
Plant Growth Promoting Effect, Gibberellic Acid and Auxin like Activity of Liquid Extract of *Caulerpa racemosa* on Rice Seed Germination

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The application of *Caulerpa racemosa* seaweed extract to determine growth promoting effect on different concentration in rice seed *in vitro* germination as a model system was evaluated. Assessment was made by measuring germination rate, length/growth of coleoptile, root length/initiation and the second leaf sheath of rice. The germination rate of 10% seaweed concentration was comparable to the commercial GA and was significantly higher than water. The 10% Seaweed Liquid Extract (SLE) concentration recorded 6.5 % increase in seed germination over control. The highest coleoptile length and leaf blade length mm/seedling were recorded in the 10% SLE of *C. racemosa* treatment and the higher concentrations showed decreasing trend. Root initiation showed increased in 10% concentration, while, highest root mm/seedling and coleoptile length mm/seedling were observed at 10% seaweed extract concentration.

KeyWords: *C. racemosa*, SLF, germination rate, coleoptile, root initiation and second leaf sheath.

Introduction

Seaweeds form an integral part of marine coastal ecosystems. They include the macroscopic, multicellular marine algae that commonly inhabit the coastal regions of the world's oceans where suitable substrata exist. The benefits of seaweeds as sources of organic matter and fertilizer nutrients have led to their use as soil conditioners for centuries (Blunden and Gordon 1986; Metting *et al.*, 1988). Some 15 million metric tons of seaweed products are produced annually (FAO 2006), a considerable portion of which is Eco-friendly and help to curtain high investment needed for chemical fertilizers. Seaweeds are rich source of growth promoting substances (Sylvia *et al.*, 2005) such as

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IAA, kinetin, zeatin and gibberellins (Zodape *et al.*, 2010) auxins and cytokinins (Zhang and Ervin, 2004); metabolic enhancers; macro and micro elements (Strik *et al.*, 2003), amino acids, vitamins and beneficial results from their use in crop plants like early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress and enhanced post-harvest shelf life of seeds (Hankins and Hockey, 1990; Bluden, 1994).

Seaweed components such as macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid (ABA)-like growth substances affect cellular metabolism in treated plants leading to enhanced growth and crop (Crouch and van Staden 1993a). Although many of the various chemical components of seaweed extracts and their modes of action remain unknown, it is plausible that these components exhibit synergistic activity (Fornes *et al.*, 2002; Vernieri *et al.*, 2005).

Although many of the various chemical components of seaweed extracts and their modes of action remain unknown, it is plausible that these components exhibit synergistic activity (Fornes *et al.*, 2002; Vernieri *et al.*, 2005). With this background studies the application of *C. racemosa* seaweed extract to determine growth promoting effect on different concentration in rice seed *in vitro* germination as a model system.

Materials and Methods

Preparation of Seaweed Liquid Extract. The marine green alga *C. racemosa* was collected from the coastal region of San Fabian, Pangasinan. The collected plants were cleaned with seawater to remove sand and epiphytes. The collected samples were washed thoroughly using tap water and distilled water to remove the salt from the surface of the sample. The water was drained off and thallus was spread on blotting paper to remove the excess water.

One kilograms of seaweed was finely chopped and boiled in 1 L of distilled water for an hour and then extract were filtered through muslin cloth. The filtrate was allowed to cool at room temperature and thereafter, through Whatmann No. 41 (pore size 20-25 μm) filter paper. Different concentrations of seaweed liquid extract (10, 25, 50 and 75%) were prepared by diluting with distilled water (Sathya *et al.*, 2010). The seaweed extract was stored at 4°C for further studies.

Seed Germination. Viability test was carried out prior to the test by soaking rice seeds in distilled water for two hours. Floating seeds were discarded and the viable seeds were used for the test. Fifty viable rice seeds were placed in a dish lined with filter paper flooded with 5 ml of different concentrations. Then were incubated at 25-28 °C and allowed to germinate for

seven days. The number of seeds was counted and the length of coleoptiles was measured using a vernier caliper.

Leaf Sheath Elongation. For the elongation of the second leaf sheath, rice seeds were allowed to germinate in a dish lined with filter paper flooded with distilled water. After 7 to 10 days of incubation, seeds with 1mm coleoptiles was transferred to a filter paper immersed in 9ml of different treatment and control solutions. The seeds were allowed to grow under ordinary daylight conditions at 25-28 °C. In each treatment 0.5 ml of distilled water was added every 24 hours. The second leaf sheath was measured using a vernier caliper after 7 days of incubation.

