

Article

Impact of Freshwater Macroalga (*Cladophora glomerata*) Extract on the Yield and Morphological Responses of *Glycine max* (L.) Merr.

Sylvia Lewandowska ^{1,*}, Krzysztof Marczewski ¹, Marcin Kozak ² , Naoko Ohkama-Ohtsu ³ ,
Magdalena Łabowska ⁴ , Jerzy Detyna ⁴  and Izabela Michalak ⁵ 

¹ Department of Genetics, Plant Breeding and Seed Production, Faculty of Life Sciences and Technology, Wrocław University of Environmental and Life Sciences, Plac Grunwaldzki 24A, 50-363 Wrocław, Poland; krzysztof.marczewski@upwr.edu.pl

² Institute of Agroecology and Plant Production, Wrocław University of Environmental and Life Sciences, Plac Grunwaldzki 24A, 50-363 Wrocław, Poland; marcin.kozak@upwr.edu.pl

³ Institute of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu-shi, Tokyo 183-8509, Japan; nohtsu@cc.tuat.ac.jp

⁴ Department of Mechanics, Materials and Biomedical Engineering, Faculty of Mechanical Engineering, Wrocław University of Science and Technology, Smoluchowskiego 25, 50-372 Wrocław, Poland; magdalena.labowska@pwr.edu.pl (M.Ł.); jerzy.detyna@pwr.edu.pl (J.D.)

⁵ Department of Advanced Material Technologies, Faculty of Chemistry, Wrocław University of Science and Technology, Smoluchowskiego 25, 50-372 Wrocław, Poland; izabela.michalak@pwr.edu.pl

* Correspondence: sylvia.lewandowska@upwr.edu.pl



Citation: Lewandowska, S.; Marczewski, K.; Kozak, M.; Ohkama-Ohtsu, N.; Łabowska, M.; Detyna, J.; Michalak, I. Impact of Freshwater Macroalga (*Cladophora glomerata*) Extract on the Yield and Morphological Responses of *Glycine max* (L.) Merr. *Agriculture* **2022**, *12*, 685. <https://doi.org/10.3390/agriculture12050685>

Academic Editor: Cinzia Margherita Berteà

Received: 30 March 2022

Accepted: 6 May 2022

Published: 11 May 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: The objective of this study was to investigate the response of Japanese and Polish soybean varieties to algal extract in terms of yield and other agronomic traits. A field experiment was conducted in 2019 at Pawlowice Experimental Station near Wrocław city (Poland) to study the effect of a foliar spray of the algal extract prepared from freshwater *Cladophora glomerata* on the growth of two varieties of soybean (*Glycine max* (L.) Merrill.)—Polish Erica and Japanese Enrei—cultivated under Polish environmental conditions. The foliar spray was applied once during the beginning of the soybean flowering (BBCH65) at one concentration (20%) of algal extract. The research showed that the foliar application of *C. glomerata* extract significantly enhanced the yield parameters. The plant height, first pod height, number of first branches, 1000-seed weight and yield were significantly higher compared with the control. The home variety Erica was better adapted to the climatic conditions in Poland than the Japanese variety Enrei. The study confirms that a foliar spray of algal extracts could be a promising option to increase soybean yield. The enhanced growth of the crop may be due to the presence of growth-promoting substances occurring in the algal extract.

Keywords: soybean; cultivars; yield; *Cladophora glomerata* extract; biostimulation

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is the most important legume, and is the fourth crop next to rice, wheat and maize in terms of the world crop production. The rapid increase in soybean acreage in the late 20th and early 21st centuries is due to the need for fodder protein. In 1961, the global soybean acreage was less than 24 million hectares, and in 2018 it was 126 million hectares, with production rising from 27 to more than 320 million tonnes [1]. It is a globally important crop providing fixed nitrogen (N) and high-quality protein and oil [2–5]. Soybean seeds are desirable because they contain, in g·kg^{−1}: total protein from 330 to 450, crude fat from 180 to 240, crude fibre from 55 to 80, lecithin from 15 to 25, and additionally isoflavones from 427 to 2743 µg·g^{−1}, which are biologically active compounds. It should be noted that the biological value of soy protein is similar to that of chicken egg.

It is used in human nutrition and as a feed for livestock. Soybean meal is of particular importance in animal nutrition, and the demand for it is steadily increasing.

The domination of soybean cultivation by three countries (the USA, Brazil, Argentina) means that the diversification of plant protein sources, including soybean meal, is the main purpose for Polish agriculture. The improvement of national plant protein sources, their production, their marketing and their use in feed have been one of the programmes in Polish agriculture. The main idea of the initiative was to develop conditions to minimize imports of feed protein (mainly post-extraction soybean meal) by about 50%, thus increasing the biological and functional value of vegetable protein from domestic raw materials. At the same time, in the event of a threat to the food security of the modern world, as a result of a sudden cataclysmic event or as a consequence of a global protein crisis [6], it is reasonable to create conditions to ensure national protein security [7] through an abundance of our own seed, new varieties and cultivation technologies, optimal feed recipes, and the animal feeding system, as well as the seed production and marketing systems.

In Europe, soybean cultivation is gaining popularity due to the possibility of the versatile use of seeds not only in the feed industry but also for food [8–13], chemical [14], or pharmaceutical applications [15]. Soybean is also an important source of traditional staple foods such as tofu, natto, miso, and soy sauce in Japan. The unique cuisine, and geographical and historical isolation of Japan probably shaped the distinct agro-morphological characteristics of Japanese soybean differently from those of continental soybean. Because Enrei is a major cultivar (9% of the total soybean cultivation area in Japan in 2014) with seeds of high quality for food processing, a previous study sequenced the whole genome of Enrei as a representative Japanese cultivar [16].

