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Effect of marine algae based polysaccharides from *porphyridium* sp. as bio-stimulant on wheat plant

Saurabh K. Tiwari[1], Mahadev Gaikwad[2], Vinod Nagle[2], Santanu Dasgupta[2] and Virendrakumar Gupta[1]*
[1]Polymer Synthesis & Catalysis Group, Reliance Research and Development Center, Reliance Industries Limited, Navi Mumbai-400701, India
[2]Germplasm & Cultivation Group, Reliance Research and Development Center, Reliance Industries Limited, Navi Mumbai-400701, India
virendrakumar.gupta@ril.com

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Abstract

In the present study exopolysaccharide (EPS) from marine red algae has been studied as bio-stimulant for agricultural use. Algal exopolysaccharide with Magnesium chloride was applied as nutrient for wheat plant. Exopolysaccharide addition of 0.5-1 wt% showed enhancement in the amino acid content, Chlorophyll content and relative water content in wheat plant. Based on the present study it can be concluded that combination of EPS and $MgCl_2$ can be recommend as bio-stimulant to enhancing the photosynthetic activity in commercial crops. Exopolysaccharide from Porphyridium has a great potential and can be used as bio-stimulator for agriculture application.

Keywords: Exopolysaccharide, Marine red algae, Bio-stimulant, Wheat plant, Amino acid, Chlorophyll content, Relative water content.

Introduction

The climatic change leading to the biotic and abiotic stresses in the agriculture crops[1]. In this framework, the chemical-based fertilizers and harmful pesticides are used to enhance agricultural production to meet the requirement of the rising world population. Currently farmers are applying chemical based stimulants to improve the quality of fruits or vegetables which persists within them even after harvest resulting in amplified Maximum residue level (MRL) and Post-harvest interval (PHI) of crop. To avoid such residual chemicals in the final product it is necessary to find out safe and non-harmful substitute for stimulants which will not appear in any residual form after harvesting in the product. These chemical-based growth promoters are serious threat to human health and environment. Harmful impact and intensification of these synthetic chemical in environment, demands modern agriculture to adopt novel ecofriendly alternatives[2]. Development of innovative strategies based on bioresources has proven to be efficient for enhanced crop performance. Plant Bio-stimulant (PBS) are the ingredients that in minute quantities promote and enhance plant growth[3,4]. Extracts of different macroalgae represent an essential category of organic bio-stimulants[5]. Among all the brown macroalgae are the most familiar seaweed that is used in agriculture as a commercial products[7].

Microalgal crude extracts, polysaccharides, fatty acids, phenolic compounds, osmolytes, vitamins, phytohormones, and hormone-like compounds are reported to be engaged in communicating plant response to abiotic stress[5,6]. Seaweed extracts were reported to enhance in seedling growth, flowering, fruit setting, and fruit color as well as provide tolerance to the abiotic stresses[7]. Predominantly, seaweeds are harvested from the natural habitats, which hampers the consistent standard raw material quality. It has been also seen that chemical characteristic of seaweeds depends on its temporal and special distribution, nutrient availability, environmental conditions and time of harvesting[8]. Considering above limitations of seaweed Biostimulants, the scientific community and the industries are looking at the controlled production of microalgae for bio-stimulant applications. *Chlorella*, *Dunaliella*, *Nannochloris* and *Spirulina* have been used to improve the crop yield in agriculture. *Chlorella vulgaris* extracts were reported to stimulate various growth parameters in vegetables crops[9]. Aqueous extracts of *Chlorella ellipsoidea*, *Spirulina maxima*[10] and *Nannochloris*[11] mitigated salt stress impact on wheat and tomato respectively. El Arroussi *et al.* reported that the exopolysaccharides from *Dunaliella salina* helps to tolerate different salinity levels in Tomato plant by producing various metabolites against oxidative stress[12].

Algal polysaccharide has been used for the improvement of soil microbial communities ultimately enhancing crop productivity. Microalgal polysaccharides also provide organic carbon to beneficial microbes and helps in formation of biofilms in rhizosphere[13]. Polysaccharides also helps in solubilization, mineralization, and bioavailability of macro and micronutrients to the plants consequently improving crop performance[14]. Various reports suggest that algal exopolysaccharides can be a source of bio-stimulants for crop improvement and protection against biotic as well as abiotic stress which is sustainable sources and also renewable[15-17]. In the present study we investigated Sulphated Polysaccharides (SPs) extracted from marine red algae *Porphyridum* to improve wheat plant growth promotion in comparison with commercially available Hoagland solution and $MgCl_2$.

