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Studies on the effect of natural extract of some marine algae on *in vitro* growth and development of banana micropropagules

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Abstract

The current work aims to provide a cost-effective and reliable micropropagation method of banana using some natural extract of unexplored potential seaweeds. We tried natural extract of eight different seaweeds (*Caulerpa racemosa, Gracilaria edalis, Caulerpa paspaloides, Ulva lactuca, Sargassum tenerrimum., Caulerpa sertularioides, Sargassum wightii, Gracilaria corticata*.) to check their role in *in vitro* growth and development of micropropagules and their potential in replacing commercial plant growth regulators (PGRs) in banana micropropagation. The *in vitro* developed shoots were allowed to grow on modified MS medium supplemented with different seaweed extracts and regularly subcultured on the same medium throughout the experiment. The growth and biochemical parameters were recorded and compared with micropropagules growing in a controlled environment (*i.e.*, MS medium supplemented with/ without standard PGRs). We found a significant increase in shoot length (highest in *S. tenerrimum, i.e.* double as compared to negative control), shoot number (highest in *G. edalis*, *i.e.,* three fold as compared to negative control) and fresh weight (*S. tenerrimum*) of the banana micropropagules when grown under the influence of Liquid Seaweed Extract (LSE). This is comparable to growth observed in standard control conditions (*i.e.* positive control). However, addition of LSE of *G. corticate, C. paspaloides* and *S. wightii* did not show any noteworthy effect on shoot length, shoot number and fresh weight. Total chlorophyll and other biomolecule accumulation in test propagules varied with types and concentrations of LSE. *S. tenerrimum* proved to be overall good growth promoter, as it caused maximum accumulation of chlorophyll (0.202±0.02 mg g^{-1}) and protein (105±0.45 mg g^{-1}) and significant and encouraging results in phenol, carbohydrates and proline. Our results show that seaweed can be potential source of beneficial natural compounds and it can be a cheaper option in banana micropropagation technology.

Key words: Seaweed, micropropagation, banana, low-cost alternatives

Introduction

The banana (*Musa sp*.) is one of humankind's important and oldest fruits and is valued for its nutritional properties and economic role. Based on its gross value, it is the world's fourth most widely consumed food crop after rice, wheat, and corn. It is commonly grown in the tropics and subtropics in all agricultural systems. In India, bananas account for 32.39% (2020-2021) of total fruit production. Multiplication of bananas by traditional methods increases the chance of disease in plants, and these methods are also very tedious and time-consuming. These problems in banana cultivation can be overcome by tissue culture technology, which is an exclusive tool for producing banana plants in less time.

Moreover, micropropagation also helps to develop pathogen-free and healthy plants that can be directly planted to fields (Joshi and Purohit, 2012). In the last two decades, the commercial tissue culture has witnessed a revolution, and the technique has been converted into a successful industry-oriented method, especially for ornamental and horticultural plants (Patil *et al.*, 2021). However, there are a few limitations of this technology when compared to the traditional methods of plant propagation. For example, commercial micropropagation is a capital-intensive sector since it requires various expertise, infrastructure, sophisticated equipment, and controlled environmental conditions. Consequently, the unit cost per plant can be excessive in some cases (Teraiya *et al.*, 2023). Therefore, business growth in developing countries like India has been limited, with only well-funded institutions and large corporations thriving while smaller units exit the market. Studies have aimed to reduce production costs in large-scale conditions to address this issue. They've explored replacing expensive medium ingredients, such as agar-agar, a major contributor to costs, with alternatives like guar gum, cotton fiber, xanthan gum, isabgol, and corn/potato starch (Nirmal *et al.*, 2023; Chauhan *et al.*, 2018) Similarly, high-grade analytical salts have been swapped with low-grade raw salts, and pure sucrose (as a carbon source) with cheaper sugars. Reducing the costs of agar-agar and sucrose, which constitute 49.61% and 38.49% of standard MS medium preparation, has been a primary research focus (Patil *et al.*, 2021). Synthetic plant growth regulators (PGRs), such as 6-Benzylaminopurine (6-BA), have been overlooked in the cost analysis of standard MS medium production. These PGRs contribute significantly to production costs, with 6-BA alone accounting for 7.78% of costs and other components totaling 4.12% (Chauhan *et al.*, 2018).

