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**Effect of Seaweed Extracts on the Growth of Seedling Roots of Soybean  
(*Glycine max* (L.) Merr.) Seasonal Changes in the Activity**

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**KEYWORDS**

*Neorhodomela larix*,  
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*Glycine max* (L.);  
*Codium fragile*.

**A B S T R A C T**

Effect of water extracts of red algae *Neorhodomela larix*, *Tichocarpus crinitus*, of brown algae *Saccharina japonica*, *Sargassum pallidum*, and green algae *Ulva fenestrata* and *Codium fragile* on the length of seedling roots of soybean (*Glycine max* (L.) Merr.) was studied. It is shown that the aqueous extracts of algae in varying degrees have a positive effect on the length of the roots of soybean seedlings. The highest stimulatory effect showed extracts of the green alga *Codium fragile* collected in November 2011 at concentrations of  $10^{-5}$  gSW mL<sup>-1</sup>, and the roots on 18.0% were longer than the control roots.

**Introduction**

The seaweeds resources are intensively used to improve harvest quantity and quality in agriculture and horticulture. The beneficial effects of seaweed products on the cultured plants are well documented. They improve seeds germination, seedlings development, increase plant tolerance to environmental stresses (Zhang and Ervin, 2004; 2008), and enhance plant growth and yield (Hong et al., 2007; Zodape et al., 2008; Khan et al., 2009; Kumari et al., 2011). More over seaweeds are used as soil amendment (Gandhiyappan and Perumal, 2001), in pests control (Hong et al., 2007)

and plant disease management (Jayaraj et al., 2008). Liquid extracts obtained from seaweeds have gained importance as foliar sprays and soil drench for many crops including various grasses, cereals, flowers and vegetable species. Also they apply to stimulate seedling germination and rooting. At present one of the most promising applications of seaweeds is their use as plant biostimulants. For example, aqueous extracts of *Sargassum johnstonii* at concentration from 0.1 to 0.8% (w/v) that is equivalent 1–8 mgSW mL<sup>-1</sup> used as foliar spray and soil drench enhanced vegetative

growth (plant height, shoot length, root length, and number of branches) and reproductive parameters (flower number, fruit number, and fresh weight) of tomato (Kumari et al., 2011). Also foliar application of 15% (v/v) aqueous extracts prepared from fresh *Kappaphycus alvarezii* resulted in 57% increase of *Glycine max* yield compare to the control and intensified nutrient uptake by soybean (Rathore et al., 2009). The introductory soaking of *Triticum aestivum* seeds in 20% (0.2 mgSW mL<sup>-1</sup>) extracts of *Sargassum wightii* for 24 h gave an 11% increase in seed germination, a 63% enhance in number of lateral roots and 46% increase in shoots length in compare to control (Kumar and Sahoo, 2011).

From the literature it is known that the chemical structure and biological activity of extracts from seaweed vary harvest season algal material. So, for example, it is shown, that toxicity of extracts of sea seaweed in November above, than in June (Marti et al., 2004) and the highest antimicrobial activity detected from December to January (Manilal et al., 2009). Were also shown seasonal changes in the content of plant growth regulators in the extracts of marine algae (Featonby-Smith and Van Staden, 1984; Blunden et al., 2010; Papenfus et al., 2012).

Thus the aim of our studies was to investigate the effect of seaweeds extracts on the growth of seedling roots of soybean (*Glycine max* (L.) Merr.) as well as its dependence from the season of algae collecting.

These investigations are of practical significance for determining the optimal period for algae harvesting in order to produce extracts with maximal stimulatory action on the agricultural plant and to find

the most effective concentration of the extracts for use in agriculture.

## **Materials and Methods**

### **Algae collection**

Two species of red algae (*Neorhodomela larix* (Turner) Masuda and *Tichocarpus crinitus* (S.G.Gmelin) Ruprecht), two brown alga (*Sargassum pallidum* (Turner) C. Agardh and *Saccharina japonica* (Areschoug) C.E. Lane, C. Mayes, Druehl & G.W. Saunders) and two green algae (*Ulva fenestrata* Postels et Ruprecht and *Codium fragile* (Suringar) Hariot) were collected at depth 0.5–1 meter at mid of November 2011 and in January, May and August 2012. Algae were washed with sea-water and hard brush to remove macroscopic epiphytes and sand particles, and then with tap water to remove adhering salt. Samples were air-dried (26°C) during 2–4 days followed by thermostat dry at 60°C for 12h.

### **Preparation of seaweed liquid extracts (SLE)**

Dried seaweeds were hand crushed and powdered with coffee-grinder. Algae were heated with sterile distilled water in a ratio 1 : 100 (w/v) at 60°C for 45 min. Then the extracts were filtered through a filter paper and stored at 4°C for further experimental studies. The filtrates were 10<sup>-2</sup> g of dried seaweeds per milliliter (gSW mL<sup>-1</sup>) extracts. Different concentrations of SLE were prepared by diluting of these extracts with distilled water.