Methodology

Extraction of polysaccharide: Marine algae *Porphyridium sp.* was isolated from coast of Maharashtra, India. The algae was grown in 250ml Erlenmeyer flasks using artificial sea water (ASW) medium[18]. For cultivation, light intensity of 150µ moles /m^2/s was incident in 12h:12h light: dark cycle, with 3% salinity of culture medium and pH of 7.8. An optimum temperature of 25±2^{o}C and ambient 2% CO_2 was provided throughout the cultivation period. *Porphyridium* secrets *Exo*polysaccharides (EPS) in culture medium. Seven days grown culture was harvested at stationary phase by centrifugation (8000rpm for 10 min) to separate cell biomass from medium. The supernatant was subjected to exopolysaccharide extraction. Equal volume of supernatant and absolute ethanol was used to precipitate the EPS. The mixture was then stirred vigorously (3 to 5min) until a translucent viscus coagulum was formed. The resultant coagulum biomass was squeezed through nylon mesh (pore size 60µ) for dewatering. Then the harvested EPS was kept in oven at 60°C for drying. Dried EPS powder was used for further experimental work. After extraction of EPS left out ethanol was again redistilled back using the rotavapor[19].

Preparation of $MgCl_2$ and Polysaccharide solution: 0.2ppm $MgCl_2$ solution was prepared in 100ml distilled water. Dissolution rate of algal polysaccharides are low at room temperature, which increased upon keeping in boiling water bath for 5min. The dissolved polysaccharide was then mixed with rest of the solution of $MgCl_2$ and final volume made to 500 ml with distilled water. The final pH value of 5 was maintained using 0.1M HCl solution. 0.5% and 1.0% polysaccharide solution was used for this study.

Treatments: 4 different treatments were used in this experiment as follows: T 1: Hoagland solution as control, T 2: $MgCl_2$, T 3: $MgCl_2$+0.5 Polysaccharide solution, T 4: $MgCl_2$+1% Polysaccharide solution.

Seed germination: For seed germination experiment, Wheat Seeds (*Triticum aestivum* L.) were procured from local agriculture shop and before starting the experiments seeds were surface sterilized for 20min. with 1% sodium hypochlorite solution[20]. Seed germination experiments were carried out in two steps as control and treatments. In control experiment 7 Petriplates were taken with whatman filters papers in each, and named them. 5ml of following solutions were added in each petriplates. Hoagland, $NH_4H_2PO_4$, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, Micronutrient, Fe–EDTA. In treatment experiments $MgCl_2$+ 0.5 and 1% polysaccharides were used. In each petriplates 15 Wheat seeds and 5ml of Hoagland and mixture of other micro nutrient solutions (Boric acid, Manganese chloride, Zinc sulphate, copper sulphate, Molybdic acid, Ferric ethylene diaminetetra acetic acid) were added and all the experimental petriplates were kept in dark incubation condition at room temperature for seed germination. After 3 days of incubation and seed germination, all the germinated seeds from each treatment were transplanted in plastic nursery trays. Each cup of the tray was filled with mixture of soil and coco peat (60:40). For each treatment 5 cups were selected. As and when, the required quantity of nutrients from 5 to 150ml were added in different time intervals during seed germination process. All the experimental trays were covered by plastic net to protect from insects as shown in Figure-1. After 7 days of growth, 5 cups of each seedling were transplanted in separate plastic pots with 12cm diameter. Transplanted pots were kept in shade net at temperature of 25±3°C, photoperiod 12h/12h and light intensity of 300µmol m^{-2} s^{-1}. Nutrients as mentioned in treatments i.e. T1, T2, T3 and T4 were provided to experimental plants at three days interval. Non-treated plants served as control. After 20 days, plants were evaluated for further biochemical and morphological studies.

Biochemical analysis were carried out using different methods as Chlorophyll content-Arnon's method[21], protein analysis-Folin-Lowry method[22], reducing sugar analysis-Folin-wu method[23], Relative water content (RWC)[24] and α-Amino Acid-Ninhydrin reaction method[25] to understand different parameters related to the Wheat plant growth.

Figure-1: Seed germination of wheat plant.