Growth of Coleoptiles. Rice seeds were soaked in water for two to three hours. Seeds were placed in a microwavable disk with filter paper (Whatman No.1). Then incubated 35 cm below white fluorescent light for 3 to 4 hours. Then the seeds were incubated in a dark room maintained at room temperature for three to four days. Under green light, 9 mm sections of the coleoptiles (about 3 mm from the tip) was cut and was placed in a 1.5 ml test solutions made up of sucrose buffer and the test substance (under green light). Then, the coleoptiles were allowed to grow for 20 hours in the dark at 25 °C. Coleoptiles were measured using a vernier caliper.

Root Initiation. For root initiation, rice seeds were soaked in distilled water for 2 hours. Then placed in a microwavable disk with filter paper (Whatman No. 1) wet with distilled water. Then allowed to germinate for 3 to 5 days. Roots were cut 1mm from the stem and placed in a test tube with 9 ml of test and control solutions. The number of roots initiated and the length of roots was measured using a vernier caliper after five days of incubation.

Statistical Analysis. The study was laid out using Completely Randomized Design (CRD). Data were analyzed using Analysis of Variance (ANOVA) and Comparison Among Means by Duncan's Multiple Range Test (DMRT). All tests of significance was set at 5%.

Results

This study was conducted to determine the auxin and gibberellic acid like of seaweed liquid extract of *C. racemosa* on rice seed germination. Evaluation was made through by measuring germination rate, length/growth of coleoptile, root length/initiation and the second leaf sheath of rice.

The germination rate, coleoptile length and leaf blade length of rice as influenced by exposure to different concentrations of seaweed liquid extract are shown in Table 1. Germination rate at 10% seaweed concentration was comparable to the commercial GA and was significantly higher than water. The

10% SLE concentration recorded 6.5 % increase in seed germination over control. The highest coleoptile length (12.89 mm/seedling), leaf blade length (36.79 mm/seedling) were recorded in the 10% SLE of *C. racemosa* treatment. While as the concentration of the seaweed extract increases, the germination rate, coleoptile length and leaf blade length decreases.

Table 1. Mean of number of seed germination, length of coleoptile and leaf blade of rice seed

Concentration	Germination (%)	Coleoptile length (mm/seedling)	Leaf blade length (mm/seedling)
10%	96.67 ^a	12.89 ^a	36.79 ^b
25%	95 ^{ab}	11.15 ^b	26.43 ^c
50%	65.33 ^c	7.92 ^c	16.14 ^d
75%	21.67 ^d	5.51 ^d	13.39 ^e
Control (GA)	97.67 ^a	13.45 ^a	54.39 ^a
Distilled water	90.17 ^b	7.32 ^c	36.01 ^b

*Means in a column that have different letters are significantly different

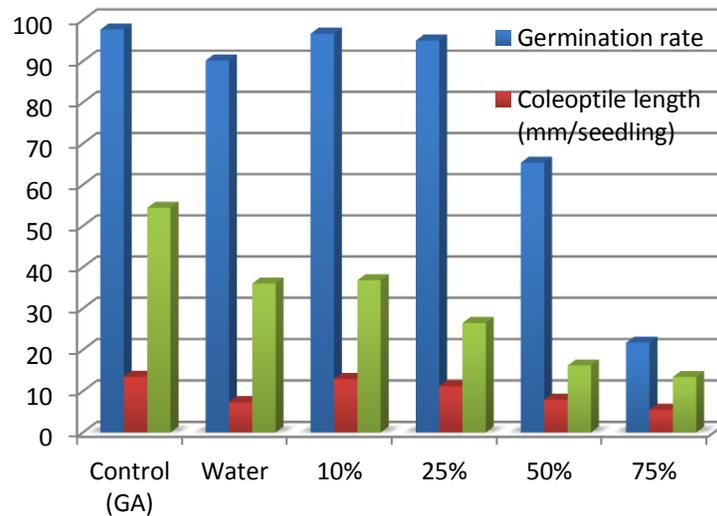
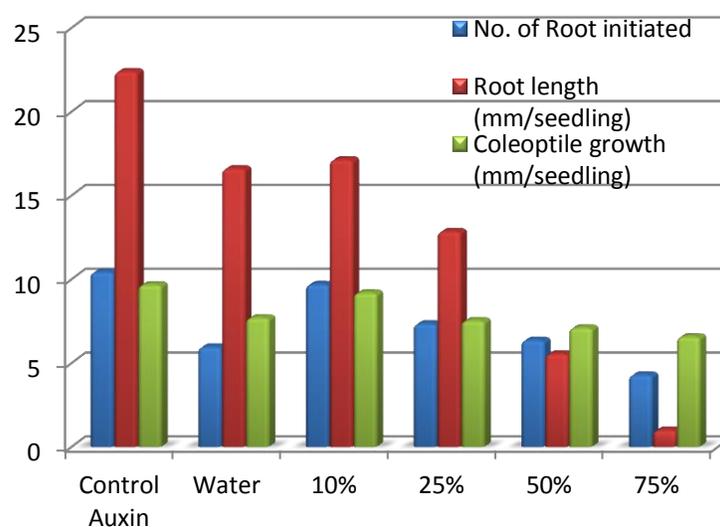


