International Journal of Research in Biosciences
Vol. 5 Issue 2, pp. (1-9), April 2016
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Comparative analysis on the effect of seaweed liquid extracts and commercial plant growth regulators on *in vitro* propagation of *Bacopa monnieri*

Govindan Pothiaraj, Rajadas Sam Ebenezer, Ebenezer Johnson Christdas, Harshavardhan Shakila*
Department of Molecular Microbiology, School of Biotechnology, Madurai Kamaraj University, Madurai, Tamilnadu, PIN-625021, INDIA

(Received March 07, 2016, Accepted March 29, 2016)

Abstract

Employment of commercial Plant Growth Regulators (PGRs) is inevitable in plant tissue culturing, but it makes the process costlier. As a natural substitute with well-known plant growth promoting activity, seaweeds act as a plentiful source of these regulators. In this study, the plant growth enhancing property of Seaweed Liquid Extracts (SLEs) from *Gracilaria edulis* and *Sargassum wightii* was compared with that of the commercial PGRs during the *in vitro* propagation of *Bacopa monnieri*, a plant of high medicinal value. 0.1mg/ml of Naphthalene Acetic acid (NAA) and 1.0mg/ml of Kinetin were used to compare the plant growth promoting activity of commercial PGRs with Seaweed Liquid Extracts. Supplementation of MS media with different proportion of SLEs from *S. wightii* and *G. edulis* significantly enhanced the growth of *Bacopa monnieri*. 30% and 40% liquid extracts from *S. wightii* and *G. edulis* respectively enhanced shoot and root proliferation and increased the survivability of the propagated plant. Our results demonstrated that seaweed liquid extracts are more effective in enhancing the *in vitro* propagation of *B. monnieri* than commercial PGRs. Hence, the seaweed extracts as a source of the plant growth regulators are suggested as a better alternative for the commercial PGRs used for *in vitro* propagation of *Bacopa monnieri*.

Keywords: Plant growth regulators, Seaweed extracts, *Bacopa monnieri*, *Gracilaria edulis*, *Sargassum wightii*.

Introduction

Bacopa monnieri (L.) Pennell (*B. monnieri*) commonly called as Brahmi is a creeping annual plant belonging to the family *Scrophulariaceae,* found throughout the Indian subcontinent in wet, damp and marshy areas. It has been used as a panacea for various diseases including cardiac, respiratory malfunctions and especially for neural disorders like insomnia, insanity, depression, psychosis, epilepsy and stress [1,2]. It contains Saponin, an active triterpenoid as its principal compound. This Saponin includes Bacosides A, B, C and D which are commonly known as “memory chemicals” [3, 4]. *B. monnieri* is an accepted neurotonic which is used to enhance memory, intellectual and cognitive functions. Apart from cognitive enhancement, it is also used as an anti-inflammatory[5], an analgesic[6], an antipyretic[7], sedative[8] and as an antiepileptic agent[9] in Ayurvedic medicines. Because of its high medicinal value, demand is also high [10]. To meet this high demand, more than 90% of plant species used by industries are collected from the naturally existing wild source, 70% of which is by unorganized harvesting, leading to the extinction of the plant. As a result, *B. monnieri* was listed as an endangered species by the International Union for Conservation of Natural and National Resources[11]. To bring in the hope of reviving these plants, *in vitro* propagation using cost effective

and readily available Plant Growth Regulators (PGRs) could be an effective alternative. Apart from the above well-known facts, this plant can also be genetically engineered to be used as a bioreactor for producing products of medicinal importance and also as a platform for the edible vaccine (Johnson *et al.,* unpublished data).

Despite the fact that large magnitude of seaweeds has been explored as a source of food, as industrial raw materials and in therapeutic and botanical applications for centuries [12], they are often regarded as an under-utilized bio-resource. In the context of organic farming, seaweeds substituted synthetic commercial fertilizers. Field application of these seaweed extracts is also simpler since it can directly be applied as a foliar spray or as powdered manure [13, 14]. Apart from agricultural benefits, seaweed extracts hosts various other qualities which make it applicable in plant tissue culturing (PTC) also. In recent trends, PTC plays an important role in the mass propagation of disease-free varieties and in the regeneration of medicinally important plants which are on the verge of extinction. Seaweeds were found to be the richest source of PGRs like auxins [15] and other growth promoters like IAA (Indole Acetic Acid) and IBA (Indole-3-butyric acid), Cytokinins, trace elements like Fe, Cu, Zn, Co, Mo, Mn, and Ni, vitamins and amino acids [16] and shown to be successfully enhancing the yield of commercial crops like *Vigna mungo* (black gram) [17].

