for 10 min, and the absorbance of clear supernatant was measured at 645 nm and 665 nm using Varian UV-Vis Spectrophotometer Model Cary 500 (Varian, Australia).

Proline estimation

Proline content of each sample was determined by following the method described by Bates *et al.*[35] with slight modification. 100 mg powder was taken into 1.5 ml micro-centrifuge tubes, and then 1.2 ml of 3 % sulphosalicylic acid was added to precipitate proline. The sample was mixed and centrifuged at 13000 rpm for 10 min, and the filtrate was transferred to a fresh 1.5 ml tube. To 500 ml filtrate, 500 ml distilled water, 1 ml glacial acetic acid and 1 ml of ninhydrin reagent [2 % (w/v) ninhydrin in acetone] was added and incubated at 90 °C for 1 h. Samples were cooled on ice and extracted with 2 ml of toluene by vortexing. The upper solvent phase was used to measure absorbance at 520 nm. Proline concentration was calculated using the standard curve plotted against absorbance versus proline concentration.

Extraction and analysis of glycinebetaine

Glycinebetaine was determined employing the method of Shaw *et al.*[36] with slight modification. To 50 mg powder, 500 μl of sterilised distilled water was added and then centrifuged at 15000 rpm for 10 min. 250 μl of supernatant was transferred to 1.5 ml micro-centrifuge tube containing 250 μl 2N H_2SO_4 and incubated on ice for 2 h, and later added 200 μl of cold KI-I_2 reagent (17.5 g I_2 and 20 g KI prepared in 100 ml of distilled water) and mixed thoroughly. The samples were incubated at 4 °C overnight and later centrifuged at 13000 rpm for 15 min. The supernatant was carefully removed with the help of a pipette, leaving the betaine periodide complex on the sides and bottom of the tube. The residue was re-suspended in 1,2-dichloroethane and transferred to a 10 ml graduated test tube, diluting with washings to 9 ml. Samples were incubated in the dark for 2 h, and the absorbance was read at 365 nm. Glycinebetaine concentrations of unknown samples were calculated against a standard curve prepared from serial dilution of glycinebetaine.

Analysis of carbohydrates

Total sugar content in samples was determined by the phenol-sulfuric acid method of Dubois *et al.*[37]. For

analysing the total sugars, 5 mg dried homogenised powder was taken in a 10 ml centrifuge tube and added 10 ml of 5 % trichloroacetic acid (in water) and then incubated in hot water bath at 90 °C for 3 h. During incubation, the samples were gently shaken periodically at an interval of 30 min. Following the incubation, the contents were allowed to cool down to room temperature and centrifuged at 12000 rpm for 10 min. Aliquot of 200 µl supernatant from each sample was transferred to a separate test tube and then added 1 ml 5 % phenol (w/v) and 5 ml concentrated sulphuric acid and mixed thoroughly. The tubes were allowed to cool down to room temperature for 30 min and the absorbance was read at 490 nm on spectrophotometer. The sugar content in the sample was calculated using the standard curve prepared from serial dilution of glucose standard.

Statistical analysis

All statistical analyses were performed using the SPSS software package (version 28.0 on a Microsoft Windows operating system). Each parameter was tested three times, and their means and Standard Deviation (SD) were calculated. One-way analysis of variance (ANOVA; $p < 0.05$) was used to determine the significance of differences in the means of growth conditions, chlorophyll content, proline content, glycinebetaine content and total carbohydrate content in different culture salinities. When differences were detected in ANOVA, Tukey's Honestly Significant Difference (HSD) Post-Hoc analysis[39] was performed to identify the source of significance.

Results

Growth responses of *G. oxyspermum* to varying salinities

In the present study, *G. oxyspermum* was gradually acclimatised to different salinities ranging from 32 ‰, 16 ‰, 8 ‰, 4.8 ‰ and 1.6 ‰ over a 35 days period to achieve consistency in growth and minimise salinity shock arising from sudden exposure. The growth of fronds varied across the salinity gradient tested and showed a characteristic linear relationship with decreasing salinities (Fig. 1). Though the growth differences remained more or less the same for the first six days across all salinity treatments (except 1.6 ‰), it was more pronounced from day 9 onwards. Growth was comparatively greater in fronds grown in lower salinities than higher ones. The fronds grown in 1.6 ‰ salinity invariably showed higher growth than others from the beginning. Nevertheless, the growth recorded for 32 ‰ and 16 ‰ salinity remained the same throughout the study and ranged from 1.4 – 2.5 and 1.2 – 2.5 fold increase, respectively. Similar growth pattern was also observed for 8 ‰ and 4.8 ‰ ranging between 1.3 and 2.9 fold increase. The overall growth obtained at the end of the growth experiment for different salinities (32 ‰ – 1.6 ‰) ranged between 2.5 and 3.7-fold increase over initial biomass.