In 2021, the area under soybean cultivation in Poland was 23,000 ha. The production and experimental results obtained in southern Poland in recent years indicate that soybean is currently at the same level of production as maize was in the mid-1960s. The main limiting factor is the low progress of breeding. According to an American study, the success of soybean growing depends on a combination of the weather conditions, soil type, and genotype [17]. Finding genotypes that tolerate unfavourable weather conditions well is essential for further progress in breeding work [18,19]. Breeding should be directed at increasing the height and quality of seed yield, the height of the first pod setting, the resistance of the plants to lodging, pod dehiscence, and seed shedding under drought conditions. A reduction in genetic diversity may result in increased susceptibility to newly emerging diseases or pests, and may result in the reduction of yields [20,21]. Soybean is characterized by a wide range of variability based on morphological and physiological traits, which are modified by environmental conditions [22]. The direct introduction of soybean genotypes from other countries into cultivation in Poland is risky [23] because the varieties transferred to Polish climatic conditions change the course of their developmental stages [24], especially those related to flowering and pod setting. It is worth emphasizing that biological progress in new soybean genotypes includes: (1) a reduction in the plant response to photoperiodism, (2) an increase in vigour, which results in a higher number of seeds obtained per 1 m², (3) a longer stay-green period, which is positively correlated with an increase in dry seed weight, and (4) a higher resistance to pathogens.

In the USA, soybean cultivation is concentrated between 43° and 45° northern latitude, while in Poland from 49° to 54° northern latitude, where climatic conditions are not favourable for the cultivation of this species. In contrast, in Japan soybean is grown from 30° to 45°, and various suitable cultivars have been developed for wide regions. Undoubtedly, the limiting factors of soybean growing are the temperature requirements and its response to the day length. Most soybean varieties are short-day plants, and are generally the best yielding genotypes. In varieties intended for cultivation in higher latitudes, it has been shown that a day length of more than 16 h at the beginning of flowering delays the next developmental stages, and the plants may not mature before the autumn frosts. However, photoperiodically tolerant forms are also known, which start flowering with a day length of up to 24 h [25–28]. With a long day and a high temperature,

most soybean varieties react like plants at a low temperature, and with a low temperature and a short day most of them behave like long-day plants [29–31]. It has been shown that the photoperiod and temperature interact with the genotype to control soybean growth and development throughout the whole growing season [18,32–34].

In order to increase the soybean yield and improve its agronomic traits, the application of algal extracts was proposed. These extracts are known as biostimulants of plant growth, the function of which is to enhance nutrition efficiency, increase the resistance to biotic and abiotic stress, and improve crop quality traits [29,31,35–40]. As a raw material for the production of the algal extract, the easily available freshwater macroalga *Cladophora glomerata* was selected. This alga is known to be rich mainly in carbohydrates, but also contains proteins, micro- and macroelements, vitamins, phytohormones, and polyphenols, etc., which can stimulate plant growth and development [41]. So far, little is known about the agricultural use of freshwater macroalgae. Most of the research is carried out on seaweed extracts used as plant growth biostimulants.

The aim of the field experiment conducted in 2019 in Poland was to assess the response of two soybean varieties of different origins—Japanese (cv. Enrei) and Polish (cv. Erica)—to the foliar application of the algal extract. Soybean is often called the miracle crop; it is cultivated worldwide due to its high protein content providing all of the essential amino acids. What is more, the world population is constantly increasing, and a sufficient supply of food is a fundamental issue for the world. The research hypothesis assumed that the foliar spray of the algal extract would significantly improve the soybean yield parameters. It was therefore desirable to determine the influence of the algal extract used as a plant growth biostimulant on the length of the vegetative and generative development of soybean varieties, the formation of its morphological traits, and the seed yield.

2. Materials and Methods

2.1. Soybean Varieties

The choice of the cultivar to grow is one of the key decisions affecting the yields to be harvested. This particularly applies to soybean, a relatively new species for the farmer, which has not been cultivated in Poland on a larger scale. Two globally popular soybean cultivars were selected for this study, differing by origin and maturity group, in order to test their reaction to various soil and weather conditions, as well as to the foliar application of the algal extract. These varieties were characterized by a high variability of flowering-related traits, as noted during the plant observations. The soybean genotypes studied in the experiment came from different regions of the world. According to the American classification, the Japanese cultivar Enrei belongs to maturity group IV: relatively late, harvested in Japanese temperature conditions, usually in October. It has a very high protein content (43%) and a high fat content (19%). Enrei has yellow seeds with a pale mark and relatively high weight (32 g). This variety is characterized by moderate resistance to diseases, but a weakness to soybean cyst nematode. Additionally, it is suitable for making tofu. It is recommended for cultivation in Japan, especially in the middle west of the country. In contrast, according to the Polish classification, Erica is an early cultivar, harvested at the end of August or the beginning of September, and uniformly ripening. This variety is characterized by a high protein content (38–40%) and a high fat content (20%). The seed yield is clearly above average for varieties of similar maturity. Erica has yellow seeds with a pale mark and high resistance to seed dropping. This variety possesses good resistance to diseases, especially to *Sclerotinia* stem rot. Erica is recommended for cultivation in Poland, especially in the northern part of country. The seeds tested in the present study came from a harvest in 2018, and were certified. The seed material for Erica was obtained from DANKO (a Polish plant breeding station), while Enrei was provided by the Tokyo University of Agriculture and Technology (Japan).