The Seaweed-derived products were previously reported as the potential source of plant growth promoting substances which makes the in *vitro* plant tissue propagation much simpler and cheaper by replacing commercially available growth promoters. Incorporation of seaweed extracts in PTC growth media can be perfectly done without compromising the effect of sterilization as it is thermostable [18]. Seaweed extracts have already been used in the *in-vitro* mass propagation of *Lycopersicon esculentum* L with 75% increased survivability [19] and in *Solanum melongena* L [20]. Based on the above information available, this study was done to analyze the plant growth promoting activity of seaweed liquid extracts from *Gracilaria edulis* and *Sargassum wightii* in comparison with the traditional PGRs.

Materials and Methods

Surface Sterilization of Explants

Before use, the explants were thoroughly washed under running tap water for 15minutes to remove dirt. It was then rinsed in 10% SDS for ten minutes with intermittent shaking and then with sterile distilled water for two to three minutes. To remove fungal contaminants, 5mg/ml Bavistin treatment was done and again washed with sterile distilled water. After washing, the explants were subjected to surface sterilization as mentioned in the table no.1.

Table 1: Surface sterilizing agents which were used in a different concentration with varying time of exposure to remove contaminants from explants of *Bacopa monnieri*

Sterilizing agent	Concentration (%)	Exposure Time
Mercuric chloride	0.01-0.1%	2-3 minutes
Sodium hypochlorite	0.01-1%	2-3 minutes
Ethanol	70%	30-40 seconds

Initiation of aseptic cultures

Initiation medium was prepared by supplementing Murashige and Skoog (Murashige and Skoog 1962) “basal medium” [21] (defined as MS medium supplemented with sucrose and gelling agent, but in the absence of phytohormones) with sucrose (3%; commercial grade; Hi-Media), agar (0.8%; commercial grade; Supreme Hi-Media, India) and clerigel 0.1%. The pH of the medium was adjusted to 5.8 with 1N NaOH (analytical grade; Hi-Media) and 1N HCl (Analytical grade; Hi-Media) before autoclaving at 121°C and 15 psi pressure for 20 minutes. The surface sterilized nodal segments were inoculated and cultured in this initiation medium under the culture conditions as given below for two to three weeks.

Shoot multiplication using commercial PGRs

The *in vitro* sterile culture obtained from two to three weeks old cultures on initiation medium were inoculated onto the MS medium supplemented with commercial plant growth regulators. For callus formation and improved frequency of shoot induction and growth, the auxins (Naphthalene Acetic acid (NAA), Indole Acetic Acid (IAA), Indole Butyric Acid (IBA)) were tested in combination with cytokinins (6-Benzyl Aminopurine (BAP), Thidiazuron (TDZ) and Kinetin). NAA at 0.1mg/ml concentration was kept as standard and MS media with varying combinations of kinetin, BAP, and TDZ at the concentrations of 0.5, 0.75 and 1.0 mg/ml, respectively were prepared and sterilized by autoclaving at 121^0C for 20 minutes at 15 psi pressure. Sub-culturing was done at an interval of about ten days. After third sub-culturing, the explants were observed for induction of roots and shoots, its length and biomass. All the experiments were carried out in triplicates.

Preparation of Seaweed Liquid Extracts (SLE)

Marine red seaweed *Gracilaria edulis* and brown algae *Sargassum wightii* were collected from the coastal area Villoondi Theertham situated in between Pamban and Ramaeswarm, Tamilnadu, India (9.288°N 79.313°E) during the month of May and June 2014. The seaweeds were identified based on their morphological characters. The Collected seaweeds were brought to the laboratory in sea water after washing to remove sand, macroscopic epiphytes, and other dirt. In the laboratory, the seaweeds were washed in running tap water twice and finally with distilled water and shade-dried for seven days. Dried seaweeds were ground into fine powder using a mechanical blender and stored in airtight containers for further use. Concentrated aqueous extracts were prepared by hot extraction method using water as the solvent. Powdered seaweeds were dispersed in double the volume of water (1:2 w/v) and boiled for two hours. The extracts were filtered first using a muslin cloth and then with Whatman no.1 filter paper. Filtrates were then autoclaved, cooled and stored at 4^oC untill further use.

***In vitro* propagation of *Bacopa monnieri* using SLEs**

The nodal segments of size 2 to 3 cm, from established lines were inoculated on MS medium supplemented with varying concentration of SLEs from 10% to 70% without altering the concentration of MS media components (Murashige and Skoog 1962). The pH of the media was set at 5.7. To maintain growth and vitality, cultures were sub-cultured in fresh medium every ten days. The average numbers of shoots and roots per explants were recorded after the third subculture and the experiments were carried out in triplicates. MS media without any SLE - was employed as the control. Throughout the study, the culture conditions were maintained as mentioned below.