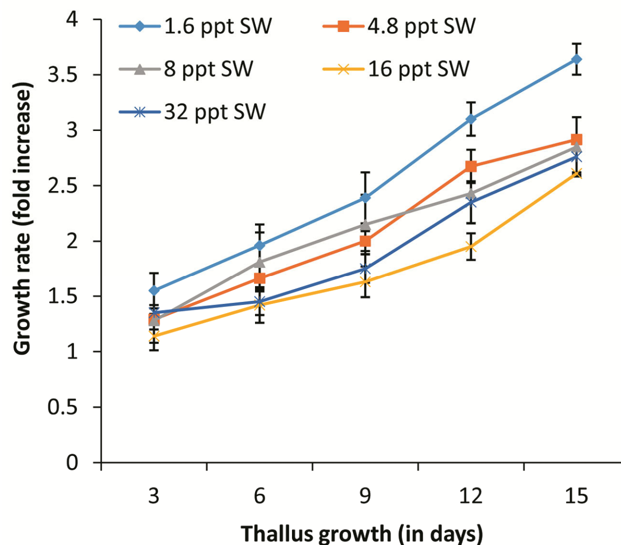

Fig. 1 — Growth of *G. oxysperma* in different salinities for 15 days period. Bars are standard deviation of two independent observations

The microscopic observation of fronds showed variations in cell morphology, cell size and intercellular space across the salinity treatments used (Fig. 2). Interestingly, the intercellular spaces and cell size decreased with increasing salinities. Further, the fronds cultured in 1.6 ‰ salinity were found to have unevenly distributed elongated cells with low density. The fronds disintegrated when grown at less than 1.6 ‰ salinity over 10 days period, indicating the requirement of critical amounts of salt for its survival and sustenance.

Chlorophyll content

Total chlorophyll content showed a distinct trend of increase with increasing salinity from 1.6 to 32 ‰ salinity (Table 1). The increase in chlorophyll content for 32 ‰ salinity was a factor of 2.9 over the lowest salinity (1.6 ‰) value. The mean total chlorophyll values ranged from 2.8 $mg.g^{-1}$ (±0.41 $mg.g^{-1}$) – 8.0 $mg.g^{-1}$ (±0.1 $mg.g^{-1}$) for 1.6 ‰ and 32 ‰ salinity, respectively. However, the chlorophyll content among 8 – 32 ‰ salinity varied narrowly from 6.6 $mg.g^{-1}$ (±0.6 $mg.g^{-1}$) – 8.0 $mg.g^{-1}$ (±0.08 $mg.g^{-1}$).