2.2. Production of the *Cladophora glomerata* Extract

The algal extract was produced from a freshwater macroalga—*Cladophora glomerata*—collected from the surface of the pond (Tomaszów, Poland, October 2016). After air-drying, the biomass was milled (Retsch GM300 grinding mill, Haan, Germany) and sieved to produce the fraction with a size lower than 400 µm. The algal extract was obtained by Ultrasound-Assisted Extraction. For this reason, an ultrasound homogenizer (UP 50H; Hielscher Ultrasonics, Teltow, Germany) was used, which worked under the following conditions: 50 W, ultrasonic frequency 30 kHz, amplitude 100, 30 min. The concentration of the biomass in the distilled water was 40 g/L. After extraction, the mixture was centrifuged at a speed rate of 4000 rpm for 10 min. The obtained supernatant was treated as 100% extract, which was later diluted with distilled water to obtain the 20% concentration. According to the previous study, this concentration best stimulated soybean growth (the germination percentage, chlorophyll and carotenoids content, plant length, and dried biomass weight) in germination tests among the tested concentrations: 20, 40, 60, 80 and 100% [36].

2.3. Field Experiment

The field study was conducted in 2019 at Pawlowice Experimental Station near Wrocław, belonging to the Institute of Agroecology and Plant Production of Wrocław University of Environmental and Life Sciences in Poland (51°10'35" N, 17°07'13" E). A two-factor field experiment with four replications was set up using the split-plot method. The factors tested were the following:

Algae extract (factor A)—main plot:

I—Control—H₂O (distillated water)

II—20% *Cladophora glomerata* extract used as a foliar spray

Cultivars (factor B)—subplot:

Enrei

Erica

In total, 60 seeds of good quality (good germination and purity) were sown on 1 m² at a depth of 3 cm, and the plot size was 15 m². The row spacing was 30 cm. Wheat was a previous crop. The soybean was sown at the end of April 2019 on soil belonging to the autogenic soils section, of the brown-earth order, the grey-brown podzolic type, subtype typical, formed from light clay on medium clay, included in the good wheat agricultural suitability complex, class III b. The soil had good physical and chemical properties, and thanks to the considerable thickness of the humus horizon (30–40 cm), deep ploughing could be carried out without fear of bringing up too much of the subsoil to the surface.

In soil, the content of available forms of phosphorus (P) and potassium (K) was determined in 2019 using the Egner–Riehm method, and the content of available magnesium (Mg) was determined using the Schachtschabel method. The nutrient content in the soil varied (d.m.): phosphorus as P₂O₅—medium to very high (63.9 mg·kg^{−1}); potassium as K₂O—medium (161 mg·kg^{−1}); and magnesium as MgO—medium to very high (90.0 mg·kg^{−1}). The soil pH (potentiometric method) measured in 1 M KCl was slightly acidic, at 5.9. At the beginning of flowering (10 July 2019), a foliar spray of macroalgal extract (20% concentration) was applied once in the field.

The soybean was harvested in the middle of September in 2019, and was dried out to 14% moisture. The chlorophyll content in the soybean seedlings was measured using a nondestructive method. For the measurements, a SPAD chlorophyll meter (Konica Minolta, Tokyo, Japan) was used, which provided a relative index of the chlorophyll content in the seedlings.

In 2019, the thermal conditions during the growing season were favorable for soybean development: during April and June–September, the temperatures were above the multi-year averages from 5.2 °C (June) to 0.8 °C (September), while in May they were lower than the multi-year average by 2 °C (Table 1). In the April–September period, the mean temperature was higher than the multi-year average for this period by 1.8 °C, and the total precipitation was lower by 47.3 mm. Only in May was the sum of the precipitation

higher than the multi-year average by 50% (25.5 mm). In the April–September period, the total precipitation was 47.3 mm lower in comparison to the multi-year averages. In the April–September period, the average temperature was higher than the multi-year average for this period by 1.8 °C, and the total precipitation was 85% of the multi-year average.

Table 1. Decade, monthly mean air temperatures (°C), and sums of rainfall (mm) in Pawlowice near Wroclaw (Poland).

Year		2019											
Decade	Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
		Temperature (°C)											
	I	0.7	1.8	6.7	10.5	9.5	20.9	17.7	20.2	16.3	10.1	7.8	2.4
	II	1.0	4.1	6.1	8.3	11.6	23.0	17.6	19.0	13.0	13.9	7.2	3.6
	III	−2.7	3.8	7.2	13.5	14.8	22.3	22.4	21.4	13.9	9.9	6.2	3.6
	Monthly mean	−0.9	3.2	6.7	10.8	12.1	22.1	19.3	20.3	14.4	11.2	7.1	3.2
	Multi-year means for 1981–2010	−0.8	0.3	3.8	8.9	14.4	17.1	19.3	18.3	13.6	9.1	3.9	0.2
		Rainfall (mm)											
	I	26.0	16.6	10.3	0.0	9.4	1.3	3.2	13.6	34.4	7.6	4.7	3.1
	II	26.3	2.7	8.4	5.2	20.6	4.2	1.5	38.6	3.9	3.2	28.2	1.6
	III	3.9	8.4	3.8	19.0	46.8	21.5	39.8	7.6	3.7	7.3	1.6	10.4
	Total sums	56.2	27.7	22.5	24.2	76.8	27.0	44.5	59.8	42.0	18.1	34.5	15.1
	Multi-year means for 1981–2010	31.9	26.7	31.7	30.5	51.3	59.5	78.9	61.7	45.3	32.3	36.6	37.4

2.4. Statistical Analysis

The results were statistically elaborated using Statistica ver. 13.0 (TIBCO Software Inc., Tulsa, OK, USA). The normality of the distribution of the experimental results was assessed using the Shapiro–Wilk test. The homogeneity of the variances was evaluated with the Brown–Forsythe test. On this basis, a statistical test was selected, which was used to investigate the significance of the differences between the tested groups. Differences between two groups for normal distribution and homogeneous variances were examined with the *t*-test, and those between more than two groups with one-way analysis of variance (ANOVA) were assessed using the Tukey test. For non-normal distribution, the Mann–Whitney U test or the Kruskal–Wallis test, respectively, was used. The results were considered significantly different at $p < 0.05$.