Culture Condition

All cultures were maintained at 25±2°C, under a 16-hour photoperiod with a light intensity of approximately 47.25 µmol m^{-2} s^{-1} (3,500 lux) provided by cool, white fluorescent tubes (40 W; Philips, New Delhi, India). Relative humidity was maintained around 60%-70%.

Hardening

Post twenty days of third sub-culturing, the plantlets with well-developed roots and shoots were removed from the culture jars and washed thoroughly with sterile distilled water to remove all traces of medium attached to the roots. They were then transplanted into plastic jars containing autoclaved soil and vermiculate (Varsha Enterprises, Bangalore, India) (1:1 w/w).

Statistical Analysis

The entire study was carried out in triplicates. The number of shoots, roots induced, and biomass were recorded after third sub-culture. All data were analyzed for significant difference using analysis of variance with 95% confidence level. ANOVA was calculated using Graphical Prism software (GraphPad Software Inc., La Jolla, CA). Calculation of the mean value and graphical representations were done using Microsoft Excel version 2010.

Results

Establishment of Explants

Aseptic cultures of the explants of *B.monnieri* were propagated on MS media with commercial PGRs, liquid extracts from the seaweeds *G.edulis* and *S.wightii*. After the third sub-culture, the explants were transferred to plastic pots for hardening (Figure 1 and Figure 2). Seaweed liquid extracts increased the survivability. The shoots of plants induced by SLEs were healthy and also high in number.

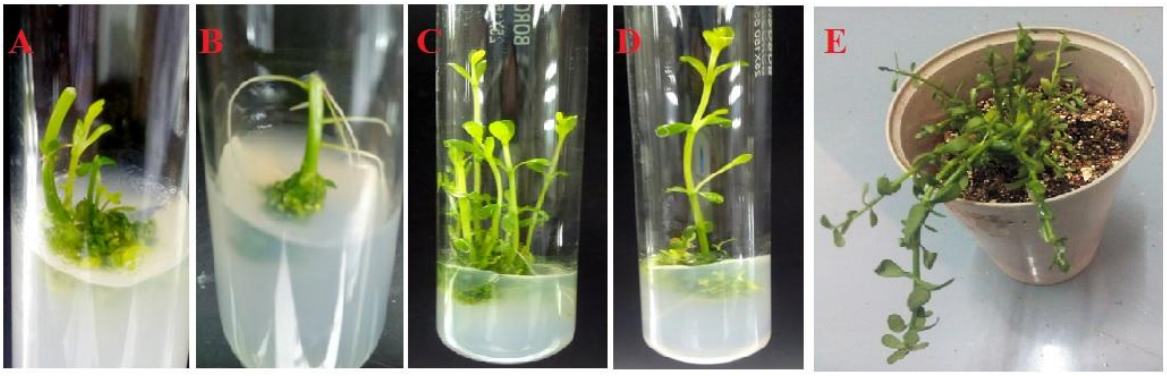

Figure 1: *In-vitro* propagation of *B. monnieri* on MS media with commercial PGRs: (A) Inoculated explants on MS media with no supplements (B) Callus and multiple induction (C) Multiple shoot generation by MS media with Kinetin (1mg/L) and NAA (0.1mg/L) (D) Reduced Shoot Induction by MS media with BAP (1mg/L) and NAA (0.1mg/L) (E) Hardened and acclimatized plant

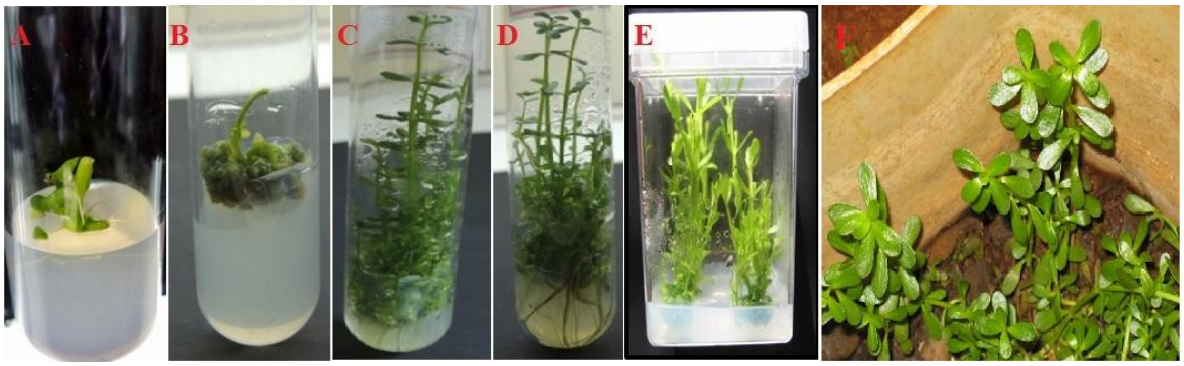

Figure 2: *In-vitro* propagation of *B.monnieri* on MS media with SLEs: (A) Inoculated explants on MS media with no supplements (B) Shoot bud regeneration (C) Induction of multiple shoots (D) Induction of multiple roots and shoots (E) Superfluous growth of sub-cultured explants (F) Hardened and acclimatized plant

Effect of PGRs on Shoot and Root Induction

Among different combinations of PGRs, basal MS medium supplemented with both 0.1 mg/ml of NAA and 1.0 mg/ml of Kinetin had the best induction of roots and shoots both in number and length.

