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SHORT COMMUNICATION in PHYSIOLOGY

Effects of *Chelidonium majus* and *Ascophyllum nodosum* Extracts on Growth and Photosynthesis of Soybean

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Abstract

Herbal extracts used in agriculture are formulated from plants (or other organisms) as alternatives to synthetic chemicals that could have adverse effects on growers, consumers, or the environment. In this study, the effects of two herbal extracts on soybean were assessed: *Chelidonium majus* (C7: Celext 07) and *Ascophyllum nodosum* (ST: Stimulagro). A standardized approach for germinating seeds and monitoring early seedling growth for 15 days (SOP-Soybean) was used to evaluate the effects of these extracts. Growth characteristics, chlorophyll content, and photosynthetic rate were measured on the fifteenth day after sowing (DAS). A combination of *C. majus* and *A. nodosum* (C7+ST), both 1 g L^{-1}, was the most beneficial treatment and significantly increased shoot height (13.2%), dry mass (10.7%), and photosynthetic rate (20.3%). In a separate experiment, foliar application of the same compounds was performed on the tenth and twentieth DAS, with sampling on the thirtieth DAS. Foliar applications with 1 g L^{-1} of *A. nodosum* (ST) significantly enhanced the dry mass (23.5%), and the photosynthetic rate was increased even at 10 days after application (22.5%). Therefore, seedling exposure to *C. majus* (C7) and *A. nodosum* (ST) and foliar applications of *A. nodosum* (ST) stimulated the growth and development of soybean. These natural compounds seem to have the potential to act as growth stimulants for soybean and should be tested for their capacity to improve field growth and yield.

Keywords
biomass; chlorophyll; photosynthetic rate; *Glycine max*; seaweed; celandine

1. Introduction

Soybean, owing to its dietary, industrial, medicinal, and economic importance, has become a major crop in Canada, with a seeded area of 2.61 million ha (Soy Canada, 2019). To maximize the production of soybean, various chemical fertilizers are being used. However, they pose some health and environmental risks; contaminants may be present and excess fertilizers may enter the water table (Loan et al., 2018). The environmental risks may involve degradation of soil quality and agricultural nonpoint pollution from overuse of chemical fertilizers (Liu & Diamond, 2005; Yin et al., 2018). To minimize the retention of harmful chemical residues in the soil, producers are nowadays using naturally occurring and, hence, biodegradable options in agriculture to increase crop yield (Savci, 2012; Siddiqui et al., 2011). In this study, we have presented the use of two extracts containing natural compounds, with potential for the growth and development of soybean.

Chelidonium majus, known as celandine, is a well-known medicinal herb and widely used against various diseases (Aljuraisy et al., 2012; Ciric et al., 2008; Monavari et al., 2012). Extracts from its leaves, flowers, and roots can be used as a tonic to stimulate the production of bile and pancreatic digestive enzymes (Papuc et al., 2012). The extracts have been shown to be safe and effective for veterinary and human use (Cho et al., 2006).

Seaweeds (e.g., *Ascophyllum nodosum*) are widely used in both agriculture and horticulture (Crouch & van Staden, 1994; Elansary et al., 2016; Karthikeyan & Shanmugam, 2016; Wang et al., 2016). Previous studies have reported the use of seaweeds as biostimulants to improve the growth, yield, and quality of maize and rice in India (Layek et al., 2018, 2019). Some studies have also reported the positive effects of seaweed extracts on soybean (Martynenko et al., 2016; Rathore et al., 2009). In the present study, we examined the effects of Celext 07 and Stimulagro on the growth and physiology of soybean.

2. Material and Methods

2.1. Plant Material and Herbal Extracts

Soybean seeds of the cultivar OAC Champion were used.

Herbal products: (***i***) C7: Celext 07 (root extract of *C. majus*) and (***ii***) ST: Stimulagro 0-0-6 (liquid extract of *A. nodosum*) were used for the experiment. Aqueous dilutions of the two extracts for the treatments (seed and foliar) included (g L^{-1}) 0.5, 1, and 2 of C7, ST, and C7:ST (1:1). Distilled water was used for preparing the dilutions, and a water control was used.