3. Results and Discussion

The analysis of the relationship between the yield and yield-related traits in plants allows us to describe how the yield is determined by these traits, and to provide knowledge about their quantitative role in yield formation. It can be used in plant yield structure studies, in order to facilitate the selection of high-yielding genotypes [42–44].

In the present study, we examined the reactions of two soybean varieties—Japanese (cv. Enrei) and Polish (cv. Erica)—grown in Polish climatic conditions to the application of the macroalgal extract. The obtained results are presented in Table 2. Generally, all of the studied parameters were improved in both varieties after the application of *Cladophora glomerata* extract. As shown in the previous study, the ultrasound-assisted extraction of this macroalga enabled the extraction of K, Na, Cu, P, S, and Mg, which can stimulate soybean growth. Additionally, this extract also contains polyphenols, which can increase the plant resistance to the abiotic stress [39]. Ashour et al. (2021) confirmed this hypothesis: hot pepper (*Capsicum annuum*) treated with seaweed extract rich in phytochemicals like phenolics,

flavonoids, ascorbic acid with good antioxidant activity presented higher amounts of these compounds in the biomass, which could have a positive effect on the immune response against biotic and abiotic stress [38].

Table 2. Comparison of the soybean traits in the control group and the algal extract-treated group.

Parameter	Enrei		Erica	
	Control	Extract	Control	Extract
Plant height (cm); Mean $\pm$ SD **	84.8 $\pm$ 7.1 ^a	89.5 $\pm$ 6.1 ^a	72.7 $\pm$ 5.8	75.2 $\pm$ 7.0
First pod height (cm); Mean $\pm$ SD **	13.1 $\pm$ 2.0	13.9 $\pm$ 1.8	10.0 $\pm$ 1.8	10.2 $\pm$ 1.8
Number of first branches; Median *	3	3	3	3
Chlorophyll (SPAD); Median *	15.3 ^a	35.2 ^a	13.1 ^a	28.2 ^a
1000 seed weight (g); Mean $\pm$ SD **	152 $\pm$ 1	155 $\pm$ 2	178 $\pm$ 1 ^a	180 $\pm$ 1 ^a
Yield (t/ha); Mean $\pm$ SD **	2.10 $\pm$ 0.08	2.22 $\pm$ 0.13	3.58 $\pm$ 0.11 ^a	3.89 $\pm$ 0.04 ^a

^a To determine the statistically significant differences (at $p < 0.05$) for the Enrei variety between the control group and the extract group, and for the Erica variety between the control group and the extract group (comparison of two groups). * For the comparison of the median between the two groups, the Mann–Whitney U test was used. ** For the comparison of the mean between the two groups, the *t*-test was used.

3.1. Effect of the Macroalgal Extracts on the Soybean Height

Table 2 presents the height of two varieties of soybean, Enrei and Erica, in the control and experimental groups (with the algal extract). The first statistical analysis was performed to compare two groups: the Enrei Control vs. the Extract, and then the Erica Control vs. the Extract. The Enrei height in the extract group was 5.5% higher than in the control group, and this difference was statistically significant. In the case of Erica, this difference was much smaller—only 3.4%—and was not statistically significant.

The second statistical analysis was performed in order to compare all four tested groups (ANOVA with the use of a Tukey test for normal distribution and homogenous variances, or a Kruskal–Wallis non-parametric test for non-normal distribution), and the result of this comparison is shown in Figure 1a. The lowest plant height was obtained for Erica in the control group, while the highest was obtained for Enrei treated with the algal extract. When comparing both varieties with extract treatment, the Enrei plants were higher (by 19.0%). For the soybean height, statistically significant differences ($p < 0.05$; Tukey test) were observed for the following groups: Enrei Extract and Enrei Control, Erica Control and Enrei Control, Erica Extract and Enrei Control, Erica Control and Enrei Extract, and Erica Extract and Enrei Extract.

Our findings coincide with those of earlier studies carried out on plants from the same family as soybean—*Cajanus cajan* (L.) Millsp. [30] and *Vigna sinensis* L. [31]—in which there was an increase in vegetative growth caused by the application of seaweed extract. The increased growth of these crops may be due to the presence of growth-promoting substances in the seaweed extract, such as phytohormones [30,31,37,40]. In addition, the growth-enhancing potential of seaweed extract could be attributed to the presence of macro- and micronutrients [31].

3.2. Effect of Macroalgal Extracts on the Height of the First Soybean Pod

There was no statistically significant effect of treating soybean with the extract on the height of the first pod. For the pair Enrei Control vs. Extract, the first pod height for the extract group was 6.1% higher than for the control group, and for the pair Erica Control vs. Extract it was 2.0%.