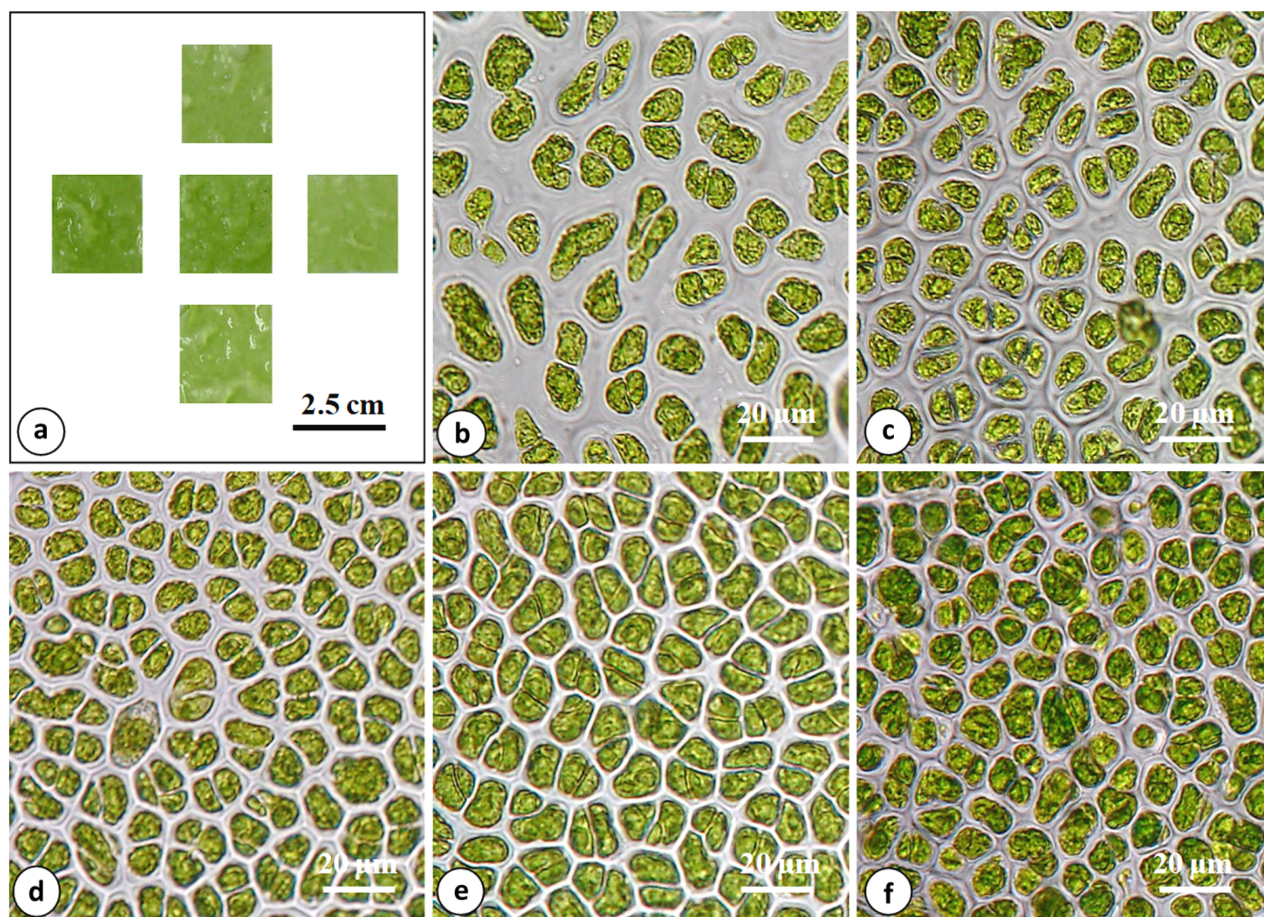

Fig. 2 — a – f) Surface view of 15 days old *G. oxysperma* thallus grown in different salinities: a) Thallus segments at the beginning of growth experiment, b – f) Microscopic view of cells of thalli at: b) 1.6 ‰, c) 4.8 ‰, d) 8 ‰, e) 16 ‰, and f) 32‰

Proline content

The proline content also increased with increasing salinity from 1.6 to 32 ‰. Nevertheless, the proline content for 1.6 to 16 ‰ showed an increase of multiple of 2 while 32 ‰ showed a multiple of about 10 over the value obtained for the lowest salinity (0.15±0.03 $mg.g^{-1}$). The fronds grown in 32 ‰ salinity showed accumulation of proline as high as 1.9±0.04 $mg.g^{-1}$ compared to other lower salinities (Table 1).

Glycinebetaine

The glycinebetaine content of fronds grown in different salinities also showed a linear relationship with increasing salinities from 1.6 – 32 ‰ and ranged from 7.6±0.30 – 17.7±0.40 $mg.g^{-1}$ (Table 1). The highest accumulation of glycinebetaine content was recorded in 32 ‰.

Total carbohydrates

The carbohydrate content of samples analysed for various salinities did not show characteristic pattern and varied between 19.7±1.5 – 26.0±1.0 % (Table 1). Although the values obtained for various salinities showed marginal variation in carbohydrate content, the fronds from 4.8 ‰ showed content as high as 26.0±1.0 %.