Marine microalgae, commonly known as seaweeds, have been used by humans since ancient times for many important applications. Earlier, the Romans, Chines and Japanese were using seaweeds as a biofertilizer and manure in agriculture and other industries. Seaweeds as a biofertilizer, promote plant growth, provide better growth under stress conditions and help in nutrient uptake from soil (Yadav *et al.*, 2016). Seaweed also contains some essential minerals (like micronutrients,

macronutrients, amino acids, and vitamins), which help the plant to grow in adverse conditions and act as a source of nutrients for crops (Yadav *et al.*, 2016). Seaweeds have garnered significant attention in plant growth due to their potential as biofertilizers. Maxicrop, in the 1940s, was among the pioneers to introduce seaweed-based liquid biofertilizers to the market, igniting interest in the growth-promoting properties of seaweeds. Researchers have identified nine different types of plant growth-promoting hormones in seaweeds, including auxins (IBA, NAA, IAA), cytokinins (BAP, Kin, trans-zeatin, 2iP), and abscisic acid (ABA) (Mori *et al.*, 2017). Studies have also detected indole-3-pyruvic acid (IPA), a key auxin biosynthesis regulator in seaweeds like *Sargassum tennerrimun*, *Kappaphcus alvarezii*, and *Gracilaria edulis*. Cytokinins such as iPR, tZR, tZ, and aromatic cytokinins like 5 topolins have been reported in species like *L. japonica*, *G. edulis*, and *S. tenerrimum*. Additionally, Gibberellic acid (GA_3) was found in *M. oxyspermum*, *G. edulis*, *S. tenerrimum*, and various *Ulva* species (Gupta *et al.*, 2011). Many studies have utilized natural seaweed extracts to create liquid biofertilizers, leading to increased shoot growth, longer roots, and improved overall plant development *in vivo*. However, the application of these extracts *in vitro* remains relatively unexplored, except for some promising reports, particularly in tomato cultivation. Seaweed-based biofertilizers hold the potential for enhancing plant growth and productivity.

In plant tissue culture, synthetic MS media typically contain various essential components, but specific nutritional requirements vary among plant species and even within plant cells. It is hypothesized that incorporating natural Liquid Seaweed Extracts (LSE) into the growth medium can potentially reduce the need for synthetic plant growth regulators (PGRs). This approach lowers production costs and serves as a natural growth enhancer in cultured conditions. This innovation could make banana micropropagation more economically viable, representing a cost-effective method for its production.

Materials and methods

Explant preparation and culture establishment: Banana (*Musa sp.*) shoot culture was initiated using healthy plant suckers collected from Sokhda farm near Vadodara (Gujarat). Suckers were initially washed with tap water to remove dirt and then sterilized in the lab with 2% Bavistin (fungicide) for 20 minutes. Afterward, they were rinsed twice with autoclaved distilled water. Further, explant sterilization was performed in a laminar airflow (LAF) bench using 1% sodium hypochlorite (NaOCl) for 10 minutes, followed by three to four washes with sterile distilled water. The explants, cut into 3-4 cm pieces, were aseptically placed on Murashige and Skoog's (1962) medium supplemented with 5.0 mg L^{-1} 6-Benzylaminopurine (BAP), 0.01 mg L^{-1} 1-Naphthaleneacetic acid (NAA), 0.8% agar, and 3.0% sucrose. Routine sub-culturing was performed every three weeks under controlled conditions: 28.0 °C temperature, 16-hour light/8-hour dark cycle, 85% humidity, and a photon flux density of 45 µmol $m^{-2}s^{-1}$ (Ahmed *et al.*, 2014).

Seaweed extraction preparation: Eight seaweed species from Bayt Dwarka, Gujarat (22° 28' 39.9" N, 69° 08' 10.8" E) were collected, cleaned, and dried at 60 °C for 72 hours. They were then ground into a powder using an electric mill. To prepare liquid seaweed extracts (LSEs), 500 g of each sample was boiled in 1L of distilled water with constant stirring for 20 minutes. The resulting solutions were filtered through muslin cloth and Whatman No. 40 filter paper and stored at 4 °C as stock solutions for experiments (Vinoth *et al.*, 2014).