Figure 1b presents the comparison of all of the tested groups. The highest first pod height was measured for the Enrei Control, and the smallest was measured for the Erica Control. For the first pod height, statistically significant differences ($p < 0.05$; Tukey test) were observed for the following groups: Erica Control and Enrei Control, Erica Extract and

Enrei Control, Erica Control and Enrei Extract, and Erica Extract and Enrei Extract. The height of the first pod for Enrei Extract was 36.3% higher than for Erica Extract

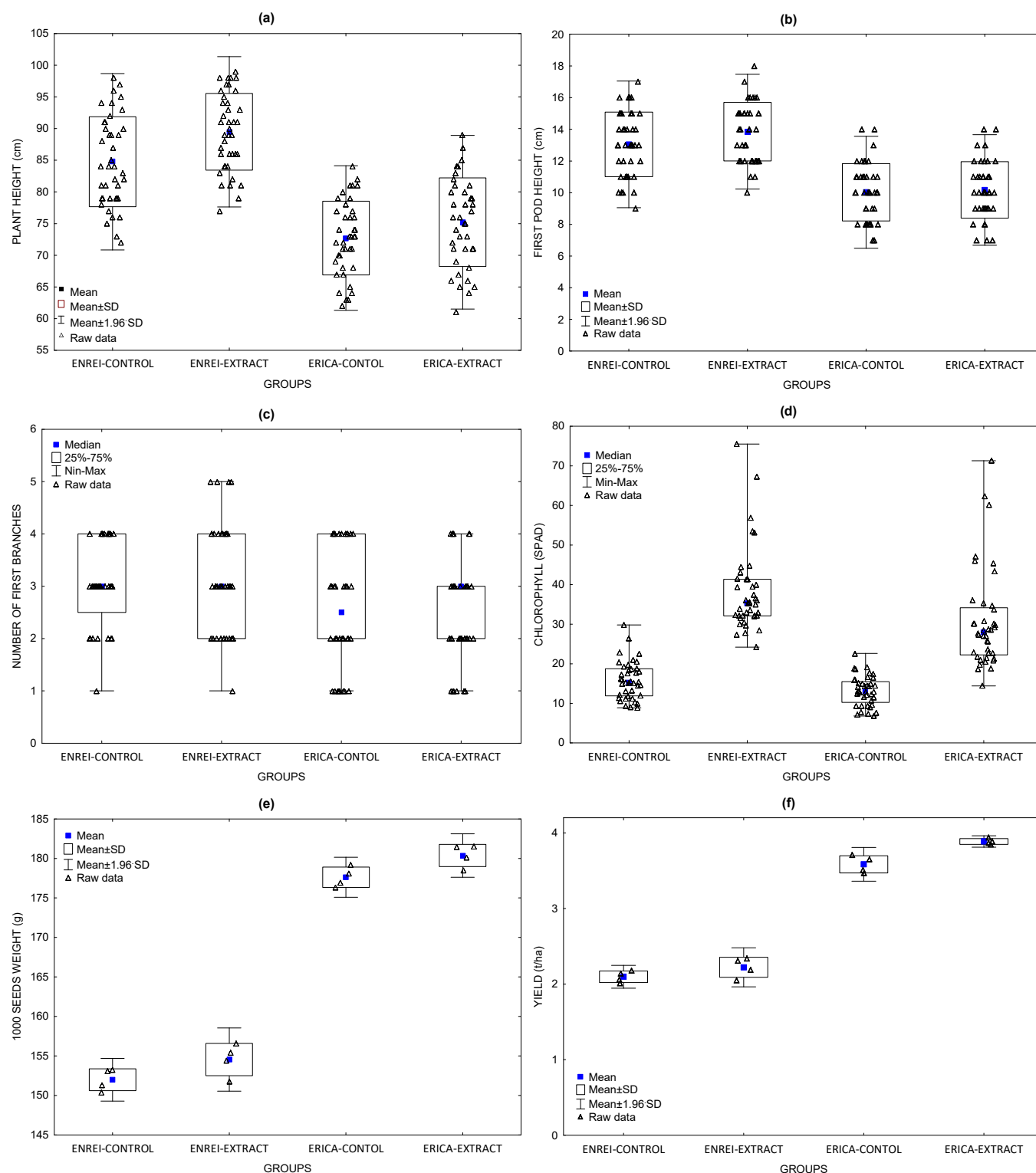


Figure 1. The effect of the algal extract on the (a) plant height (cm), (b) first pod height (cm), (c) number of first branches, (d) chlorophyll (SPAD), (e) 1000-seed weight (g), and (f) yield (t/ha) of two varieties of soybean (for data presented as the median, the Kruskal-Wallis non-parametric test was used to analyze the statistically significant differences; for data presented as the mean, the Tukey test was applied).

3.3. Effect of the Macroalgal Extracts on the Number of First Branches in Soybean

As seen in Table 2, the number of first branches was the same in all of the tested groups. For the number of first branches (Figure 1c), there were no statistically significant differences ($p < 0.05$; Kruskal–Wallis test) between the examined groups.

3.4. Effect of the Macroalgal Extracts on the Chlorophyll Content in the Soybean Seedlings

The application of algal extract significantly influenced the chlorophyll content (SPAD) in the seedlings of both varieties of soybean. In the pair Enrei Control vs. Extract and Erica Control vs. Extract, the chlorophyll content in the experimental group was more than twice that in the control group.

Figure 1d demonstrates the comparison of the chlorophyll content in all of the tested groups. For this parameter, statistically significant differences ($p < 0.05$; Kruskal–Wallis test) were observed for the following groups: Enrei Extract and Enrei Control, Erica Extract and Enrei Control, Erica Control and Enrei Extract, and Erica Extract and Erica Control. The highest chlorophyll content was measured in the Enrei Extract group, and the lowest was measured in Erica Control. When comparing both varieties treated with the extract, a higher chlorophyll content was found in the Enrei variety; it was 24.8% higher than that in Erica.

Numerous studies have also found that using seaweed extract increases the amount of chlorophyll in the leaves [29,36,38,39,41]. These results coincide with our previous work, in which 20% *Cladophora glomerata* extract increased the content of chlorophyll (SPAD measurements) in soybean seedlings cultivated in germination tests; it was higher by 9.2% than in the control group treated with distilled water [36].