2.2. Seed Treatment

Standard operating procedure (SOP): The rolled towel test by International Seed Testing Association (1985) was used with some modifications (Figure 1). The first modification involved soaking the seeds overnight in distilled water and using dry prefolded paper. The test was performed on a 60 cm × 20-cm brown paper towel cut from a roll and folded lengthwise (60 cm × 10 cm). The seeds were placed in a single row, 1 cm from the top and 5 cm apart, and held in position by folding the paper again lengthwise to cover the seeds. The paper rectangle containing the seeds was rolled along the short axis and secured with scotch tape. The roll was placed vertically, with the seeds toward the top, inside a 1-L plastic container containing 40 mL of the treatment solution. The containers were maintained in a growth chamber (Conviron environmental chamber; Winnipeg, Canada) at 25 ± 2 °C temperature, 400 µmol m^{-2} s^{-1} photosynthetic flux density, and 50% relative humidity, and 16:8 hr light:dark cycle. The paper towels were not allowed to dry out. Every morning, the residual solution was discarded, and 40 mL of fresh treatment solution was added.

2.3. Foliar Application

The soybean seeds were planted in 12-cm pots containing AgroMix G10 (Fafard, Canada) in a growth chamber (Conviron environmental chamber) at 25 ± 3 °C, 400 µmol m^{-2} s^{-1} photosynthetic photon flux density, 50% humidity, and 18:6 hr dark:light cycle. Foliar application with 0.5 g L^{-1}, 1 g L^{-1}, and 2 g L^{-1} of C7, ST, and C7:ST (1:1) treatments were performed twice: on tenth and twentieth day.

Data collection for the seed treatment experiment was performed on the fifteenth DAS (day after sowing), whereas that for the foliar application experiment was performed on the thirtieth DAS. In each case, five randomly selected plants per replicate were measured, with five replicates per treatment.

The photosynthetic rate was measured using a portable infra-red gas analyzer (IRGA-LI-6400; LI-COR; Lincoln, U.S.), and chlorophyll content of soybean leaves (fully expanded unifoliate leaf for seed treatments and fully expanded third trifoliate leaf for foliar applications) was estimated using a SPAD 502 meter (Konica Minolta Optics, U.S.) under the growth chamber conditions.

Plant height was measured from the basal node to the shoot apex by using a ruler. Fresh shoot mass and dry shoot mass were measured with an analytical balance. The shoots were harvested for fresh mass and then dried at 60 °C for 72 hr for dry mass.

