Plant Growth Promoting Properties of Four Arctic Seaweed Extracts

Tatjana Shibaeva[1,*], *Elena* Sherudilo[1], *Konstantin* Bogolitsyn[2], *Anastasiya* Parshina[2] and *Khurshed* Mamatmurodov[2]

[1]Karelian Research Centre of Russian Academy of Sciences, Petrozavodsk, Russia
[2]Northern (Arctic) Federal University named after M.V. Lomonosov, Arkhangelsk, Russia

Abstract. The objective of this study was to evaluate the effects of the White Sea brown seaweed *Ascophyllum nodosum, Fucus vesiculosus, Laminaria digitata* and *Saccharina latissimi* extracts on plant growth and biomass accumulation. Extracts were obtained by isopropanol maceration method. Growth promoting effects of all four seaweed extracts were observed at the initial growth phase of *Triticum aestivum* and *Cucumis sativus* plants. *A. nodosum* and *F. vesiculosus* extracts were more effective at medium concentrations (60, 120 and 300 mg/L) while higher concentration (1200 mg/L) decreased the growth rate and biomass accumulation in wheat plants, but not in cucumber seedlings when compared to control. Higher phenolic content in *A. nodosum* and *F. vesiculosus* extracts can possibly be a reason of lower effectiveness of extracts with high concentration. These results suggest that investigated arctic seaweeds have biostimulatory properties that affect plant growth and biomass accumulation and after intensive future studies can be exploited for elaboration of innovative products for agriculture.

1 Introduction

Seaweed biomass contains many bioactive compounds which can stimulate plant growth. The main chemical algal components that affect plant growth are phytohormones (auxins and auxin-like compounds, cytokinins, abscisic acid), polyphenols, amino acids, betaines, sterols, polysaccharides, minerals and trace elements [1, 2]. The most effective form of algal based products for agriculture are liquid extracts [1, 3]. Seaweed extracts are used in agriculture as biostimulants as a low concentration is required to induce a positive response in plant growth and increase plant tolerance to various stress factors [4]. The beneficial effects of seaweed extracts on plant growth can be attributed to direct and indirect stimulation mechanisms [5]. Seaweed extracts are able not only to improve crop growth, but also to increase nutrient uptake, photosynthesis, yield, quality and plant tolerance to abiotic and biotic stress [1, 6].

Currently plant biostimulants are considered as a full-fledged class of agri-inputs and highly attractive business opportunity for major actors of the agroindustry [7]. There is a number of commercially available algal based products used in modern agriculture [8].

* Corresponding author: shibaeva@krc.karelia.ru

Russia has large reserves of different seaweeds, which are traditionally harvested in the Far East (Japanese Sea), on the Kuril Islands, Sakhalin, in Primorye, on the mainland coast of the Sea of Okhotsk and in the White Sea. Studies of kelp and fucus algae from the White Sea, Japanese Sea and Yellow Sea confirmed the general similarity of their chemical composition with that characteristic of these taxonomic groups: polysaccharides, vitamins, fatty polyenoic acids, auxins and gibberellins, almost the same primary and secondary metabolites, a rich composition of micro- and macroelements [9-11]. This allows assuming that brown seaweeds from the White Sea are suitable resource for the production of brown seaweed extracts with the properties of biostimulants. The extracts from the White Sear brown seaweeds are already commercially exploited and getting increasing attention in food, cosmetics and pharmaceutical industries. So far, seaweed based agricultural products from the White Sea are fertilizers and soil amendment products, but not bioactive extracts.

For the elaboration of innovative products for agriculture the information about bioactive compounds in seaweed extracts and their effects on plant growth and biomass accumulation is essential [12, 13]. Currently, commercially available algal extracts are liquids and most of them were produced by conventional extraction techniques. The composition and concentration of the bioactive compounds in seaweed extracts depend on their structure and content in seaweed cells, but a key role plays the method of their extraction [14].

In the view of the above-mentioned the objective of this study was to evaluate the effects of the White Sea brown seaweed *Ascophyllum nodosum, Fucus vesiculosus, Laminaria digitata* and *Saccharina latissima* extracts (obtained by isopropanol maceration method) on plant growth and biomass accumulation.