Experimental design: In our experiment, we assessed the impact of seaweed extracts on banana micropropagule growth. We selected eight seaweed species (*C. racemosa*, *Gracilaria edulis*, *Caulerpa paspaloides*, *Ulva Lactuca*, *Sargassum tenerrimum*, *Caulerpa sertularioides*, *Sargassum wightii*, and *G. corticata*) and tested various concentrations of liquid seaweed extract (LSE) ranging from 10% to 50% (v/v). We included positive and negative controls (standard MS medium with and without plant growth regulators) for comparison. Each experiment was conducted in 200 ml culture bottles containing 50 ml of medium with a pH of 5.8. After autoclaving and solidification, five aseptically inoculated shoots were placed in each bottle. The bottles were transferred to a growth room for 126 days, involving six subcultures. We maintained six replicates per experiment, with three repetitions of each treatment. At the experiment's end, micropropagules were evaluated using various growth factors and biochemical analyses.

Measurement of growth parameters: We measured shoot length, total shoot count, and biomass (fresh and dry weight) of propagules. Fresh weight was assessed with an electronic balance, and shoots were dried at 62 °C until a constant weight was reached to determine dry weight.

Chlorophyll contents: The chlorophyll contents in the micropropagules of banana were calculated per the protocol Arnon described (1949). Green shoots (500 mg) were crushed in 80% acetone under darkness, and the extract was centrifuged at 10,000 rpm. The supernatant was then used for absorbance measurements using a Shimadzu UV-1800 spectrophotometer (Japan).

Total phenols: Phenol content was assessed following Mahadevan's (1975) method. 500 mg of shoots were crushed in 70% methanol centrifuged at 10,000 rpm for 15 minutes, and the clear supernatant was used. In a test tube, it was mixed with diluted Folin-Ciocalteu reagent and sodium carbonate. After brief heating and cooling, the blue product was diluted, and phenol concentration was measured at 650 nm using a Shimadzu UV-1800 spectrophotometer with a caffeic acid standard.

Total carbohydrates: Total carbohydrate estimation followed Tandon's (1976) method. One-gram fresh shoot samples were crushed in 0.1 M phosphate buffer (pH 7.0) centrifuged at 10,000 rpm for 15 min. Then, 15 µL supernatant was mixed with 4.0 mL of 0.2% anthrone reagent in concentrated H_2SO_4 and boiled in a water bath for 5 min. Absorbance at 610 nm was measured after green colour development, with a standard curve prepared using varying glucose concentrations.

Total protein: To estimate total protein, we followed the method described by Bradford (1976). One ml of supernatant (methanolic extract suitably diluted and centrifuged) was taken and mix with 5.0 ml Bradford reagent (Coomassie Brilliant Blue G-250 dye) and absorbance was taken at 595 nm. The protein concentration was calculated by using a standard curve of bovine serum albumin protein.

Total proline: Total proline was determined following Bates *et al.*'s (1973) method. One gram of fresh tissue was crushed in 10 mL of 3.0% aqueous sulphosalicylic acid and centrifuged at 10,000 rpm for 15 minutes. Two mL of appropriately diluted supernatant was mixed with 2.0 mL of glacial acetic acid and 2.0 mL of freshly prepared ninhydrin. The mixture was boiled for 1 hour, then cooled on ice. After adding 4.0 mL of toluene, the pink toluene layer was separated, and transmittance was measured at 520 nm using a Shimadzu UV-1800 spectrophotometer with a standard curve prepared using varying concentrations of L-proline.

Result and discussion

India's agri-biotechnology industry, focusing on horticulture, plays a significant role in the economy by supplying plantlets to over 100 countries. The Department of Biotechnology certified about 100 plant tissue culture-based industries under the "National Certification System for Tissue Culture Raised Plants (NCS-TCP)" in 2016, a significant increase from just one in 1985. However, this number has declined to 69 in the last decade (source: https://dbtncstcp.nic.in/Recognised-TCPUs). Notably, bananas, guavas, date palms, and ornamental plants are preferred for commercial production *via* tissue culture. Improving micropropagation protocols, especially for bananas, is crucial, although the higher production cost remains a challenge compared to traditional methods. Researchers have often tried to cut production costs in tissue culture by altering gelling agents and carbon sources, while the expense of synthetic plant growth regulators (PGRs) has been overlooked. This study investigated the potential of seaweed extracts as more economical alternatives to synthetic PGRs.