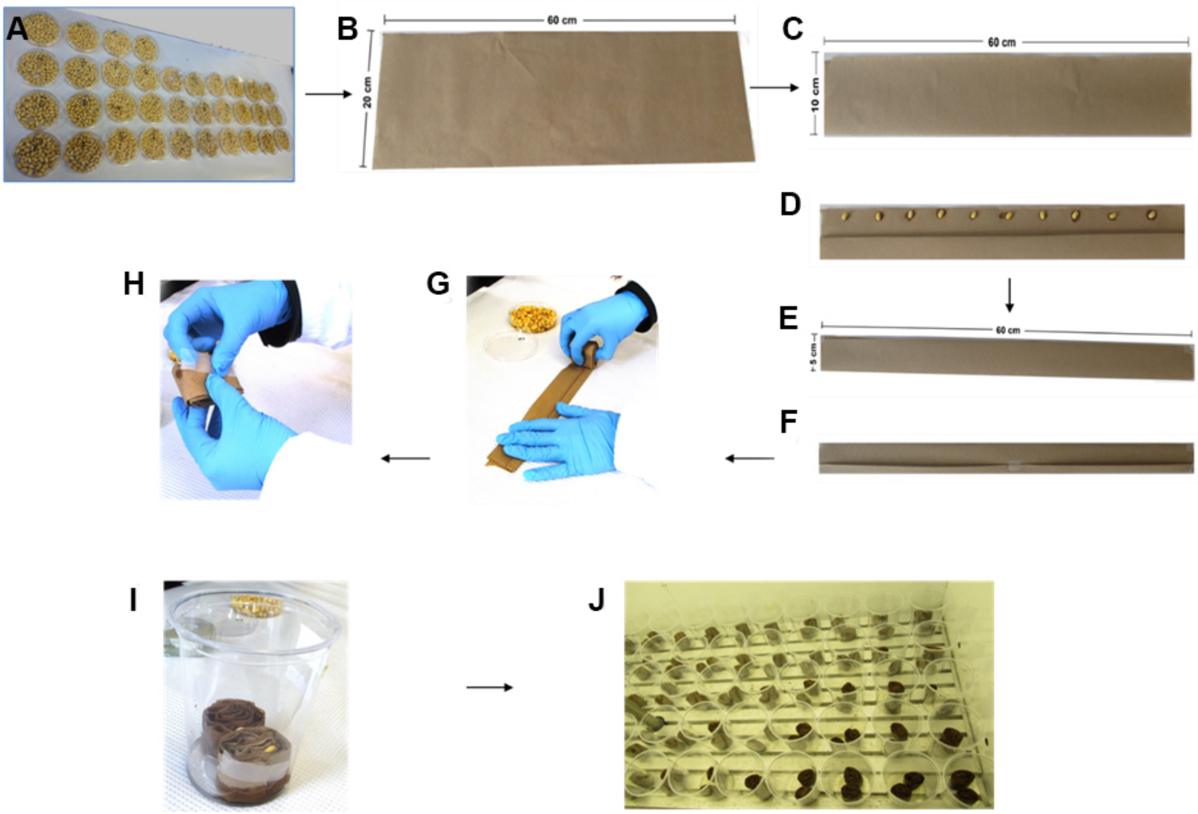

Figure 1 Schematic representation of SOP-Soybean.

2.4. Statistical Analysis

Analysis of variance was performed, followed by the post hoc Newman–Keuls multiple comparison test (GraphPad Prism 5.01).

3. Results

3.1. Seed Treatment

The treatment with C7 and ST had a positive effect on the growth and physiological parameters of soybean. The combined treatment of 1 g L^{-1} C7+ST significantly increased plant height by 13.2%. A significant increase in dry mass (by 16.1%) was observed. Other treatments (1 g L^{-1} C7 and 0.5 g L^{-1} ST) increased the dry mass to a significant (12.9%) but lesser extent. However, the photosynthetic rate was significantly enhanced by only the combined treatment of 1 g L^{-1} C7+ST (by 20.4%; Table 1).

3.2. Foliar Application

The foliar application with C7 and ST also had a positive effect on the growth characteristics of soybean. The dry mass significantly increased by 23.5% after the 1 g L^{-1} ST treatment and 18.4% after the 1 g L^{-1} C7+ST treatment. The chlorophyll content significantly increased by 20.3% and 18.2% after foliar application of 1 g L^{-1} ST and 2 g L^{-1} ST, respectively. The photosynthetic rate was enhanced by 22.5% after the 1 g L^{-1} ST treatment (Table 2). An increase in the dry mass was observed in the treatments in which an increase in the chlorophyll content and photosynthetic rate was found.

4. Discussion

The results indicate the positive effects of *C. majus* and *A. nodosum* on dry matter accumulation and photosynthesis in soybean. Seed treatment and foliar application

Table 1 Effect of Celext 07 (C7) and Stimulagro (ST) on soybean at fifteenth day after sowing using SOP-Soybean.

Treatment	Shoot height (cm)		Fresh mass (g)		Dry mass (g)		Chlorophyll content (SPAD units)		Photosynthetic rate (μmol m^{-2} s^{-1} CO_2)	
Control	22.7		1.02		0.31		33.4		7.31	
0.5 g L^{-1} C7	23.1	(1.8%)	1.08	(5.9%)	0.31	(0.0%)	33.6	(0.6%)	7.58	(3.7%)
1 g L^{-1} C7	24.5	(7.9%)	1.12	(9.8%)	0.35*	(12.9%)	33.5	(0.3%)	7.92	(8.3%)
2 g L^{-1} C7	24.3	(7.0%)	1.11	(8.8%)	0.32	(3.2%)	33.6	(0.6%)	7.31	(0.0%)
0.5 g L^{-1} ST	25.3	(11.5%)	1.13	(10.8%)	0.35*	(12.9%)	34.2	(2.4%)	7.89	(7.9%)
1 g L^{-1} ST	24.2	(6.6%)	1.12	(9.8%)	0.32	(3.2%)	34.6	(3.6%)	7.82	(7.0%)
2 g L^{-1} ST	24.1	(6.2%)	1.10	(7.8%)	0.32	(3.2%)	34.2	(2.4%)	7.60	(4.0%)
0.5 g L^{-1} C7+ST	24.2	(6.6%)	1.10	(7.8%)	0.33	(6.5%)	34.6	(3.6%)	8.27	(13.1%)
1 g L^{-1} C7+ST	25.7*	(13.2%)	1.13	(10.8%)	0.36*	(16.1%)	35.1	(5.1%)	8.80*	(20.4%)
2 g L^{-1} C7+ST	24.9	(9.7%)	1.08	(5.9%)	0.32	(3.2%)	35.2	(5.4%)	8.68	(18.7%)