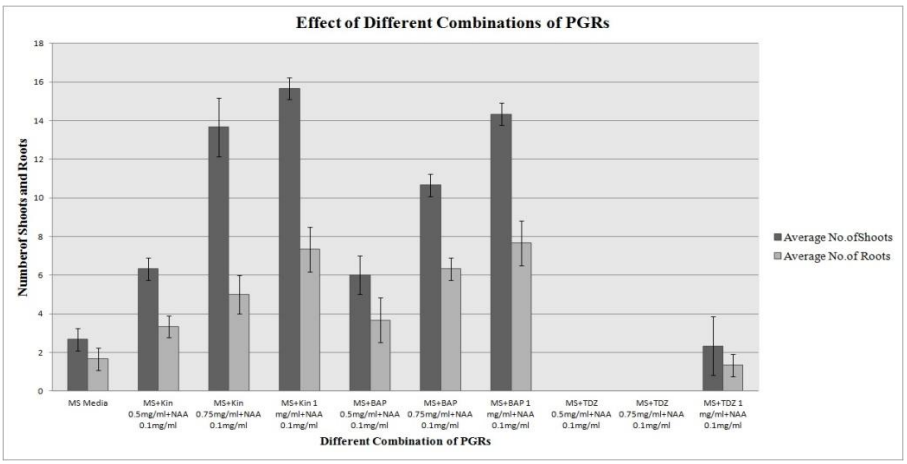

Figure 3: Effect of different combinations of commercial PGRs on induction of roots and shoots. MS media with 0.1mg/ml of NAA and 1.0mg/ml of Kinetin induced more number of roots and shoots. ($p<0.05$)

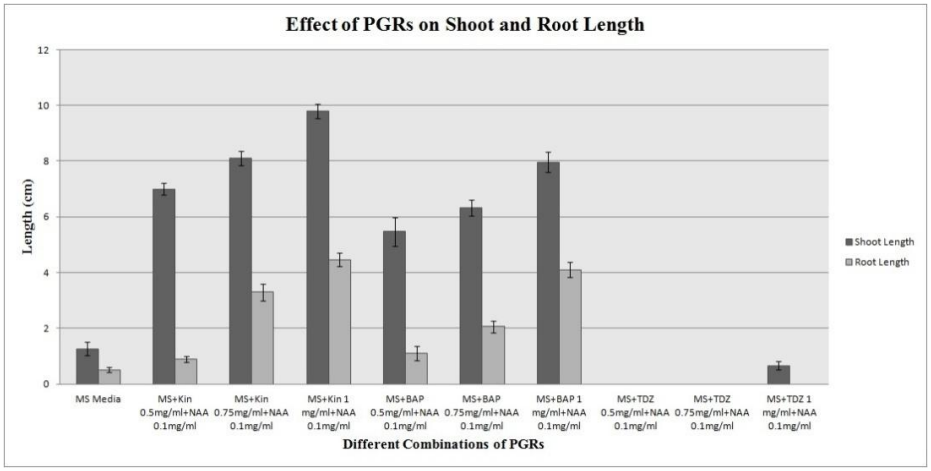

Figure 4: Effect of different combinations of commercial PGRs on length of roots and shoots. MS media with NAA 0.1mg/ml and Kinetin 1mg/ml produced longer roots and shoots than other combinations of PGRs. ($p<0.05$)