Discussion

Most intertidal benthic marine macroalgae employ an array of complex physiological and biochemical mechanisms for acclimation to fluctuating environmental conditions in their habitats[39]. The results of the present study reveal the growth of *G. oxysperma* in relation to varying salinities and associated biochemical changes for adaptation. *Gayralia*, being an estuarine form, exhibited its innate abilities to tolerate and grow well in a wide range of salinities of 1.6 – 32 ‰ with growth optima at 1.6 ‰ indicating its potential for mariculture in brackish waters of India. Fong *et al.*[40], while studying the seasonal succession pattern and dominance between *Ulva expansa* (Satchel) Satchel & Gardner and

Table 1 — Mean chlorophyll content, proline content, glycinebetaine content and total sugar content of *G. oxysperma* thallus during 15 days of experimentation with varying salinities (3rd day, 6th day, 9th day, 12th days and 15th day of sampling/measurements)

Sampling/Measurements (days)	Salinity	Chlorophyll	Proline	Glycinebetaine	Total sugars
	1.6 ‰	1.78±0.11[a]	0.07±0.01[a]	5.95±013[a]	0.30±0.01[a]
	4.8 ‰	2.69±0.09[a]	0.17±0.01[b]	7.99±0.09[b]	0.31±0.01[a]
3rd day	8 ‰	3.99±0.13[bc]	0.28±0.05[c]	9.37±0.14[c]	0.32±0.03[a]
	16 ‰	4.18±0.12[c]	0.35±0.02[c]	10.12±0.21[cd]	0.34±0.02[a]
	32 ‰	4.34±0.24[c]	0.79±0.03[d]	11.49±0.14[d]	0.36±0.01[a]
	1.6 ‰	1.93±0.20[a]	0.09±0.02[a]	6.11±0.02[a]	0.34±0.09[a]
	4.8 ‰	2.99±0.11[a]	0.21±0.05[b]	8.23±0.06[b]	0.33±0.02[a]
6th day	8 ‰	4.62±0.21[bc]	0.32±0.03[b]	10.46±0.04[b]	0.35±0.05[a]
	16 ‰	4.95±0.14[c]	0.41±0.09[c]	11.31±0.16[c]	0.35±0.01[a]
	32 ‰	5.21±0.13[c]	0.86±0.10[d]	13.61±0.09[d]	0.38±0.08[a]
	1.6 ‰	2.01±0.33[a]	0.10±0.02[a]	6.71±0.12[a]	0.34±0.01[a]
	4.8 ‰	3.43±0.21[a]	0.27±0.05[b]	8.90±0.15[bc]	0.35±0.05[a]
9th day	8 ‰	5.09±0.3[a]	0.38±0.04[b]	12.29±0.10[c]	0.39±0.06[a]
	16 ‰	6.32±0.14[a]	0.46±0.09[c]	12.53±0.09[c]	0.36±0.08[a]
	32 ‰	6.68±0.22[a]	0.93±0.08[d]	15.20±0.17[d]	0.41±0.01[a]
	1.6 ‰	2.43±0.11[a]	0.11±0.01[a]	7.03±0.09[a]	0.35±0.02[a]
	4.8 ‰	4.07±0.16[a]	0.31±0.02[b]	9.76±0.06[b]	0.39±0.03[a]
12th day	8 ‰	5.89±0.19[a]	0.43±0.01[bc]	13.01±0.22[c]	0.40±0.01[a]
	16 ‰	6.84±0.18[a]	0.59±0.05[c]	13.20±0.09[cd]	0.39±0.05[a]
	32 ‰	7.40±0.11[a]	1.10±0.03[d]	16.54±0.11[d]	0.43±0.04[a]
	1.6 ‰	2.75±0.41[a]	0.15±0.03[a]	7.6 ±0.30[a]	0.41±0.03[a]
	4.8 ‰	5.03±0.32[b]	0.4±0.02[b]	10.5±0.12[b]	0.49±0.06[a]
15th day	8 ‰	6.64±0.27[b,c]	0.57±0.09[b]	13.64±0.15[c]	0.43±0.02[a]
	16 ‰	7.08±0.31[c]	0.83±0.04[c]	14.72±0.26[c]	0.41±0.02[a]
	32 ‰	7.86±0.08[c]	1.9±0.04[d]	17.7±0.40[d]	0.39±0.09[a]

Values in the rows denoted by a different letter indicate significant difference at $P \leq 0.05$ in one-way ANOVA. Mean average value ± SD obtained from three replicates. Means followed by the same letter is not significantly different at 5 % level

Enteromorpha intestinalis (Linnaeus) Nees in coastal lagoons and estuaries reported that *E. intestinalis* with differential salinity tolerance and greater N uptake abilities dominated over *U. expansa* during low salinity and high nutrient regimes.