Figure 1. Mean of number of seed germination, length of coleoptile and leaf blade of rice seed

Table 2. Mean number on root initiated, root length and coleoptile growth of rice seed

Concentration	No. of Root initiated	Root length (mm/seedling)	Coleoptile growth (mm/seedling)
10%	9.69 ^a	17.13 ^b	9.19 ^a
25%	7.34 ^b	12.85 ^c	7.53 ^b
50%	6.36 ^b	5.55 ^d	7.10 ^{bc}
75%	4.29 ^c	1.00 ^e	6.57 ^c
Control (Auxin)	10.43 ^a	22.39 ^a	9.67 ^a
Distilled water	5.96 ^{bc}	16.60 ^b	7.70 ^b

*Means that have different letters are significantly different

**Figure 2.** Mean number on root initiated, root length and coleoptile growth of rice seed

Root initiation shows increased in 10% concentration 9.69 is comparable to control 10.43 while, highest root length 17.13 mm/seedling and coleoptile length is 9.19 mm/seedling. In the present investigation, the plants treated with lower concentration of seaweed liquid fertilizer (SLF) increased the vegetative growth. Erulan *et al.*, (2009) reported that SLF at low concentrations enhanced the growth parameters such as shoot length, root

length, leaf area, fresh weight, dry weight and moisture content. Biochemical parameters like chlorophyll 'a' and 'b', protein, sugars, starch.

Occurrence of growth regulators and their effect alter the physiological attributes (Temple and Bomke, 1989) and presence of growth promoting substances including auxins, gibberellins in seaweed (Zhag *et al.*, 1997) have been reported. (Zhag *et al.*, 1997) speculate higher concentration plant showed decreasing trend due to high solute affect osmolarity that inhibits growths.

Dicussions

Liquid extracts of marine algae are used in agriculture and horticulture. There is an extensive remunerations that have been circulated about these extracts. It includes increased in crop yields, flower set and fruit production and many more. Thus, this study was conducted.

A study on the evaluation of auxin and gibberellic acid like activity was undertaken. The germination rate, coleoptile and leaf blade length, growth of coleoptiles, the length of roots and the number of roots initiated were measured. At germination rate 10% seaweed concentration is comparable to the commercial GA and significantly higher than water. The 10% SLE concentration of recorded 6.5 % increase in seed germination over control. In a similar trend the application of lower concentration of SLE has increased the α -amylase and β -amylase activities of seeds of *Vigna mungo* at lower concentration of *C. scalpelliformis* (25%). The similar trend was recorded in *Vigna sinensis* (Sivasankari *et al.*, 2006c). The increase in α and β amylase activities at lower concentration may be due to the presence of growth promoting substance like gibberellins.

The highest coleoptile length (12.89 mm/seedling), leaf blade length (36.79 mm/seedling) were recorded in the 10% SLE of *C. racemosa* treatment and the higher concentrations showed decreasing trend. Root initiation shows increased in 10% concentration 9.69 is comparable to control 10.43 while, highest root length 17.13 mm/seedling and coleoptile length is 9.19 mm/seedling.

The increased growth parameters at lower concentration may be due to the presence of higher levels of N, P, K, high amount of Mg. in the seaweed extract of *C. racemosa*. Sivakumar and Gandhi (1973) reported similar effect of SLF prepared from *Sargassum wightii* on *Vigna*. Similar observations were also reported in earlier studies of (J Paul *et al.*, 1997), on protein s, total sugars and amino acids.

Auxins stimulate the occurrence of adventitious roots in the majority of plant species, if they have at least some natural capacity to root. Root

promoting properties of auxins provide for the fact that they are commonly used in vegetative propagation.

Conclusion

This study is conducted to determine the auxin and gibberellic acid like activity of seaweed liquid extract of *Caulerpa racemosa* on rice seed germination. Based on the results of the study, the following are being concluded:

Germination percentage, 10 and 25% concentration increased in germination rate which is comparable to the commercial gibberellic acid, thus a potential gibberellic acid like activity. Length of coleoptile, at 10% had increased the length of coleoptiles which is comparable to the commercial gibberellic acid and 25% is significantly higher than water, while, No. of roots initiated, only at 10% concentration exhibited auxin like activity through increasing the number of roots among all the concentrations used, only 10% concentration exhibited auxin like activity through coleoptiles elongation.

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