2 Materials and methods

2.1 Collection of seaweeds

Four arctic brown seaweeds *Ascophyllum nodosum* (L.) Le Jolis*, Fucus vesiculosus* L.*, Laminaria digitata* (Huds.) Lamouroux and *Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl, et G. W. Saunders were freshly collected from the Rebalda Bay near the Solovetsky Islands in the White Sea in August 2021 by the Arkhangelsk Seaweed Factory LLC . The collected seaweeds were brought to the laboratory, cleaned and were given a quick freshwater rinse to remove surface salts. Subsequently the seaweeds were air dried at 40°C. The dried samples were finely powdered and sieved. The material obtained was stored in sealed vessels in the dark at 20°C until use. The fraction with the particle size of 0.25–0.50 mm was used for further study.

2.2 Preparation of seaweed extracts

Seaweed samples were macerated with 40 % isopropanol at 60°C for 1 h under constant stirring. Then they were centrifuged at 10 595 g for 5 min and supernatant (extract) was collected. Obtained extracts were vacuum-dried at 40°C and stored at -4°C.

2.3 Chemical analyses of seaweed extracts

Protein content in extracts was measured by following the Bradford method [15] using bovine serum albumin as a standard. Proteins were expressed as percentage of algal fresh weight.

The content of polyphenolic compounds was determined according to modified Folin-Ciocalteu colorimetric method [16]. The absorbance changes of reaction of the blue colour product were measured by UV-spectrophotometer (Spekol UV 1300, Analytic Jena, Germany) at 765 nm. Phloroglucinol (≥99,0 %, Sigma Aldrich, USA) was used as a standard for calibration curve.

Extraction and estimation of chlorophyll. The samples were centrifuged at 3270 g for 15 min. Absorbance of the extract was read at 663 and 645 nm for chlorophyll a and chlorophyll b, respectively using UV-spectrophotometer.

Carotenoids content of seaweeds was determined spectrophotometrically at 480 nm according to [17] using the same extract used for chlorophyll estimation.

Alginates content in extracts was measured by UV-spectroscopy after the reaction with Alcian blue dye (ApliChem, Italy) resulting in precipitation of alginates according to the method described by [18]. The amount of unbounded dye was used for alginates quantification.

Mannitol content was measured according to the method described by [19]. Method is based on the reaction between dissolved mannitol and added copper ions in strong alkaline medium which produce the insoluble residue. The quantity of mannitol was calculated based on the optical density of solutions at 597 nm after the reaction.

Polysaccharide content was determined by a colorimetric reaction of reducing sugars with 3,5-dinitrosalicylic acid (98 %, Acros Organics, Belgium). Reducing sugars were obtained by acidic hydrolysis with 2 % HCl during 3 h at boiling temperature. Obtained mixture of sugars was neutralized and used for further analysis according to the method described by [20].

2.4 Plant material

Wheat (*Triticum aestivum* L. var. Zlata) and cucumber (*Cucumis sativus* L. var. Zozulya F1) were grown for two weeks in hydroponic vessels under controlled environmental conditions in a growth chamber at the average air temperature of 23°C and relative air humidity of 70%. Plants were grown under 14 h photoperiod with the PPFD of 150 µmol m^{-2} s^{-1}. Each vessel contained 16 wheat plants or 9 cucumber plants. Hydroponic vessels were filled with liquid extracts (dry extacts dissoved in distilled water) of four seaweeds in concentrations 30, 60, 120, 300 and 1200 mg/L. Distilled water was used as a control (0 mg/L). Two vessels per each treatment were used.

2.5 Biometric measurements

Ten seedlings of each species were randomly selected and measured to determine plant height, cotyledon (cucumber) and first leaf (wheat) area, length of the first true leaf (cucumber), root length and dry weight (DW) of shoots and roots. To determine DW samples were dried at 105°C in an oven until a constant DW was observed.

2.6 Statistical analysis

The experiment was carried out twice. The tables show mean values and their standard errors. The data were analyzed by one-way analysis of variance (ANOVA). Difference between the mean values was considered significant at $P \leq 0.05$.