3.5. Effect of Macroalgal Extracts on the Soybean 1000-Seed Weight

As seen in Table 2, the weight of 1000 seeds was comparable in both pairs: Enrei Control vs. Extract and Erica Control vs. Extract. The difference was insignificant between the control and experimental groups.

Figure 1e presents a comparison of all of the groups, for both varieties. For the 1000-seed weight, statistically significant differences ($p < 0.05$; Tukey test) were noted for the following groups: Erica Control and Enrei Control, Erica Extract and Enrei Control, Erica Control and Enrei Extract, and Erica Extract and Enrei Extract. The highest 1000-seed weight was measured for Erica Extract, and the lowest was measured for Enrei Control. Similarly, 1000 seeds collected from the Erica variety treated with extract were heavier than those from Enrei by 16.1%.

3.6. Effect of the Macroalgal Extracts on the Soybean Yield

The macroalgal extract statistically influenced the soybean yield in the Erica Control vs. the Extract pair: in the extract group, the yield was 8.7% higher than that in the control group. For the pair Enrei Control vs. Extract, this difference was 5.7%, and was not statistically significant.

Comparing the yield of two varieties of soybean measured in all of the experimental groups (Figure 1f), statistically significant differences ($p < 0.05$; Tukey test) were observed for the following groups: Erica Control and Enrei Control, Erica Extract and Enrei Control, Erica Control and Enrei Extract, Erica Extract and Enrei Extract, and Erica Extract and Erica Control. The highest yield was observed in the Erica Extract group, and the lowest was observed in the Enrei Control group. For the groups treated with the extract, the Erica variety had a higher yield than the Enrei variety (by 75.2%). It might be assumed that the Japanese Enrei variety is not well adapted to the climatic conditions in Poland.

Table 3 presents morphological traits of two varieties of soybean. Generally, the application of algal extract improved characteristics such as the number of pods per plant, the number of seeds per plant, the weight of seeds per plant, and the weight of seeds per pod in both varieties of soybeans. For Enrei, the number of pods per plant was higher in the extract group by 12.8% than that in the control group. The differences for the remaining

parameters were not so significant: the number of seeds per plant was higher but only by 1%, the seed weight per plant was higher than in the control group but only by 2%, whereas the seed weight per pod was higher in the control group.

Table 3. Agronomic traits (mean $\pm$ SD).

Parameter	Enrei		Erica	
	Control	Extract	Control	Extract
Number of pods per plant *	21.1 $\pm$ 1.2	23.8 $\pm$ 1.7	28.4 $\pm$ 1.0	31.3 $\pm$ 0.6
Number of seeds per plant **	41.3 $\pm$ 0.8	41.7 $\pm$ 0.9	56.8 $\pm$ 1.1	64.3 $\pm$ 1.5
Seed weight per plant *** (g)	6.37 $\pm$ 0.14	6.50 $\pm$ 0.14	10.1 $\pm$ 0.2	11.7 $\pm$ 0.2
Seed weight per pod **** (g)	0.303 $\pm$ 0.024	0.274 $\pm$ 0.021	0.358 $\pm$ 0.006	0.375 $\pm$ 0.003

* The number of pods per plant was counted from the sample of 10 plants from each plot (N = 4) and then reported per soybean plant. ** The number of seeds per plant was counted from the sample of 10 plants from each plot (N = 4) and then reported per soybean plant. *** The seed weight per plant was obtained from the sample of 10 plants from each plot (N = 4) and then reported per soybean plant. **** The seed weight per pod = the seed weight per plant/number of pods per plant.

Greater differences were observed between the control group and the extract for the Erica variety. The number of pods per plant in the extract group was 10.2% higher than in the control group, the number of seeds per plant was 13.2% higher, the seed weight per plant was 15.8% higher, and the seed weight per pod was 4.7% higher.

The positive effect of the algal extract on the examined parameters was more visible in the case of the Erica variety. The number of pods per plant, the number of seeds per plant, the weight of seed per plant, and the weight of seed per pod were higher by 31.5%, 54.2%, 80% and 38.9%, respectively, compared to the Enrei variety. In conclusion, the home variety Erica is more responsive to the algal extract, and it seems to be better adapted to the climatic conditions in Poland, because of its stable higher yields. According to Egli et al. (1987), an increase in the number of Maturity Groups (MG) raises the plant height, the number of nodes per plant, and the vegetative weight per unit area. The results showed that the longer vegetation period of varieties maturing later does not ensure a higher yield potential, while varieties with a shorter growing period under similar environmental conditions can have a similar yield potential [45].

4. Conclusions

The foliar spraying of *Cladophora glomerata* extract could be a promising option to increase soybean yields. Algal extracts have gained in popularity due to their potential use in organic and sustainable agriculture. The use of algal extract increased all of the growth parameters measured for soybean. In general, a gradual increase in plant height was observed with the application of *C. glomerata* extract. The plant height, first pod height, number of first branches, 1000-seed weight, and yield were significantly affected by the foliar spray of the algal extract.

Author Contributions: Conceptualization, J.D., I.M., S.L. and M.K.; methodology, S.L., M.K., I.M., K.M. and J.D.; formal analysis, I.M., S.L., M.L., J.D. and K.M.; resources, S.L., I.M., K.M., N.O.-O. and M.K.; writing—original draft preparation, I.M., S.L. and N.O.-O.; visualization, S.L., I.M. and N.O.-O.; supervision, S.L. and I.M.; writing—review and editing, S.L. and I.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a grant entitled: “Increasing productivity and sustainability of European plant protein production by closing the grain legume yield gap”, the SusCrop-ERA-NET (Grant No. SUSCROP/I/LegumeGap/01/2019) from the National Centre for Research and Development in Poland. The algal extract was produced in the framework of a grant entitled: “Eco-friendly technologies for the management of seaweed biomass for products useful for sustainable agriculture and biosorbents used for the removal of heavy metal ions from the environment”

(No. 2019/33/B/NZ9/01844) from the National Science Centre in Poland (for the preparation of algae extracts).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: Authors state no conflict of interest.