The number of shoots was found to be highest (9.32±1.83) when *Gracilaria edalis* extract was added at a 30% (v/v) concentration range (Table 1). Among the different concentrations of various other LSE tested, 30% concentration was found to be best in terms of increase in shoot number except in the case of *Caulerpa racemose* and *Sargassum tenerrimum* where 50% concentration (9.00±0.82) was found to be best. In contrast, in the case of *C. sertularioides,* 10% concentration was found to be best for the increase in shoot number (7.60±0.50). It was recorded that a higher concentration (50% v/v) of LSE always resulted in a decrease in shoot number and this can be explained by the fact that with increasing concentrations, the number of inhibitory molecules present in the LSE also increases, which subsequently interfere in growth (Chauhan *et al.*, 2018). In addition to the growth regulators, the addition of LSE also increases various other important nutrients like minerals, amino acids and vitamins in the medium, which indirectly help in the increase of shoot number (Niedz *et al.*, 2015). The shoot numbers in LSE-treated plants were comparable to those obtained in control plants, where standard PGRs were used (Table 1).

Similarly, a significant increase in shoot length was also observed with the addition of LSE however it is not comparable to control conditions where standard PGRs where added. The highest increase in shoot length was recorded in *Sargassum tenerrimum* extract (4.97±0.42 cm @ 50% v/v) treated propagules, which was more than control. In general, the 50% concentration, positively affect the increase in shoot length except in case of *Sargassum wightii* where 30% concentration was found to be best. The least increase in shoot length was observed with *G. corticata*. This may be due to the higher concentration of zinc reported in this species, which hampers the shoot length (Rosemary *et al.*, 2019). When evaluating the growth performance of *in vitro* grown plants, fresh weight (FW) and dry weight (DW) gives us a better idea. The total biomass of micropropagules can be directly connected to plant performance as a response to photosynthetic ability, nutrition, environmental conditions, and more. In our study, a significant increase in biomass was recorded when LSE of *Sargassum tenerrimum* was used @50% concentration (v/v) (FW 11.61±0.89 g and DW 2.71±0.01 g) (Table 1). Overall increase in biomass was observed @30% concentration in all LSE tested. LSE of *G. corticata* have a detrimental effect on biomass accumulation and this was recorded 2.48±0.13 g FW, and 0.56±0.04 g DW, almost equivalent to the data recorded for plant grown on medium without synthetic PGRs. No morphological abnormalities like callus formation, curling of shoots, stunted growth, or rooting were observed in LSE treated plant at this multiplication stage.

Table 1. Effect of LSE on shoot length, shoot number and biomass of banana micropropagules grown *in vitro* conditions