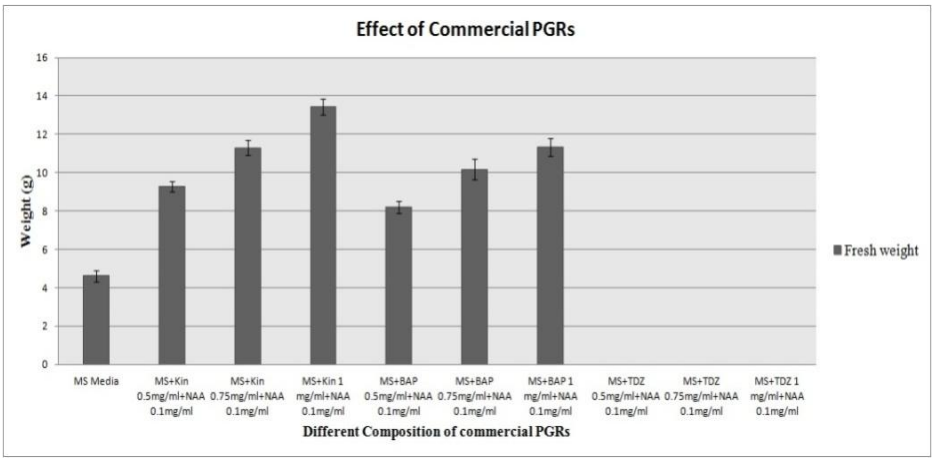

Figure 5: Effect of different combinations of commercial PGRs on biomass

This combination of PGRs induced fifteen shoots and seven roots from each explant on the average (Figure 3). The length of shoots and roots were 9.8cm and 4.5cm respectively (Figure 4). Biomasses of the induced plants were also showing similar result with 0.1mg/ml of NAA and 1.0mg/ml of kinetin (Figure 5).

Effect of SLEs on Shoot and Root Induction

Seaweed Liquid extracts had a profound effect on induction of shoots and roots of *B.monnieri.* After the third sub-culture, the nodal explants were inoculated onto control MS media and MS media supplemented with SLEs of *G.edulis* and *S.wightii* were observed for the biomass, number and length of shoots and roots induced. Medium supplemented with 40% *G.edulis* liquid extracts produced nineteen shoots and fifteen roots while 30% of *S.wightii* induced twenty five shoots and twenty roots on average (Figure 6). On the other hand, propagation on the control MS media produced on the average of only 3 shoots and 2 roots. SLEs also increased the length of shoot and roots. 40% *G.edulis* extracts produced shoots and roots of length 15cm and 7.5cm respectively while 30% *S.wightii* extracts produced 17cm and 7cm of the shoots and the roots respectively (Figure 7). When the biomass of each plant was quantified both as wet weight and dry weight, it was observed that SLEs influenced the biomass also. 40% *G.edulis* extracts and 30% *S.wightii* extracts produced increased biomass when compared to control MS media (Figure 8).

On the whole, SLEs displayed better plant growth promoting property than the commercial PGRs. MS media supplemented with 30% liquid extracts of *S.wightii* significantly influenced the *invitro* propagation of *B.monnieri* by inducing more number of shoots and roots with increased length and

biomass. The propagated plants were healthy and survivability at green house environment was also high.

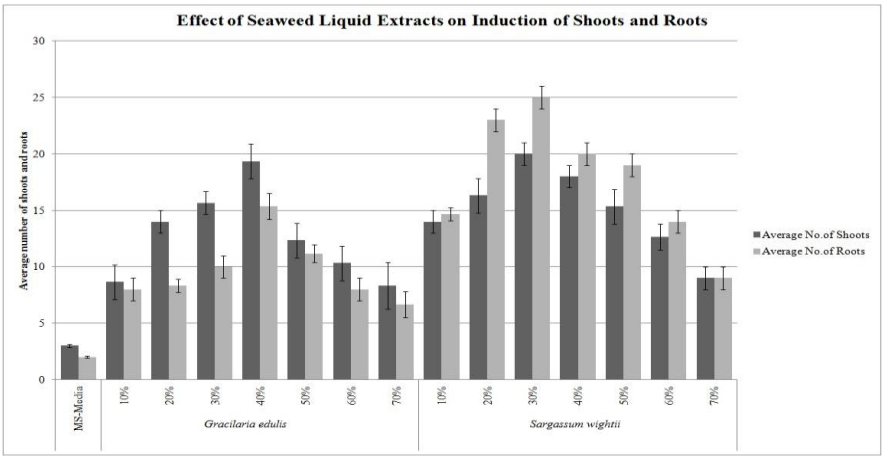

Figure 6: Effect of *Seaweed Liquid E*xtracts on induction of roots and shoots. Among the two seaweeds 30% *S. wightii* extracts induced more number of roots and shoots. Induction of shoots by 30% *S. wightii* extracts are significantly similar to 40% *G. edulis* extracts (p<0.05).