3 Results and discussion

3.1 Chemical composition of seaweed extracts

The chemical properties of different seaweed extracts were analyzed (Table 1). The seaweed extracts of *A. nodosum* and *F. vesiculosus* contained higher levels of protein, polyphenols and carotenoids and lower level of polysaccharides compared to the other two seaweed extracts. *L. digitata* contained the highest amount of total chlorophyll.

Table 1. Chemical composition of seaweed extracts, % of dry weight

Compounds	*Ascophyllum nodosum*	*Fucus vesiculosus*	*Laminaria digitata*	*Saccharina latissima*
Protein	1,42±0,07	1,07±0,13	0,22±0,02	0,17±0,02
Polyphenols	5,89±0,15	4,71±0,17	0,12±0,01	0,20±0,02
Chlorophyll	0,008±0,001	0,007±0,001	0,015±0,001	0,005±0,001
Carotenoids	0,023±0,001	0,016±0,001	0,009±0,001	0,008±0,001
Alginates	1,58±0,01	1,47±0,01	1,15±0,02	0,97±0,01
Mannitol	8,03±0,34	10,92±0,05	8,93±0,31	5,22±0,24
Polysaccharides	5,84±0,03	5,37±0,37	8,6±0,40	7,21±1,15

3.2 Plant growth and biomass accumulation

The growth and productivity of wheat and cucumber seedlings were significantly affected by all four seaweed extracts. *A. nodosum* and *F. vesiculosus* extracts were more effective at medium concentrations (60, 120 and 300 mg/L) while higher concentration (1200 mg/L) decreased the growth rate and biomass accumulation in wheat plants, but not in cucumber seedlings when compared to control (Table 2, 3). Cucumber plants treated by 1200 mg/L seaweed extracts in most cases outperformed control and other treated plants in terms of plant height, leaf area, root length, shoot DW and root DW. The present results are in agreement with those who recorded that the low concentrations of seaweed extracts promoted the seedling growth [1-8, 12-13].

Dramatic decrease in values of growth characteristics in wheat plants treated by high concentration *A. nodosum* and *F. vesiculosus* extracts can be due to their much higher polyphenolic content (Table 1). Phenolics synthesized by brown seaweeds are among the most numerous and important bioactive compounds. They have been shown to promote shoot and root lengthening and development of adventitious roots in plants by regulating the activity of auxins [21]. Therefore, it is possible that high concentration of phenolics may inhibit plant growth in sensitive plant species.

Table 2. Growth characteristics of *Triticum aestivum* plants treated by four seaweed extracts

Parameter	Seaweed extract concentration, mg/L					
	0	30	60	120	300	1200
	Ascophyllum nodosum					
Plant height, mm	185±3	191±4	202±5	205±10	220±5	154±5
First leaf area, mm^2	329±10	330±18	340±15	335±13	360±16	308±12
Root length, mm	45±1	50±5	63±2	69±2	67±2	27±1
Shoot DW, mg	26.6±1.1	27.5±1.1	27.6±1.1	27.7±0.8	28.7±0.9	21.0±0.8
Root DW, mg	8.8±0.4	9.4±0.5	9.2±0.5	9.4±0.4	10.2±0.5	11.9±0.6
Fucus vesiculosus						
Plant height, mm	181±7	198±6	214±5	222±8	221±13	149±8
First leaf area, mm^2	321±76	374±22	372±22	395±19	398±18	224±11
Root length, mm	159±7	207±5	208±5	137±6	48±6	29±1

Shoot DW, mg	21.5±1.4	22.9±1.2	24.9±0.6	26.7±1.3	28.3±2.0	17.8±0.9
Root DW, mg	9.8±0.6	14.3±0.7	14.1±0.5	14.2±0.6	10.5±0.4	9.2±0.4
Laminaria digitata						
Plant height, mm	150±5	150±5	161±6	178±5	195±7	186±7
First leaf area, mm^2	200±11	206±8	221±11	226±4	242±8	212±7
Root length, mm	128±6	130±6	133±5	121±5	108+6	65±7
Shoot DW, mg	22.5±1.4	20.9±1.5	20.5±1.5	23.2±0.8	27.5±1.3	26.5±1.4
Root DW, mg	8.4±0.4	8.5±0.5	8.9±0.5	10.1±0.7	9.5±0.4	8.2±0.4
Saccharina latissima						
Plant height, mm	174±8	181±12	197±6	200±7	195±7	182±8
First leaf area, mm^2	174±14	176±10	209±7	242±16	294±12	290±10
Root length, mm	88±4	90±4	120±3	90±4	71±2	61±6
Shoot DW, mg	24.3±2.5	24.8±2.4	25.1±1.6	22.8±1.5	21.4±1.2	22.0±1.4
Root DW, mg	8.8±1.3	10.8±0.6	11.0±0.5	10.1±0.3	8.4±0.8	8.4±0.5