Liquid Seaweed Extract (LSE)	Concentration of LSE (% v/v)	Shoot length (cm)	Shoot number (mean)	Fresh weight (g)	Dry weight (g)
C. racemosa	10	1.900^{b}	5.000^{abcde}	4.310^{cde}	0.980^{def}
	30	2.700^{def}	5.600^{cdef}	3.580^{bc}	0.800^{bcd}
	50	3.530^{gh}	6.800^{efg}	5.780^{ghi}	1.280^{hi}
G. corticata	10	2.800^{ef}	4.000^{abc}	4.370^{cde}	0.970^{cdef}
	30	2.850^{f}	4.800^{abcd}	3.720^{bcd}	0.820^{bcd}
	50	2.870^{f}	4.600^{abcd}	2.480^{a}	0.560^{a}
C. paspaloides	10	2.800^{ef}	4.600^{abcd}	6.240^{hi}	1.410^{ij}
	30	2.370^{cd}	5.800^{cdefg}	6.460^{i}	1.460^{j}
	50	2.770^{ef}	3.200^{a}	4.370^{cde}	0.970^{cdef}
U. lactuca	10	2.670^{def}	4.400^{abcd}	3.930^{bcd}	0.880^{bcde}
	30	2.530^{cdef}	5.000^{abcde}	5.850^{ghi}	1.310^{hij}
	50	2.770^{ef}	4.000^{abc}	3.410^{bc}	0.780^{bc}
S. tenerrimum	10	3.270^{g}	5.400^{cdef}	5.400^{fgh}	1.220^{gh}
	30	4.370^{j}	6.200^{defg}	5.160^{efg}	1.160^{gh}
	50	4.970^{k}	9.000^{h}	11.610^{k}	2.710^{l}
C. sertularioides	10	2.470^{cde}	7.600^{gh}	4.040^{bcd}	0.870^{bcd}
	30	3.470^{g}	5.200^{bcde}	4.590^{def}	1.060^{efg}
	50	3.800^{hi}	4.400^{abcd}	5.070^{efg}	1.100^{fg}
Sargassum wightii	10	1.600^{ab}	6.200^{defg}	3.830^{bcd}	0.850^{bcd}
	30	2.300^{c}	7.600^{gh}	6.410^{i}	1.470^{j}
	50	1.370^{a}	4.200^{abc}	4.360^{cde}	0.970^{cdef}
Gracilaria edalis	10	2.800^{ef}	7.200^{fg}	6.410^{i}	1.470^{j}
	30	3.870^{i}	9.200^{h}	8.060^{j}	1.800^{k}
	50				
MS medium with standard PGR		4.500^{j}	11.000^{i}	16.010^{l}	3.410^{m}
MS medium without standard PGR		2.300^{c}	3.300^{ab}	3.230^{ab}	0.750^{b}
CV		11.90	15.35	6.91	6.23
SEM		0.17	0.40	0.18	0.04
CD 5%		0.50	1.12	0.50	0.12

SEM - Standard Error Mean; CD - Critical Difference; CV - Coefficient of variation; SD - Standard Deviation; Means in the same columns followed by different letters are significantly different ($P \leq 0.05$) using the Duncan's Multiple Range Test

Regarding biochemical parameters, different LSE at different concentrations evoked varied responses (Table 2). We observed changes in terms of biomolecule accumulation. Phenolic compounds are a

vast collection of secondary metabolites formed by plants, the common component of which is the aromatic ring linked to at least one hydroxyl group. Phenolic-based secondary metabolites are required and fulfill many important roles in the plant system. These compounds protect the plants from UV radiation, interact with the environment, and offer a defense mechanism against biotic and abiotic stresses. It is also evident that the phenolics give colour to the plant and cooperate with hormones (*e.g.,* auxins) (Kołton *et al.*, 2022). When LSE was added to the medium, it showed a significant increase in the total phenol compared to the shoots growing on control synthetic PGRs. In all cases, the highest accumulation of phenolics was recorded on the maximum concentration of LSE (*i.e.,* 50% concentration). Although all seaweed extracts showed a similar response, a slightly higher accumulation of phenolics was observed in *C. paspaloides* (1.31±0.03 mg g^{-1} Fresh Tissue @ 10% concentration).

The increased accumulation of phenols in tissue culture propagules can be attributed to external glucose in the growth medium, which is facilitated by LSE and is responsible for this effect. A similar observation was recorded in Scarlet Rose by Amorim (1977), where an exogenous supply of glucose in culture medium resulted in an increased accumulation of phenols. Another important reason for increased phenol accumulation is due to increased proline biosynthesis. It is clearly observed in Table 2 that the accumulation of phenol and proline are correlated and directly proportionate. Our results are in accordance with the observation of Shetty (2004) that the proline-linked pentose phosphate pathway stimulate shikimate and phenylpropanoid pathwa, leading to the stimulation of phenol biosynthesis in cell. There are two views on role of phenols under *in vitro* growth and development of plants. One opinion states that the phenols depress plants' *in vitro* proliferation and growth while others talk about the opposite (Arnaldos *et al.*, 2001). Role of phenols in controlling the interaction between PGRs and averting the abscisic acid promoted cell senescence under *in vitro* conditions has also been reported (Feucht and Treutter 1996), which are in agreement with our observation and hypothesis that an increase in phenol is not hampering growth of propagules.