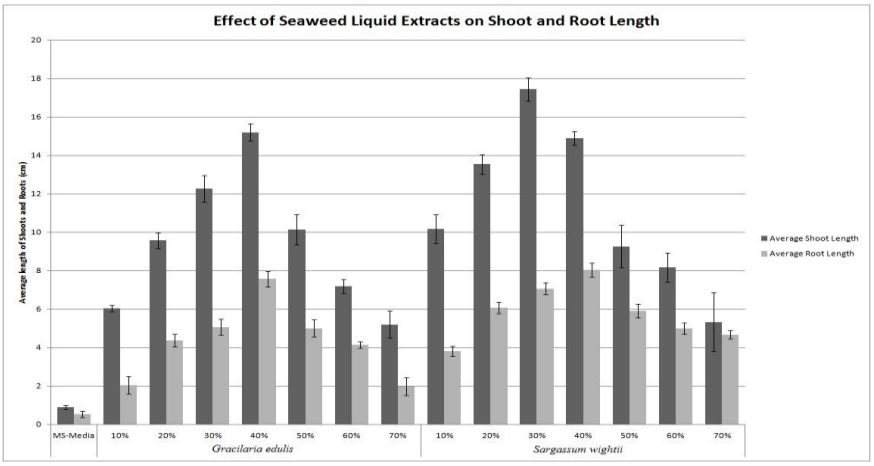

Figure 7: Effect of SLEs on length of induced roots and shoots. 30% of *S.wightii* extracts produced more number of roots and shoots with increased length (p<0.05)

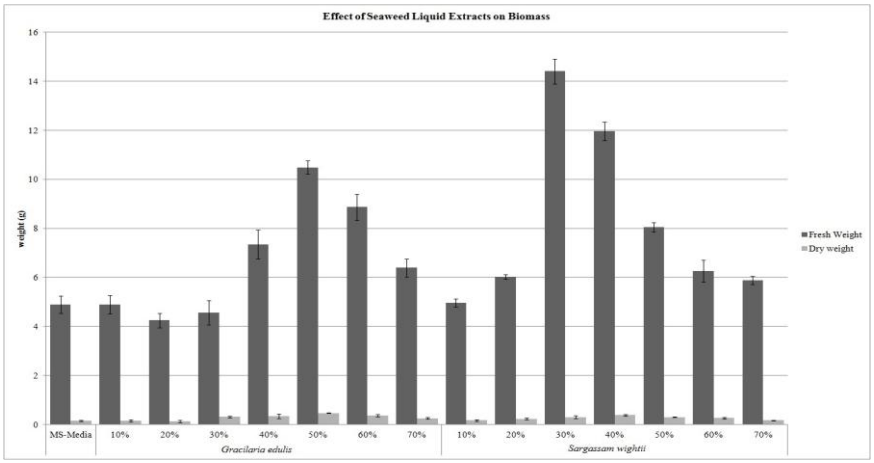

Figure 8: Effect of *SLEs* on biomass. 30% of *S. wightii* influenced the *in-vitro* propagation of *B. monnieri* by inducing more number of roots and shoots with increased biomass. (p<0.05)

Discussion

Seaweeds are the richest natural source of macro elements [22] and growth promoters [15, 16]. The presence of precursors of plant growth promoters [23], essential polysaccharides [24], zeatines [18], and betaines [25, 26] were also previously reported from seaweeds. These findings attracted the researchers to use seaweed extracts at varying concentrations for the *in vitro* propagation of commercially and medicinally important plants. In this study, we compared the plant growth promoting property of seaweed liquid extracts from *G. edulis* and *S. wightii* with that of the commercial plant growth regulators like NAA, BAP, Kinetin and TDZ. Among the different combinations of commercial PGRs, MS media supplemented with 0.1mg/ml of NAA and 1mg/ml of Kinetin induced shoots and roots significantly.

Basal MS media supplemented with either 30% *S. wightii* extracts or 40% *G. edulis* enhanced the growth and biomass of *B. monnieri* than the commercial PGRs. However, the further increase in the concentration of SLEs showed a negative effect. Our findings are concordant with the previous reports on the effect of seaweeds on the growth of *Solanum lycopersicum* L. as observed by Rosalba Mireya *et al.,* [27] and findings by Vinoth *et al.,* [19] on the *in vitro* propagation of *Lycopersicon esculentum*. The reason may be the increase in pH upon addition of excess seaweed extracts as it was clearly pointed out by Arnon and Johnson [28].

The most important benefit of using seaweed liquid extracts is that it can be applied to the field in any form either by spraying or applying granules and it is independent of the further addition of macro- and micro-elements [29]. Our results demonstrated the increase in shoot length, root length and biomass of *B. monnieri,* when SLEs were supplemented with the MS media. An analogous effect was also observed in tomato with the extracts from *S. johnstonii* [30]. Similarly, in a field study, foliar spray of seaweed extracts increased height and growth of tomato [31] and even in nematode infested soils [32]. The Root-to-shoot ratio is also increased by SLEs and thereby enables the plant to gain more access to nutrients present in the environment and increase the survivability and maturity. This may be augmented due to the presence of plant hormones in seaweeds [33].

Plant growth promoting activities of seaweed concentrates in fields have already been demonstrated in various crops. Very few reports are available on the use of seaweeds for *in vitro* propagation. Our findings strongly recommend the aqueous seaweed extracts from *G.edulis* and *S.wightii* as an effective replacement for expensive commercial plant growth regulators used for *in vitro* mass propagation of plants like *B.monnieri*.