This may explain the dominance and larger thallus size of *G. oxyspermum* found in brackish water habitats than in oceanic waters. Further reduction of salinity < 1.6 ‰ caused disintegration of fronds in 10 days period, indicating the potential role of Na^+ ions for osmotic adjustment. Therefore, it is evident from this study that salt at critical levels in ambient waters is crucial for the survival of *Gayralia*. Similar results were obtained by Kakinuma *et al.*[41] during thermal stress. In *E. intestinalis*, an estuarine algae, at 0 ‰ practical salinity unit, loss of pigmentation occurred and both wet and dry biomass declined[42].

The cell morphology, cell size and intercellular spaces varied across the salinity gradient tested. The unique cell distribution pattern as well as morphology observed in fronds at 1.6 ‰ salinity, could be a kind of cellular adaptation that might check the transpiration/ evaporation rate at high concentrations of salt in the tissues.

Generally, photosynthetic activity is suppressed under salt and water stresses[43]. However, in sea asters, which grow naturally in salt marshes and coastal areas, water stress affects photosynthetic activity more severely than salt stress[44]. The increased chlorophyll content in *Gayralia* could compensate for the damaged photosynthetic activity arising from physiological drought caused by salt stress. *Ulva pertusa* also showed accumulation of photosynthetic pigments with increasing salinity[41]. In *Dunaliella*, high salinity enhanced photosynthetic CO_2 assimilation and diverted carbon and energy resources for synthesising glycerol, a major osmotic element[45].

During environmental stress, the use of ions for osmotic adjustment is energetically more favourable than the biosynthesis of organic osmolytes.

Nevertheless, plants accumulate organic osmolytes since high concentrations of inorganic ions cause protein denaturation[46]. Several higher plants, marine algae and bacteria accumulate organic solutes to equalise external osmotic pressure. During stress treatment, different cellular enzymes and osmolytes are produced to antagonise the osmotic pressure in the cytosol[28,47]. Proline, glycinebetaine and mannitol have been reported to function as cytosolic osmotic and interacting with cellular macromolecules such as enzymes and stabilise their structure and function, serve as an energy source after the release of stress, and as a regulator of cellular redox status[28]. Many researchers found accumulation of proline in plants exposed to salt stress. Proline has increased markedly in *Gayralia* thallus with increasing salinity in the medium. It showed about 10-fold increases in 32 ‰ salinity than 1.6 ‰. Similarly, the proline has also been reported to accumulate in several micro- and macro-algae in response to elevated salinity like in *Ulva fasciata*[22,39], *Nostoc muscorum*[27], and *Ulva pertusa*[48].

Glycinebetaine (N,N,N-trimethylglycine-betaine) and trehalose act as osmoprotectants by stabilising the quaternary structures of proteins and highly ordered states of membranes. Although, a detailed study on glycinebetaine tolerance mechanism in higher plants has been reported, limited information is available with regard to marine algae. In *Gayralia*, glycinebetaine content increased linearly with the increasing concentration of salinity. In phytoplankton, this compound appears to function as a true compatible salute without inhibiting enzyme functions and stabilising macromolecules even at high intracellular concentrations[49,50].

The concentration of soluble sugars, in general, increases or remains constant under stress conditions[51]. The hydroxyl group of sugar alcohols substitutes the OH group of water to maintain the hydrophilic interactions with the membrane lipids and proteins. In this study, the total sugar level in tissue remained constant under varying salt concentrations.

Conclusion

The growth of *G. oxysperma* to varying salinities (1.6 – 32 ‰) was found optimal at 1.6 ‰. Unique cell distribution pattern and morphology was observed in fronds at 1.6 ‰ salinity. The total chlorophyll, proline and glycinebetaine content was found higher at a higher salinity regime *i.e*. 32 ‰, while the total sugar level in tissues remained constant under varying salt concentrations. The acquisition of growth in *Gayralia* during salt stress is regulated by the high expression of osmoprotectants. Therefore, it would be interesting to study the molecular mechanism of stress tolerance by isolating and characterising the genes conferring salinity tolerance in *Gayralia*.