References

1. Faostat. 2018. Available online: <http://www.fao.org/faostat/en/#data/QC/visualize> (accessed on 1 January 2022).
2. Holmberg, S.A. Soybeans for cool temperature climates. *Agric. Hort. Genet.* **1973**, *31*, 1–20.
3. Kamp, J.A.L.M.; van Berkum, S.; Timmer, R.D.; van Reeuwijk, P. *Verkenning Naar de Mogelijkheden van Eiwithoudende Teelten in Europa*; Wageningen University & Research: Wageningen, The Netherlands, 2010.
4. Kusano, M.; Baxter, I.; Fukushima, A.; Oikawa, A.; Okazaki, Y.; Nakabayashi, R.; Bouvrette, D.J.; Achard, F.; Jakubowski, A.R.; Ballam, J.M.; et al. Assessing metabolomic and chemical diversity of a soybean lineage representing 35 years of breeding. *Metabolomics* **2015**, *11*, 261–270. [CrossRef]
5. Carrera, C.S.; Dardanelli, J.L.; Soldini, D.O. Chemical compounds related to nutraceutical and industrial qualities of non-transgenic soybean genotypes. *J. Sci. Food Agric.* **2014**, *94*, 1463–1469. [CrossRef]
6. Häusling, M. The EU Protein Deficit: What Solution for a Long-Standing Problem? (2010/2111(INI)). (European Parliament, 2011). Available online: <https://www.europarl.europa.eu/sides/getDoc.do?type=REPORT&reference=A7%E2%80%93932011%E2%80%93930026&language=%20EN> (accessed on 12 September 2014).
7. Podleśny, J. Rośliny strączkowe w Polsce—Perspektywy uprawy i wykorzystania nasion. *Acta Agrophys.* **2005**, *6*, 213–224.
8. Lee, H.; Garlich, A. Effect of overcooked soybean meal on chicken performance and amino acid availability. *Poult. Sci.* **1992**, *71*, 499–508. [CrossRef] [PubMed]
9. Ingram, D.; Parsons, S.; Parsons, C.M. In Methods for determining quality of soybean meal important. *Feedstuffs* **1999**, *71*, 10–11.
10. Xu, S.; Liu, X.; Wie, J.; Ma, L.; Sun, Z. Effects of different fermented soybean meals on growth performance and intestinal morphology in piglets. *Feed Ind.* **2013**, *21*, 1–9.
11. De Visser, C.L.M.; Schreuder, R.; Stoddard, F.L. The EU's dependency on soya bean import for the animal feed industry and potential for EU produced alternative. *Oilseed Fat Crops Lipids* **2014**, *21*, 1–8. [CrossRef]
12. Brown, P.B. Soybean Meal in Aquaculture. Soybean INFO. March 2002. Available online: www.soymeal.org (accessed on 1 January 2022).
13. Willis, S. The use of soybean meal and full fat soybean meal by the animal feed industry. In Proceedings of the 12th Australian Soybean Conference, Bundaberg, Australia, 2–6 February 2003; pp. 1–8.
14. Biermann, U.; Friedt, W.; Lang, S.; Lühs, W.; Machmüller, G.; Metzger, J.O.; Klaas, M.R.; Schäfer, H.J.; Schneider, M.P. New syntheses with oils and fats as renewable raw materials for the chemical industry. *Angew. Chem. Int. Ed.* **2000**, *39*, 2206–2224. [CrossRef]
15. Karczmarszuk, I. Soja—roślina ze wszech miar użyteczna. *Wiadomości Zielar.* **1999**, *41*, 6–7.
16. Ohya, T.; Tewari, K.; Ishikawa, S.; Tanaka, K.; Kamiyama, S.; Ono, Y.; Hatano, S.; Ohtake, N.; Sueyoshi, K.; Hasegawa, H.; et al. Role of nitrogen on growth and seed yield of soybean and a new fertilization technique to promote nitrogen fixation and seed yield. Open access peer-reviewed chapter in a book. In *Soybean—The Basis of Yield, Biomass and Productivity*; BoD—Books on Demand: Norderstedt, Germany, 2017. [CrossRef]
17. Haeghele, J.W.; Below, F.E. The Six Secrets of Soybean Success: Improving Management Practices for High Yield Soybean Production. 2013. Available online: <http://cropphysiology.cropsci.illinois.edu/documents/2012%20Six%20Secrets%20of%20Soybean%20Success%20report.pdf> (accessed on 8 November 2015).
18. Summerfield, R.J.; Lawn, R.J.; Qi, A.; Ellis, R.H.; Roberts, E.H.; Chay, P.M.; Brouwer, J.B.; Rose, J.L.; Shanmugasundaram, S.; Yeates, S.J.; et al. Towards the reliable prediction of time to flowering in six annual crops. II. Soybean (*Glycine Max*). *Exp. Agric.* **2008**, *29*, 253–289. [CrossRef]
19. Szyrmer, J.; Federowska, B. Kierunki badań i hodowli roślin soi. *Biul. IHAR* **1975**, *3–4*, 3–7.
20. Wilcox, J.R.; Boerma, H.R.; Specht, J.E. Soybeans: Improvement, production, and uses. In *American Society of Agronomy, Crop Science Society of America, Soil Science Society of America*; Boerma, H.R., Specht, J.E., Eds.; Agronomy: Madison, WI, USA, 2004; Volume 16, pp. 303–416.
21. Hyten, D.L.; Choi, I.Y.; Costa, J.M.; Cregan, P.B.; Nelson, R.L.; Song, Q.; Specht, J.E.; Shoemaker, R.C.; Zhu, Y. Impacts of genetic bottlenecks on soybean genome diversity. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 16666–16671. [CrossRef]
22. Jaranowski, J.; Muszyński, A.; Skorupska, H.; Torz, L. Charakterystyka zmienności i ważniejszych pod względem gospodarczym cech użytkowych soi (*Glycine max* L.). In *Poznańskie Towarzystwo Przyjaciół Nauk, Wydział Nauk Rolniczych i Leśnych; Prace Komisji Nauk Rolniczych i Komisji Nauk Leśnych*; Poznań, Poland, 1984; Volume LVII, pp. 149–159.
23. Nawracała, J.; Konieczny, G. Możliwości wykorzystania zmienności introdukowanej, mutacyjnej i rekombinacyjnej w hodowli soi. *Hod. Roślin—I Kraj. Konf.* **1997**, *XXII*, 91–94.