Conclusion

In the present study, plant growth promoting activity of commercial PGRs and seaweed liquid extracts of *G. edulis* and *S. wightii* was analyzed and compared. Among the different combinations of commercial PGRs, 0.1mg/ml NAA and 1mg/ml Kinetin yielded significant growth. However, further increased growth and survivability of *B. monnieri* were achieved by supplementing the MS media with Seaweed liquid Extracts. Among the seaweeds, *Sargassum* displayed better root and shoot inducing ability. Comparatively, the growth promoting activity was higher with SLEs rather than with PGRs. Hence we suggest the use of the SLEs as a better and cheaper alternative to commercially available PGRs for the *in vitro* propagation of commercially important plants. However, further insight regarding its macro and micro element is necessary to have a better understanding about SLEs and their plant growth promoting activity.

Acknowledgement

Authors thank Dr. M. Ramesh, Associate Professor, School of Biotechnology, Alagappa University for providing the Plant and their suggestions for aseptic techniques. The authors also thank Ms. Agilda and Mr. Kennedy for their help in collecting the seaweeds. The Authors thank the University Grants Commission, India for financial support.

References

1. Howes M.J.R. and Houghton P.J., Traditional medicine for memory enhancement. In: Ramawat KG (ed) Herbal drugs: Ethnomedicine to modern medicine, Springer, Berlin, 239–291 **(2009)**

2. Rajani M. *Bacopa monnieri*, a nootropic drug. In: Ramawat KG, Merillon JM (eds) Bioactive molecules and medicinal plants, Springer, Berlin, 175–195 **(2008)**

3. Rastogi S., Mehrotra B.N. and Kulshreshtha D.K., Proceedings of IV international Congress of Ethnobiology, Deep publications, New Delhi, 93 **(1994)**

4. Sivaramakrishna C., Rao C.V., Trimurtulu G., Vanisree M. and Subbaraju G.V., Triteropenoid glycosides from *Bacopa monnieri,* Phyto Chemistry, 66, 2719-2728 **(2005)**

5. Shabana Channa, Ahsana Dar, Shazia Anjum, Muhammad Yaqoob and Atta-ur-Rahman, Anti-inflammatory activity of *Bacopa monnieri* in rodents, Journal of Ethnopharmacology, 104(1-2), 286-289 **(2006)**

6. Hemayet Hossain, Md Sariful Islam Howlader, Shubhra Kanti Dey, Arpona Hira and Arif Ahmed. Evaluation of analgesic, anti-diarrhoeal and cytotoxic activities of ethanolic extract of *Bacopa monnieri* (L), British Journal of Pharmaceutical Research, 2(3), 188-196 **(2012)**

7. Srinivasa Rao Bammidi, Sharan Suresh Volluri, Seema Chaitanya Chippada, Sumanjali Avanigadda and Meena Vangalapati. A Review on Pharmacological Studies of *Bacopa monnieri*, Journal of Chemical, Biological and Physical Sciences, 1(2), 250-259 **(2006)**

8. Tripathi Y.B., Chaurasia S., Tripathi E., Upadhyay A. and Dubey G.P., *Bacopa monnieri* Linn. as an antioxidant: mechanism of action, Indian Journal of Experimental Biology, 34(6), 523-526 **(1996)**

9. Priyaverma, Poonamsingh and Behrose S. Gandhi, Prophylactic efficacy of *Bacopa monnieri* on Decabromodiphenyl Ether (pbde-209)-induced alterations in oxidative status and spatial memory in mice, Asian Journal of Pharmaceutical and Clinical Research, 6(3), 242-247 **(2013)**

10. Chandra Gurnani, Vikram Kumar, Shinam Mukhija, Abha Dhingra, Sohan Rajpurohit and Poonam Narula. *In vitro* regeneration of Brahmi (*Bacopa monneiri* (l.) Penn.) - A threatened medicinal plant, Kathmandu University Journal of Science, Engineering and Technology, 8(1), 97-99 **(2012)**

11. Subashri B. and Justin Koil Pillai Y. *In-vitro* regeneration of *Bacopa monnieri* (l.) pennel.-a multipurpose medicinal plant, International Journal of Pharmacy and Pharmaceutical Sciences, 6(4), 559-563 **(2014)**

12. Khan Wajahatullah, Usha P. Rayirath, Sowmyalakshmi Subramanian, Mundaya N Jithesh *et al.,* Seaweed Extracts as Biostimulants of Plant Growth and Development, Journal of Plant Growth Regulation, 28(4), 386-399 **(2009)**