Table 2. Effect of Liquid Seaweeds Extracts (LSE) on accumulation of biomolecules in *in vitro* grown micropropagules of banana

Name of different LSE	Concentration of LSE (% v/v)	Total phenol content (mg g^{-1} fresh tissue)	Total carbohydrate content (mg g^{-1} fresh tissue)	Total protein content (mg g^{-1} fresh tissue)	Total proline content (mg g^{-1} fresh tissue)
C. racemosa	10	1.030^{abc}	111.300^{abcdef}	61.000^{ab}	18.000^{de}
	30	1.230^{defg}	121.300^{efgh}	63.000^{abc}	19.000^{ef}
	50	1.300^{fgh}	135.770^{gh}	70.000^{bcd}	21.000^{gh}
G. corticata	10	1.230^{defg}	116.470^{cdef}	75.000^{def}	15.000^{b}
	30	1.450^{hi}	117.270^{defg}	77.000^{def}	21.000^{gh}
	50	1.530^{i}	120.370^{efgh}	78.000^{defg}	28.000^{j}
C. paspaloides	10	1.300^{fgh}	95.570^{ab}	80.000^{defgh}	17.000^{cd}
	30	1.470^{hi}	97.720^{abc}	82.000^{efgh}	20.000^{fg}
	50	1.520_{i}	109.700^{abcdef}	89.000^{gh}	27.000^{j}
U. lactuca	10	1.030^{abc}	94.470^{a}	72.000^{cde}	18.000^{de}
	30	1.120^{bcdef}	99.290^{abcd}	75.000^{def}	22.000^{h}
	50	1.250^{efg}	126.600^{fgh}	77.000^{def}	25.000^{i}
S. tenerrimum	10	1.240^{defg}	95.500_{ab}	83.000^{efgh}	13.000^{a}
	30	1.360^{ghi}	115.570^{cdef}	89.000^{gh}	15.000^{b}
	50	1.500^{i}	136.630^{h}	105.000^{i}	18.000^{de}
C. sertularioides	10	1.050^{abcd}	92.300^{a}	77.000^{def}	19.000^{ef}
	30	1.110^{bcdef}	111.470^{abcdef}	79.000^{defgh}	25.000^{i}
	50	1.360^{ghi}	115.780^{cdef}	80.000^{defgh}	30.000^{k}
S. wightii	10	1.140^{cdef}	111.500^{abcdef}	78.000^{defg}	16.000^{bc}
	30	1.190^{cdefg}	119.230^{efgh}	82.000^{efgh}	18.000^{de}
	50	1.250^{efg}	124.670^{fgh}	83.000^{efgh}	21.000^{gh}
G. edalis	10	1.060^{abcde}	102.800^{abcde}	79.000^{defgh}	15.000^{b}
	30	1.160^{cdef}	114.230^{bcdef}	85.000^{fgh}	17.000^{cd}
	50				
MS medium (+standard PGR)		0.900^{a}	125.370^{fgh}	90.400^{h}	15.000^{b}
MS medium (-standard PGR)		0.950^{ab}	92.000^{a}	58.400^{a}	25.000^{i}
CV		3.53	2.97	13.47	19.96
SEM		0.02	0.19	0.16	0.13
CD 5%		0.06	0.56	0.47	0.39

SEM - Standard Error Mean; CD - Critical Difference; CV - Coefficient of variation; SD - Standard Deviation; Means in the same columns followed by different letters are significantly different ($P \leq 0.05$) using the Duncan's Multiple Range Test

Carbohydrates are not only a source of energy for plant cells but also play many important roles in signaling pathways, photosynthesis and also in cell differentiation. In our study, we have observed a varied response in the accumulation of carbohydrates in various treated shoots. In general, the gradual increase in LSE resulted in a higher accumulation of carbohydrates, which was highest in 50% concentration in all tested LSEs. In the case of *Sargassum tenerrimum* extract, we observed 136.63±0.31 mg g^{-1} of carbohydrate accumulation at 50% concentration, which was even higher than positive control plants (Table 2). This can be explained on the basis that LSE contain some level of sugars that contribute to hexose feeding in the medium and hence more carbohydrate is accumulated. Our results are in accordance with the results obtained by Chauhan *et al.* (2018), where the addition of some natural extracts significantly increased carbohydrates in rose micropropagules.