Table 3. Growth characteristics of *Cucumis sativus* plants treated by four seaweed extracts

Parameter	**Seaweed extract concentration, mg/L**					
	0	30	60	120	300	1200
	Ascophyllum nodosum					
Plant height, mm	22.3±0.8	23.6±0.6	23.8±0.6	23.6±0.8	22.2±0.9	25.0±0.4
Cotyledons area, mm^2	116±4	165±5	195±3	205±4	256±10	302±11
True leaf length, mm	2.1±0.2	2.1±0.2	2.1±0.2	2.2±0.2	4.7±0.7	12.3±0.9
Root length, mm	23±1	27±2	28±2	43±5	49±2	48±2
Shoot DW, mg	18.8±0.1	20.5±0.1	21.7±0.1	22.5±0.2	26.5±0.2	33.1±0.2
Root DW, mg	1.7±0.1	2.4±0.1	2.6±0.1	3.2±0.2	5.7±0.5	8.5±0.7
Fucus vesiculosus						
Plant height, mm	18.3±0.6	19.3±0.8	20.9±0.8	21.1±0.7	21.0±0.9	20.7±0.8
First leaf area, mm^2	174±18	250±24	265±18	286±25	199±15	201±13
True leaf length, mm	1.8±0.3	3.5±0.3	4.3±0.5	7.1±1.1	9.3±0.9	12.2±1.3
Root length, mm	36±2	43±2	28±2	43±5	49±2	48±2
Shoot DW, mg	20.0±1.8	23.4±1.0	34.6±2.7	35.7±2.9	34.7±2.1	30.8±3.6
Root DW, mg	3.8±0.1	5.7±0.4	7.6±0.6	8.8±0.5	8.9±0.6	7.7±0.5
Laminaria digitata						
Plant height, mm	20.1±1.1	20.2±0.9	21.4±0.9	21.3±1.9	21.1±0.7	18.3±0.6
First leaf area, mm^2	148±18	159±4	192±10	208±11	268±10	230±16
True leaf length, mm	2.0±0.1	2.0±0.1	2.5±0.2	3.6±0.3	3.4±0.3	3.6±0.3
Root length, mm	39±4	58±3	59±3	60±4	65±2	57±2
Shoot DW, mg	21.8±1.3	22.4±1.8	25.8±1.5	27.8±0.3	32.3±1.7	33.3±1.8
Root DW, mg	1.8±0.2	2.3±0.3	2.4±0.2	2.8±0.3	3.5±0.3	4.1±0.3
Saccharina latissima						
Plant height, mm	19.0±1.1	19.4±0.6	22.2±1.1	22.3±1.1	22.6±0.9	24.2±0.2

First leaf area, mm^2	128±9	137±13	159±27	170±13	203±14	243±24
True leaf length, mm	1.6±0.2	2.8±0.4	3.0±0.3	3.2±0.4	4.6±0.5	6.4±1.1
Root length, mm	36±4	39±4	44±4	43±3	45±4	47±2
Shoot DW, mg	20.5±0.8	21.6±2.3	22.1±2.3	22.3±1.3	28.4±2.3	32.9±1.5
Root DW, mg	1.4±0.2	1.7±0.3	2.2±0.2	1.9±0.3	2.6±0.2	4.2±0.4

4 Conclusions

This study investigated the growth responses of wheat and cucumber seedlings on brown seaweed *Ascophyllum nodosum, Fucus vesiculosus, Laminaria digitata* and *Saccharina latissimi* extracts (30-1200 mg/L) obtained by isopropanol maceration method. The obtained results suggest that investigated arctic seaweeds have biostimulatory properties that affect plant growth and biomass accumulation and after intensive future studies can be exploited for elaboration of innovative products for agriculture.

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