Acknowledgments

We would like to thank Mr. Sheetal Patel, Secretary (SPEC), for encouragement in the present study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Statement

This is to certify that the reported work in the paper entitled “Growth and biochemical changes in response to salinity stress in *Gayralia oxysperma* (Kützing) K.L. Vinogradova ex Scagel et al. 1989 (Chlorophyceae)” submitted for publication is an original one and has not been submitted for publication elsewhere. We further certify that proper citations to the previously reported work have been given and no data/table/figure has been quoted verbatim from other publications without giving due acknowledgement and without the permission of the author(s). The consent of all the authors of this paper has been obtained for submitting the paper to the Indian Journal of Geo-Marine Sciences.

Author Contributions

MCT conceptualised the work, supervised and contributed for manuscript draft, editing and finalising. SL carried out the statistical analysis using the One-way ANOVA (Analysis of Variance) and did formal analysis.

References

1 Ohno M & Critchley A T (eds.), *Seaweed cultivation and marine ranching*, (Kanagawa International Fisheries Training Center, Japan International Cooperative Agency, Yokosuka, Japan), 1993, pp. 162.

2 Pellizzari F, Vélez-Rubio G M, Cristine-Silva M & Carranza A, The seaweeds *Myriogloea major* Asensi (Chordariacea, Phaeophyceae) and *Gayralia oxysperma* (Kützing) K.L. Vinogradova ex Scagel *et al*. (Ulvophyceae, Chlorophyta): a case of range extension in the Southwestern Atlantic Ocean? *Mar Biodivers*, (2016) 1–8. https://doi.org/10.1007/s12526-016-0447-8

3 Pise N M, Verlecar X N, Gaikwad D K & Jagtap T G, Nutraceutical properties of the marine macroalga *Gayralia*

oxysperma, *Bot Mar*, 55 (6) (2012) 581–589. http://dx.doi.org/10.1515/bot-2012-0161

4 Pellizzari F M, Absher T, Yokoya N S & Oliveira E C, Cultivation of the edible green seaweed *Gayralia* (Chlorophyta) in southern Brazil, *J Appl Phycol*, 19 (2007) 63–69. https://doi.org/10.1007/s10811-006-9111-1

5 Wilkinson M, Estuarine benthic algae and their environment: a review, *Shore Environ*, 2 (1980) 425–486.

6 Mathieson A C & Penniman C A, Species composition and seasonality of New England seaweeds along an open coastal-estuarine gradient, *Bot Mar*, 29 (1986) 161–176.

7 Deshmukhe G V K, Dhargalkar V K & Untawale A G, Life history and culture studies of *Monostraoma oxyspermum* (Kutz.) Doty (Monostromataceae; Chlorophyceae) growing in estuarine conditions along the central west coast of India, *Curr Sci*, 75 (12) (1998) 1302–1303.

8 Guiry M D & Guiry G M, AlgaeBase, World-wide electronic publication, available online at: http://www.algaebase.org; Accessed on March 2024.

9 Wong K H & Cheung P C K, Nutritional evaluation of some subtropical red and green seaweeds, Part I-proximate composition, amino acid profiles and some physico-chemical properties, *Food Chem*, 71 (2000) 475–482.

10 Norziah M H & Ching C Y, Nutritional composition of edible seaweed *Gracilaria changii*, *Food Chem*, 68 (2000) 69–76.

11 Sánchez-Machado D I, López-Hernández J & Paseiro-Losada P, High-performance liquid chromatographic determination of alpha tocopherol in macroalgae, *J Chromatogr*, 976 (2002) 277–284.

12 McHugh D J, A guide to the seaweed Industry, *FAO Fisheries Technical Paper 441*, (2003) 1-105.

13 Cassolato J E F, Noseda M D, Pujol C A, Pellizzari F M, Damonte E B, *et al.*, Chemical structure and antiviral activity of the sulfated heterorhamnan isolated from the green seaweed *Gayralia oxysperma*, *Carbohydr Res*, 343 (2008) 3085–3095. https://doi.org/10.1016/j.carres.2008.09.014

14 Kelman D, Posner E K, McDermid K J, Tabandera N K, Wright P R, *et al.*, Antioxidant Activity of Hawaiian Marine Algae, *Mar Drugs*, 10 (2012) 403–416. https://doi.org/10.3390/md10020403

15 Ropellato J, Carvalho M M, Ferreira L G, Noseda D, Zuconellib A R, *et al*., Sulfated heterorhamnans from the green seaweed *Gayralia oxysperma*: Partial depolymerisation, chemical structure and antitumor activity, *Carbohydr Polym*, 117 (2015) 476–485. https://doi.org/10.1016/j.carbpol.2014.09.089