Studies on the protein contents in *in vitro* grown shoots give us an idea of protein metabolism and its role in cell differentiation. We observed a steady increase in protein accumulation regarding increased concentrations of different LSE. The highest protein accumulation was observed @50% concentration in all LSE samples, although it is slightly lesser than the shoots grown on synthetic medium (90.4±0.25 mg g^{-1} of fresh tissue), except the shoots grown on *Sargassum tenerrimum* extract (105±0.45 mg g^{-1} of fresh tissue). The probable reason for decreased protein accumulation is the presence of protein synthesis inhibitors in crude seaweed extracts that interfere with protein biosynthesis. Moreover, the shoots growing in a positive control environment, where purified synthetic PGR are present, stimulate the cells to grow and divide; hence more protein will be synthesized and accumulated.

A similar tendency in change of proline accumulation was observed when different LSE were tried. Proline is an important amino acid considered a highly beneficial compound that protects against various stress conditions. Proline is considered an excellent osmolyte that protects three ways: i) it acts as a metal chelator, ii) it is a proven antioxidative defense molecule and iii) it is an

important signaling molecule. As a general observation, adding more LSE resulted in more proline accumulation. In all cases 50% concentration of LSE resulted in highest accumulation of proline which was higher than positive control plants (15±0.10 mg g^{-1} fresh tissue) (Table 2). The highest proline content was recorded in propagules treated with *C. sertularioides* extract @50% concentration, which was almost double compared to positive control. The possible reason behind increased proline synthesis under LSE treatment is due to several inorganic salt, acidic compounds, secondary metabolites and phenols present in seaweed extracts. Sometimes adaptive response of micropropagules in culture conditions also induce proline accumulation. Similar results were also observed by Putnik-Delić *et al.* (2012) in *in vitro* culture of sugar beet where increased salt concentration resulted in increased proline accumulation.