### **Soybean assay**

The seeds of soybean (*Glycine max* (L.) Merr.) were harvested in 2011. Seeds were germinated in rolls of filter paper

(Anisimov *et al.*, 2013a). Dry seeds (twenty seeds per strip) were spread on strips of filter paper (12 cm width and 42 cm length) previously moistened with test solution. Then the strips were rolled and placed into beakers with a small amount of test solution (100 mL). Three rolls with seeds were incubated at each concentration of algal extracts for 3 days in a thermostat at 26–27°C (in total 60 seeds per the concentration). The control seedlings were grown in distilled water. The length of the main root of the seedling was measured after incubation. The results were expressed as percentage of the control. Data were analyzed using Origin 7.0 software. Student t-test was used for analysis of significant differences of root length between control group and under different concentration of extracts. P-values less than 0.05 were considered statistically significant.

## **Result and Discussion**

It was interesting to study effects of aqueous extracts of the marine algae collected in different seasons, at the first stages of ontogeny of plants, from seedling growth of soybean, when the most visible and principal changes influencing the physiological processes of the plant productivity formation occur.

It is shown that extracts of the red alga (*Neorhodomela larix* and *Tichocarpus crinitus*), collected in different seasons, had a slight stimulatory effect on the growth of seedling roots of soybean. Extracts of the red alga *Neorhodomela larix* and *Tichocarpus crinitus*, collected in August and May 2012, showed the maximum stimulatory effect (112.0%) on the growth of seedling roots of soybean at a concentration of  $10^{-4}$  gSW mL<sup>-1</sup> respectively.

Extracts of the brown alga (*Sargassum pallidum* and *Saccharina japonica*, collected in November 2011, January, May and August 2012, did not exceed 8% .

Extracts of the green alga *Ulva lactuca*, collected in November 2011, January, May and August 2012, did not exceed 10% (Table 3). Extracts of the green alga *Codium fragile*, collected in November 2011, January, May 2012, showed the stimulatory effect (118, 110 and 112.7%) on the growth of seedling roots of soybean at a concentration of  $10^{-5}$ ,  $10^{-5}$  and  $10^{-4}$  gSW mL<sup>-1</sup> of seaweed extracts respectively.

Aqueous extracts of most algae have an inhibitory effect on the growth of seedling roots of soybean in concentrations  $10^{-2}$  gSW mL<sup>-1</sup> of seaweed extracts. The same pattern is marked by the action of seaweed extracts on the growth of seedling roots of buckwheat (Anisimov *et al.*, 2013a, b).

Most of the studied extracts of marine algae exhibit in varying degrees stimulatory effect on root growth of soybean seedlings. Thus, the peak of the stimulation of root growth was manifested at concentrations of most algae  $10^{-4}$ – $10^{-7}$  gSW mL<sup>-1</sup> of seaweed extracts. The most perspective for use as biostimulants is green algae *Codium fragile* . It should be noted that all algal extracts in concentration  $10^{-2}$  gSW mL<sup>-1</sup> inhibited development of seedling roots. This result is concordant to finding of other researchers. It was shown that dilute extracts are more effective than the concentrated ones (Sathya *et al.*, 2010; Kumar, Sahoo, 2011). Moreover, stimulatory or inhibitory effect of extracts depended and on the kind of the test plants. Thus, it was shown that high concentrations of aqueous extracts of the brown alga *Sargassum wightii* (5-25%) slowed the growth of roots of seedlings of green peas

(*Phaseolus aureus* L.) (Christobel, 2008), but increase the length of the root sprouts *Abelmoschus esculentus* (L.) Medikus by 48.8% (Jothinayagi, Anbazhagan, 2009) and wheat *Triticum aestivum* var. Pusa Gold - almost 50% (Kumar and Sahoo, 2011).

Plant hormones, most likely, control of stimulating and inhibitory effects of aqueous extracts of marine algae on the growth of soybean seedling roots. Chemical analysis of the algae and their extracts showed the presence in them a whole range of types of plant growth regulators auxin, cytokinins, gibberellins, abscisic acid, etc. (Prasad et al., 2010; Yokoya et al., 2010; Takezawa et al., 2011), which have high concentrations of inhibitory, while low - a stimulating effect on the growth of plants (Crouch and van Staden, 1993).

We found that extracts of various algae have a different effectiveness. A possible explanation for this difference is variety in composition and content of plant growth regulators in extracts of the different seaweeds. It is known that the hormone quantitative content in extracts of marine algae largely is depended on the season of algae collection (Featonby-Smith and Van Staden, 1984; Blunden et al., 2010; Papenfus et al., 2012). Thus, marked quantitative and qualitative variations in the content of such substances cytokinin (Featonby-Smith and Van Staden, 1984). During summer zeatin, ribosylzeatin and their dihydroderivatives were responsible for most of the detected activity. The cytokinin glucosides increased above the levels of free cytokinins during winter. In another study it was shows, that the there were two peaks annually, with the highest concentrations of polyamines for both Putrescine-VR 7 and Spermine-VR 8.62 during winter (June to July) and the second

peak during summer (October to February) in *Ecklonia maxima* (Papenfus et al., 2012).

## Conclusion

In conclusion, the present study showed that in any measure algal extracts stimulate roots development of soybean seedlings. The most perspective to improve seeds germination as well as yield of crop plants are green algae *Codium fragile* collected in November 2011 and May 2012. Aqueous extracts of the alga *Codium fragile* can be recommended for study in the field as growth promoters soybeans.

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