16 da Silva A C R, Duarte M E R, Noseda M D, Ferreira L G, Cassolato J E F, *et al.*, Potential Utilization of a Polysaccharide from the Marine Algae *Gayralia oxysperma*, as an Antivenom for Viperidae Snakebites, *Mar Drugs*, 16 (412) (2018) 1–14. https://doi.org/10.3390/md16110412

17 Barbara I & Cremades J, *Guia de las algas del litoral gallego*, (2nd edn), (Ayuntamiento de A Coruña, Spain), 1993, pp. 190.

18 Ganesan M, Reddy C R K, Trivedi N, Gupta V, Madhav V S, *et al.*, Seaweed resources in India - Current status of diversity and cultivation: Prospects and challenges, *Bot Mar*, 62 (5) (2019) 463–482. http://dx.doi.org/10.1515/bot-2018-0056

19 Kirst G O, Salinity tolerance of eukaryotic marine algae, *Annu Rev Plant Physiol Plant Mol Biol*, 40 (1990) 21–53.

20 Kubler J E & Davison T R, High temperature tolerance of photosynthesis in the red alga *Chondrus crispus*, *Mar Biol*, 117 (1993) 327–335.

21 Maegawa M & Sugiyama A, Relationship between heat tolerance and the vertical distribution of intertidal algae, *Suisanzoshoku*, 43 (1995) 429–435.

22 Lee T M & Liu C H, Correlation of decreased calcium contents with proline accumulation in the marine green macroalga *Ulva fasciata* exposed to elevated NaCl contents in seawater, *J Exp Bot*, 50 (341) (1999) 1855–1862.

23 Carrier-Belleau C, Drolet D, McKindsey W C & Archambault P, Environmental stressors, complex interactions and marine benthic communities, responses, *Sci Rep***,** 11 (4194) (2021) 1–14. https://doi.org/10.1038/s41598-021-83533-1

24 Reed R H, Solute accumulation and osmotic adjustment, In: *Biology of the red algae*, edited by Cole K M & Sheath R G, (Cambridge University press, UK), 1990, pp. 147–170.

25 McParland E L, Alexander H & Johnson W M, The Osmolyte Ties That Bind: Genomic Insights Into Synthesis and Breakdown of Organic Osmolytes in Marine Microbes, *Front Mar Sci*, 8 (2021) 1–20. https://doi.org/10.3389/fmars.2021.689306

26 Bartels D & Nelson D, Approaches to improve stress tolerance using molecular genetics, *Plant Cell Environ*, 17 (1994) 659–667.

27 Singh A K, Chakaravarthy D, Singh T P K & Singh H N, Evidence for a role for L-proline as a salinity protestant in the cyanobacterium *Nostoc muscorum*, *Plant Cell Environ*, 19 (1996) 490–94.

28 Delauney A J & Verma D P S, Prolhae biosynthesis and osmoregulation in plants, *Plant J*, 4 (1993) 215–223.

29 Mickelbart M V, Ejeta G, Rhodes D, Joly R J & Goldsbrough P B, Assessing the contribution of glycinebetaine to environmental stress tolerance in sorghum, In: *Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments*, An International Workshop, El Batan, Mexico. 21–25 June 1999, (International Maize and Wheat Improvement Center, El Batan, Mexico), 1999.

30 Kumar G R, Addepalli M K & Reddy C R K, Regeneration of the phallus of *Monostroma oxyspermum* (Chlorophyta) from protoplasts in axenic culture, *Phycologia*, 38 (1999) 503–507.

31 Oza R M, Joshi H V, Parekh R G & Chauhan V D, Preliminary observations on *Monostroma* sp. from the Okha coast, Gujarat, *Indian J Geo-Mar Sci*, 12 (1983) 115–117.

32 Jha B, Reddy C R K, Thakur M C & Rao U M, Seaweeds of India: The Diversity and Distribution of Seaweeds of Gujarat Coast, 1st edn, (Springer, Dordrecht), 2009, pp. 205. https://doi.org/10.1007/978-90-481-2488-6

33 Provasoli L, Media and prospects for the cultivation of marine algae, In: *Cultures and collection of algae*, Proceedings of the US/Japan Conference, Hakone, edited by Watanable A & Hatton A, *J Braz Soc Plant Physiol*, (1968) 63–75.