13. Ganapathy Selvam G., Sivakumar K. Effect of foliar spray from seaweed liquid fertilizer of *Ulva reticulate* (Forsk.) on *Vigna mungo* L. and their elemental composition using SEM – energy dispersive spectroscopic analysis, Asian Pacific Journal of Reproduction, 2(2), 119-125 **(2013)**

14. Sivasankari S., Venkatesalu V., Anantharaj M., Chandrasekaran M., Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. Bioresource Technology, 97, 1745–1751 **(2006)**

15. Crouch I. J. and Van Staden J. Identification of auxins in a commercial seaweed concentrate, Plant Physiology, 139, 590–594 **(1992)**

16. Challen S. B., Hemingway J. C. Growth of higher plants in response to feeding with seaweed extracts. Proceedings of 5th Ind. Seaweed Symposium **(1965)**

17. Kalaivanan C. and Venkatesalu V. Utilization of seaweed *Sargassum myriocystum* extracts as a stimulant of seedlings of *Vigna mungo* (L.) Hepper, Spanish Journal of Agricultural Research, 10(2), 466-470 **(2012)**

18. Finnie J. F. and Van Staden J. Effect of seaweed concentrate and applied hormones on *in vitro* cultured tomato roots, Journal of Plant Physiology, 120(3), 215-222 **(1985)**

19. Vinoth S., Gurusaravanan P. and Jayabalan N. Effect of seaweed extracts and plant growth regulators on high-frequency *in-vitro* mass propagation of *Lycopersicon esculentum* L (tomato) through double cotyledonary nodal explants, Journal of Applied Phycology., 24, 1329–1337. DOI 10.1007/s10811-011-9717-9 **(2012)**

20. Lakkakula Satish, Ramakrishnan Rameshkumar, Periyasami Rathinapriya, Subramani Pandian, Arokiam Sagina Rency, Thota Sunitha, Manikandan Ramesh. Effect of seaweed liquid extracts and plant growth regulators on in vitro mass propagation of Brinjal (*Solanum melongena* L.) through hypocotyl and leaf disc explants, Journal of Applied Phycology., DOI 10.1007/s10811-014-0375-6 **(2014)**

21. Murashige T. and Skoog F.A., Revised medium for rapid growth and bioassay with tobacco tissue cultures, Physiologia. Plantaram., 15, 473-497 **(1962)**

22. Hong D.D., Hien H.M. and Son P.N. Seaweeds from Vietnam used for functional food, medicine and bio-fertilizer, Journal of Applied Phycology, 19, 817–826 **(2007)**

23. Stephenson W. A. Seaweed in agriculture and horticulture, 3rd edn. B and G Rateaver, Pauma Valley, 241 **(1974)**

24. Rolland F., Moore B. and Sheen J. Sugar sensing and signaling in plants, The Plant Cell, 14, 185–205 **(2002)**

25. Blunden G., Cripps A.L., Gordon S.M., Mason T.G. and Turner C.H., The characterization and quantitative estimation of betaines in commercial seaweed extracts, Botanica Marina, 29, 155–160 **(1986)**

26. Ghoul M., Minet J., Bernard T., Dupray E. and Cornier M., Marine macro algae as a source for osmo-protection for *Escherichia coli*., Microbial Ecology, 30, 171–181 **(1995)**

27. Rosalba Mireya Hernández-Herrera, Fernando Santacruz-Ruvalcaba, Mario Alberto Ruiz-López Jeffrey Norrie, Gustavo Hernández-Carmona. Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.), Journal of Applied Phycology, DOI 10.1007/s10811-013-0078-4 **(2013)**

28. Arnon D.I. and Johnson C.M. Influence of hydrogen ion concentration on the growth of in higher plants under controlled conditions, Plant Physiology, 17, 525–539 **(1942)**

29. Steveni C.M., Norrington-Davies J. and Hankins S.D., Effect of seaweed concentrate on hydroponically grown spring barley, Journal of Applied Phycology, 4, 173-180 **(1992)**

30. Kumari R., Kaur I. and Bhatnagar A.K., Effect of aqueous extract of *Sargassum johnstonii* Setchell & Gardner on growth, yield and quality of *Lycopersicon esculentum* Mill, Journal of Applied Phycology, 23, 623–633 **(2011)**

31. Zodape S.T., Gupta A. and Bhandari S.C. Foliar application of seaweed sap as biostimulant for enhancement of yield and quality of tomato (*Lycopersicon esculentum* Mill.), Journal of Scientific and Industrial Research, 70, 215–219 **(2011)**

32. Featonby Smith B.C. and Van Staden J., The effect of seaweed concentrates on the growth of tomato plants in nematode-infested soil, Scientia Horticulturae, 20, 137–146 **(1983)**

33. Pramanick B., Brahmachari K., and Ghosh A., Effect of seaweed saps on growth and yield improvement of green gram, African Journal of Agriculture Research, 8, 1180–1186 **(2013)**.