* Significance at $p < 0.05$ as compared to control. Values in parenthesis show percent increase as compared to control.

Table 2 Effect of foliar application of Celext 07 (C7) and Stimulagro (ST) as compared to control on soybean at thirtieth day after sowing.

Treatment	Shoot height (cm)		Fresh mass (g)		Dry mass (g)		Chlorophyll content (SPAD units)		Photosynthetic rate (μmol m^{-2} s^{-1} CO_2)	
Control	41.8		6.3		3.91		38.74		12.4	
0.5 g L^{-1} C7	41.9	(0.24%)	6.4	(1.59%)	4.11	(5.12%)	41.93	(8.35%)	12.6	(1.61%)
1 g L^{-1} C7	42.6	(1.91%)	6.5	(3.17%)	4.2	(7.42%)	41.94	(8.38%)	12.9	(4.03%)
2 g L^{-1} C7	42.8	(2.39%)	6.45	(2.38%)	4.3	(9.97%)	42.22	(9.11%)	12.6	(1.61%)
0.5 g L^{-1} ST	45.3	(8.37%)	6.68	(6.03%)	4.39	(12.28%)	43.02	(11.16%)	13.6	(9.68%)
1 g L^{-1} ST	47.1	(12.68%)	7.09	(12.54%)	4.83*	(23.53%)	46.55*	(20.28%)	15.2*	(22.58%)
2 g L^{-1} ST	45.6	(9.09%)	6.9	(9.52%)	4.41	(12.79%)	45.74*	(18.20%)	13.9	(12.10%)
0.5 g L^{-1} C7+ST	44.9	(7.42%)	6.51	(3.33%)	4.3	(9.97%)	43.63	(12.75%)	13.5	(8.87%)
1 g L^{-1} C7+ST	46.2	(10.53%)	6.84	(8.57%)	4.63*	(18.41%)	43.47	(12.33%)	14.1	(13.71%)
2 g L^{-1} C7+ST	45.4	(8.61%)	6.63	(5.24%)	4.4	(12.53%)	43.13	(11.46%)	13.8	(11.29%)

* Significance at $p < 0.05$ as compared to control. Values in parenthesis show percent increaseas compared to control.

of both extracts increased the dry mass of soybean at relatively low concentrations over a short assessment period. To the best of our knowledge, this is the first study in which a combination of *C. majus* and *A. nodosum* was used on soybean. The response of the soybean plants to the seaweed extract was consistent with that observed in previous studies (Martynenko et al., 2016; Rathore et al., 2009). Our results showed that foliar application of 1 g L^{-1} ST and 2 g L^{-1} ST enhanced the chlorophyll content significantly. Enhancement of leaf chlorophyll content by seaweed has also been reported in grapevine (Sivasankari et al., 2006) and strawberry (Spinelli et al., 2010).

Previous studies with seaweed extract have reported enhanced photosynthetic rate as well as leaf chlorophyll content (Kumari et al., 2011). Our study also showed maximum enhancement of the photosynthetic rate (22.5%) with 1 g L^{-1} ST as well as increased chlorophyll content (20.28%) after foliar application. In the seed treatment experiment, the photosynthetic rate was increased to the maximum extent (by 20.3%) with 1 g L^{-1} C7+ST combination. This increase in photosynthesis explains the increased biomass accumulation, indicating improved carbon fixation at an early stage of growth in both experiments. Therefore, these treatments should be investigated for potentially beneficial effects on enhancing the later growth and yield of soybean. The first part of the study shows the synergistic effects of both extracts on plant growth at the earliest stage, whereas the second part of the study shows the stimulatory effects of the seaweed extract on the growth of older plants.

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