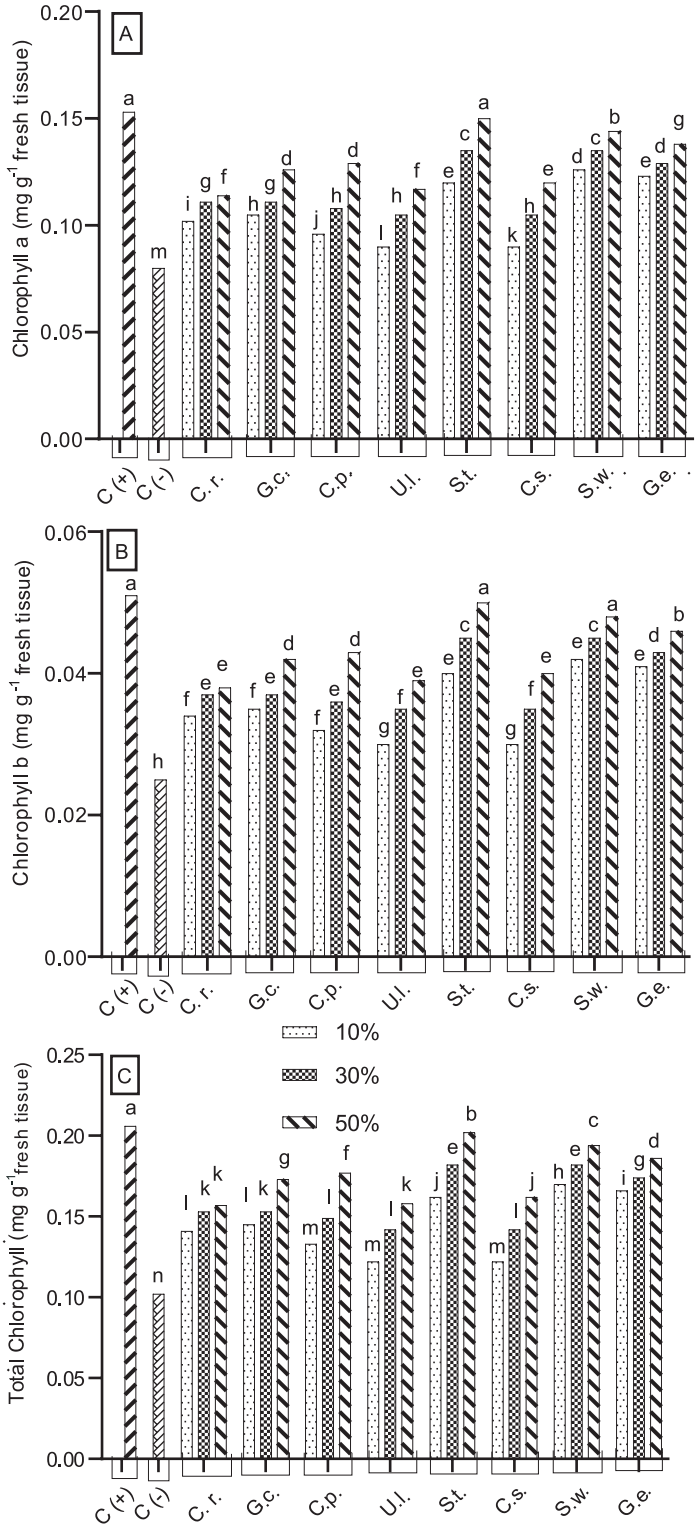

Fig. 1. Effect of different LSE on accumulation of Chlorophyll a (A), Chlorophyll b (B) and total chlorophyll (C) in banana micropropagules grown under *in vitro* conditions. C (+) represent – positive control plant grown on a standard MS medium with PGRs, C (-) represent – negative control plant grown under standard MS medium without PGRs; *C.r., G.c., C.p., U.l., S.t., C.s., S.w.* and *G.e.* represent *C. racemosa, G. corticata, C. paspaloides , U. lactuca, S. tenerrimum, C. sertularioides, S. wightii and G. edalis,* respectively.

Chlorophyll is an important photosynthetic pigment in all autotrophic organisms like algae, photosynthetic bacteria and plants, which can convert light energy into chemical energy. The chlorophyll content reflects the plant's health condition and ability to fix atmospheric carbon dioxide. In our experiment, adding LSE does not significantly affect the increase in chlorophyll content. In all the cases, the increasing concentration of LSE also resulted in a steady increase in chlorophyll, which was recorded highest in *Sargassum tenerrimum* @ 50% concentration. However, this value did not surpass that of the positive control plants. The probable reason for slightly low chlorophyll content may be due to the alkaline nature of LSE, which does not support chlorophyll synthesis. Moreover, the presence of elements in extract, like calcium, zinc, manganese, phosphorus, or copper, can bind with iron, making it available for chlorophyll synthesis (Li *et al.*, 2018).

Numerous studies have explored the use of natural extracts to enhance the *in vitro* growth of plantlets in tissue culture. Plant tissue culture media are often enriched with various natural extracts, such as protein hydrolysates, coconut milk, yeast, malt extract, ground banana, orange juice, potato extract, and tomato juice. Several natural cytokinins, like zeatin and zeatin riboside, along with cell division-promoting activity, have been identified in sweet corn extract. In the case of *Anthurium cubense*, substituting cytokinins with the cost-effective citrus fruit rind-derived Pectimorf showed improved *in vitro* growth and 90% plantlet survival during acclimatization (Montes *et al.*, 2000). Similar results were observed with *Spathiphyllum* using Pectimorf compared to the synthetic cytokinin BA (Hernandez *et al.*, 2009). Orange juice has also been successfully applied in culturing explants of various citrus species. Coconut water, known for containing ribosyl-zeatin similar to maize zeatin, has promoted embryo culture in multiple species. Other extracts from sweet lime juice and tomato fruit have also demonstrated growth-promoting properties (Maria *et al.*, 2012). While natural plant extracts have been extensively studied, the use of seaweed extracts containing growth-promoting substances like auxins, cytokinins, and betaines in tissue culture remains relatively unexplored. Some reports have highlighted seaweed extracts, including *U. lactuca*, *C. sertularioides*, *P. gymnospora*, and *Sargassum liebmannii*, as cost-effective options for tomato seedling growth under laboratory conditions. Similar seaweed-based growth studies have been conducted with tomato, wheat, soybean, and maize, but these were *ex vitro* studies. Notably, *in vitro* applications of seaweed extracts in *Lycopersicon esculentum* showed a significant increase in shoot length. Our findings align with these observations, suggesting that seaweed-derived growth promoters may positively influence *in vitro* growth of banana micropropagules, potentially serving as economical alternatives

to synthetic plant growth regulators (PGRs). However, further testing is necessary to evaluate the suitability of seaweed extracts for *in vitro* culture in a broader range of plant species, including various horticultural plants.

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