Results and discussion

ICP and CHNS analysis: In the present study, *Porphyridium* sp. belonging to the division Rhodophyta and family Porphyridiophyceae was explored for its potential bio-stimulant activities on wheat plant has shown in Figure-2. *Porphyridium* sp. is a red microalgae which is enfolded in a sulphated polysaccharide. Extracted Polysaccharide was characterized by inductively coupled plasma mass spectrometry (ICP-MS: Thermo scientific- iCAP 7000 series) and CHNS analysis

(Elementar-Vario MACRO Cube). The ICP analysis showed the presence of different minerals those are essential for plant growth as depicted in Table-1 and sulfur as shown in Table-2 evaluated by CHNS analysis. High Sulphur content (8.32%) was found in red algae extracted polysaccharide.

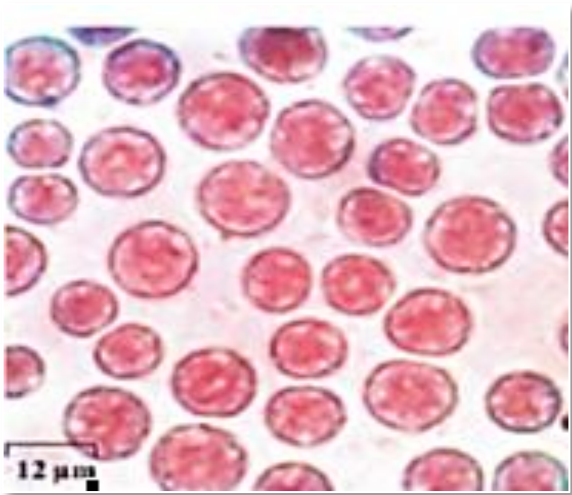

Figure-2: *Porphyridium* microphotograph at 100X magnification[26].

Table-1: ICP analysis of extracted polysaccharide from red algae.

Sample	Element	Composition (%)
Polysaccharide	Ca	14.92
	Na	5.05
	Mg	1.03
	K	0.24

Table-2: CHNS analysis of extracted polysaccharide from red algae.

Sample	N %	C %	H %	S %
Polysaccharide	1.26	9.02	2.81	8.32

Root/Shoot length: The ratio of Root/Shoot length for plant grown in Hoagland medium was 3.65. It was found to be maximum of those grown in magnesium chloride. The combination of magnesium chloride with 0.5 and 1% polysaccharide also gave good response as compare to Hoagland medium.

Chlorophyll content analysis: Plants grown in Hoagland medium (standard) showed 1.53mg of chlorophyll/100gm but plant grown in magnesium chloride using 0.5 and 1% polysaccharide has shown highest amount of chlorophyll content comparatively than standard as shown in Table-3.

Protein, sugar and α-Amino Acid analysis: Wheat plant grown in Hoagland medium (standard) 1020mg of protein /100 gm. While plant grown in magnesium chloride and different concentration of polysaccharide did not show any elevation in protein content i.e. 660mg of protein/100gm. Sugar content also analyzed in wheat plant which grown in Hoagland medium (standard) showed 300mg of sugar/100gm. Plant grown in magnesium chloride and polysaccharide containing medium showed 100mg of sugar/100gm. It was seen that plants grown in Hoagland medium (standard) showed 400mg of A.A./100gm. Plants grown using magnesium chloride with 0.5% and 1% polysaccharide have shown higher amount of amino acid i.e.480 and 560mg of A.A./100 gm respectively. Less amount of amino acid content was observed in magnesium chloride medium. i.e. 320mg of A.A./100gm of plant material.

Relative water content (RWC): The relative water content in all leaves was almost 78% for $MgCl_2$+1% polysaccharide which is found higher among all formulation as shown in Figure-3.

Table-3: Growth parameters of wheat plant.

Treatments	Root/Shoot (cm)	Protein (mg of protein/100 gm of plant material)	Reducing Sugar (mg of sugar /100gm of plant material)	α-Amino Acid (mg of A.A. /100gm of plant material)	Chlorophyll content (mg of chlorophyll /100gm of plant material)	Relative water content (RWC in %)
T1	3.65± 0.18	1020± 5	300	400	1.53± 0.01	62±1
T2	4.6±0.72	660± 2.8	100	320	2.09± 0.05	72±2
T3	3.92±0.09	660± 3.5	100	480	2.37± 0.01	62±0.58
T4	3.56±0.33	600± 5	100	560	2.78± 0.01	78±0.58

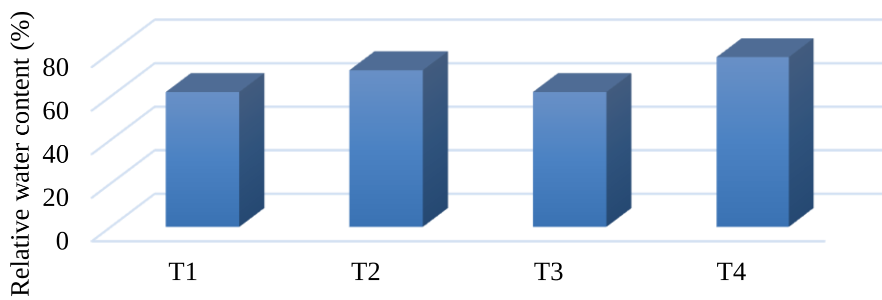

Figure-3: Relative water content in wheat plant.