34 Arnon D I, Copper enzyme in isolated chloroplasts, polyphenoloxidase in *Beta vulgaria*, *Plant Physiol*, 24 (1949) 1–15.

35 Bates L S, Waldren R P & Tease I D, Rapid determination of proline for water stress studies, *Plant Soil*, 39 (1973) 205–207.

36 Shaw B, Thomas T H & Cooke D T, Responses of sugar beet (*Beta vulgaris* L.) to drought and nutrient deficiency stress, *Plant Growth Regul*, 37 (2002) 77–83.

37 Dubois N, Gilles K A, Hamilton J K, Rebers P A & Smith F, Colorimetric method for determination of sugars and related substances, *Annal Chem*, 28 (1956) 350–356.

38 Sokal R R & Rohlf F J, *Biometry: the principles and practice of statistics in biological research*, (WH Freeman, New York), 1981, pp. 859.

39 Liu C H, Shih M C & Lee T M, Free proline levels in *Ulva* (Chlorophyta) in response to hypersalinity: Elevated NaCl in seawater versus concentrated seawater, *J Phyco1*, 36 (1) (2000) 118–119. https://doi.org/10.1046/j.1529-8817.2000.99127.x

40 Fong P, Boyer E K, Desmond J S & Zedler J B, Salinity stress, nitrogen competition and facilitation: What controls seasonal succession of two opportunistic green macroalgae, *J Exp Mar Biol Ecol*, 206 (1996) 203–221.

41 Kakinuma M, Coury D A, Kung Y, Itoh S, Kozawa Y, *et al.*, Physiology and biochemical responses to thermal salinity stresses in a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta), *Mar Biol*, 149 (2006) 97–106. https://doi.org/10.1007/s00227-005-0215-y

42 Kamer K & Fong P A, Fluctuathag salinity regime mitigates the negative effects of reduced salinity on the estuarine macroalga, *Enteromorpha intestinalis* (L.) link, *J Exp Mar Biol Ecol*, 254 (2000) 53–69. https://doi.org/10.1016/s0022-0981(00)00262-8

43 Scemann J R & Critchley C, Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L, *Planta*, 164 (1985) 151–162.

44 Ueda A, Kanechi M, Uno Y & Inagaki N, Photosynthetic limitations of a halophyte sea aster (*Aster tripolium* L) under water stress and NaCl stress, *J Plant Res*, 116 (2003) 65–70. https://doi.org/10.1007/s10265-002-0070-6

45 Liska A J, Shevchenko A, Pick U & Katz A, Enhanced photosynthesis and redox energy production contribute to salinity tolerance in *Dunaliella* as revealed by homology-based proteomics, *Plant Physiol*, 136 (1) (2004) 2806–2817. https://doi.org/10.1104/pp.104.039438

46 Timasheff S N, A physiochemical basis for the selection of osmolytes by nature, In: *Water and life*, edited by Somero G N, Osmond C B & Bolts C L, (Springer Verlag, Berlin), 1992, pp. 70–84.

47 Suescún-Bolívar L P, Traverse G M I & Thomé P E, Glycerol outflow in *Symbiodinium* under osmotic and nitrogen stress, *Mar Biol*, 163 (2016) p. 128. https://doi.org/10.1007/s00227-016-2899-6

48 Kakinuma M, Kuno Y & Amana H, Salinity stress responses of a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta), *Fish Sci*, 70 (2004) 1177–1179. https://doi.org/10.1111/j.1444-2906.2004.00921.x

49 Gröne T & Kirst G O, The effect otnitrogen deficiency, methionine and inhibitors of methionine metabolism on the DMSP content of *Tetraselmis subcordiformis* (Stein), *Mar Biol*, 112 (1992) 497–503.

50 Nishiguchi M K & Romero G N, Temperature- and concentration dependence of compatibility of the organic osmolyte B-dimethylsulfoniopropionate, *Cryobiol*, 29 (1992) 118–24.

51 Pinheiro C, Chaves M M & Ricardo C P, Alterations in carbon and nitrogen metabolism induced by water deficit in the stems and leaves of *Lipinus albus* L, *J Exp Bot*, 52 (2001) 1063–1070. https://doi.org/10.1093/jexbot/52.358.1063