Discussion: In agriculture field, macroalgal polysaccharide had been used extensively to produce bio-stimulant while microalgal polysaccharide remain under exploitation. Guzman-Murillo *et al.* depicted that exopolysaccharides taken out from *Phaeodactylum tricornutum* and *D. salina* triggered the germination of pepper plant under salt stress conditions[27]. In addition, *D. salina* polysaccharides indicated the encouraging potential to trigger germination, endurance and growth of wheat and tomato plants at salt stress conditions[28]. The polysaccharides obtained from various microalgae and cyanobacteria were used on *Solanum lycopersicum* plants and compared biostimulant effects based on its effects on root length, shoot and nodes number. According to the Rachidi *et al*. 1mg /L of *Porphyridium* sp. polysaccharide extract boosted the carotenoid content and NAD-GDH activity in *Solanum* plant as compared to the control[29]. In other set of studies they have also seen the stimulatory effect of microalgae polysaccharide on germination and seedling growth of *Solanum lycopersicum* plants[30]. Mutale-joan, *et al* reviewed the microalgae based polysaccharides and its possible use in agriculture as a potential feasible substitute for nutrient uptake, enhanced crop production and flexibility to environmental stress[31]. Mutale-joan *et al* screened liquid extracts of 18 different algae from different groups to use as bio stimulant for the plant growth. They have seen the remarkable improvement in root and shoot length which is approximately 112% and 53% respectively[20]. El-Naggar *et al.* studied the extraction and characterization of the soluble fraction of *Chlorella vulgaris* based polysaccharides to synthesize silver nanoparticles. They have observed its stimulatory effect on germination and seedling growth of *Triticum vulgare*, *Phaseolus vulgaris* plant[32].

Current study indicates the positive correlation between concentration of EPS used as biostimulant by increasing the α-amino acids, chlorophyll content and relative water content (Table-3). Different abiotic and biotic stresses can affect the plant growth. In this regards the osmotic stress experienced due to drought and salinity pose serious threat inhibiting growth and productivity[33]. However, in the present study treatment T4 showed the relative water percentage of 78% which is higher as compared to other treatments. This is in conformity with the Clarke and McCaig and Schonfeld *et al.* as there is a correlation between yield stability and relative water content monitored in the wheat plant[33,34]. In one more study Tahara *et al*. observed the relationship between the grain yield and relative water content in winter wheat and in tetraploid wheat under drought stress[35]. According to the Sinclair and Ludlow, the plant metabolism is also associated with the relative water content in the leaf[36]. According to Hassanzadeh *et al.* the relative water content and chlorophyll b and total chlorophyll has positive and significant correlations and these results are comparable with the present study as the relative water content and increase in total chlorophyll content[37]. In addition to this it also indicates increment in the photosynthetic activity[38].

Higher amino acid content in the treated plant as compared to control plants observed in present study (Table-3). The aggregation of amino acids in the plants promotes regulation of ion transport and osmolyte[39]. That means the use of exopolysaccharides can play important role in the plant metabolism through increasing the amino acids like proline in the plant and also help in the drought stress[31]. So treatment T3 and T4 can be recommend as biostimulant to enhance the photosynthetic activity in plants.

Conclusion

Elemental analysis showed higher amount of sulphur content present in polysaccharide extracted from red algae. It is known to have immunomodulatory activities which could be useful in various applications like stimulation of immune system and other antiviral product development. The extracted polysaccharide from same algae has been studied as a plant bio-stimulant. *Porphyridium* EPS in different concentration with $MgCl_2$ was found to be effective in stimulating plant growth and other essential parameters. Addition of exopolysaccharides improves the amino acid content, chlorophyll content and relative water content in wheat plant. Based on the present study it can be concluded that $MgCl_2$ with 0.5% and 1% exopolysaccharide from red algae can be recommended as a safe and eco-friendly bio-stimulant to enhance productivity. This will lead towards the establishing sustainable and ecofriendly agriculture. This area can be explored more in depth for the advancement of this product for enhanced productivity.

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