24. Jaranowski, J.; Konieczny, G.; Muszyński, A.; Skorupska, H.; Torz, L. Charakterystyka zmienności ważniejszych pod względem gospodarczych cech użytkowych soi. *Zesz. Probl. Post. Nauk. Rol.* **1983**, *253*, 7–24.
25. Criswell, J.G.; Hume, D.J. Variation in sensitivity to photoperiod among early maturing soybean strains. *Crop Sci.* **1972**, *12*, 657–660. [[CrossRef](#)]
26. Polson, D.E. Day-Neutrality in Soybeans. *Crop Sci.* **1972**, *12*, 6. [[CrossRef](#)]
27. Runge, E.C.A.; Odell, R.T. The relation between precipitation, temperature and the yield of soybeans on the Agronomy South Farm, Urbana, Illinois. *Agron. J.* **1960**, *52*, 245–247. [[CrossRef](#)]
28. Shanmu-Gasundaram, S.; Tsou, S.C.S. Photoperiod and critical duration for flower induction in soybean. *Crop Sci.* **1978**, *18*, 598–601. [[CrossRef](#)]
29. Hassan, S.M.; Ashour, M.; Sakai, N.; Zhang, L.; Hassanien, H.A.; Ammar, G.A.G.; Ammar, G. Impact of seaweed liquid extract biostimulant on growth, yield, and chemical composition of cucumber (*Cucumis sativus*). *Agriculture* **2021**, *11*, 320. [[CrossRef](#)]
30. Mohan, V.R.; Venkataraman Kumar, V.; Murugeswari, R.; Muthuswami, S. Effect of crude and commercial seaweed extract on seed germination and seedling growth in *Cajanus cajan* L. *Phykos* **1994**, *33*, 47–51.
31. Sivasankari, S.; Venkatesalu, V.; Anantharaj, M.; Chandrasekaran, M. Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. *Bioresour. Technol.* **2006**, *97*, 1745–1751. [[CrossRef](#)]
32. Cober, E.R.; Voldeng, H.D. A new soybean maturity and photoperiod-sensitive locus linked to E1 and T. *Crop Sci.* **2001**, *41*, 698–701. [[CrossRef](#)]
33. Heatherly, L.G.; Elmore, R.W. Managing inputs for peak production. In *Soybeans: Improvement, Production, and Uses*, 3rd ed.; Specht, J.E., Boerma, H.R., Eds.; Agronomy Monographs; American Society of Agronomy: Madison, WI, USA, 2004; pp. 451–536.
34. Major, D.L.; Anderson, I.C.; Johnson, D.J.; Tanner, J.W. Effects of day length and temperature on soybean development. *Crop Sci.* **1975**, *15*, 174–179. [[CrossRef](#)]
35. Lewandowska, S.; Michalak, I. Effect of algal extracts on germination ability of different varieties of soybean. In *Seed and Seedlings*; XIV Scientific and Technical Seminar; 978-80-213-2922-5; Czech University of Life Sciences Prague: Prague, Czech Republic, 2019; pp. 84–90.
36. Dziergowska, K.; Lewandowska, S.; Mech, R.; Pol, M.; Detyna, J.; Michalak, I. Soybean germination response to algae extract and a static magnetic field treatment. *Appl. Sci.* **2021**, *11*, 8597. [[CrossRef](#)]
37. Mooney, P.A.; Van Staden, J. Algae and cytokinins. *J. Plant. Physiol.* **1986**, *123*, 1–21. [[CrossRef](#)]
38. Ashour, M.; Hassan, S.M.; Elshobary, M.E.; Ammar, G.A.G.; Gaber, A.; Alsanie, W.F.; Mansour, A.T.; El-Shenody, R. Impact of commercial seaweed liquid extract (TAM[®]) biostimulant and its bioactive molecules on growth and antioxidant activities of hot pepper (*Capsicum annuum*). *Plants* **2021**, *10*, 1045. [[CrossRef](#)]
39. Dziergowska, K.; Wełna, M.; Szymczycha-Madeja, A.; Chęćmanowski, J.; Michalak, I. Valorization of *Cladophora glomerata* biomass and obtained bioproducts into biostimulants of plant growth and as sorbents (biosorbents) of metal ions. *Molecules* **2021**, *26*, 6917. [[CrossRef](#)]
40. Blunden, G. Agricultural uses of seaweeds and seaweed products. In *Seaweed Resources in Europe: Uses and Potential*; Guiry, M.D., Blunden, G., Eds.; John Wiley and Sons: Chichester, UK, 1991; pp. 65–81.
41. Michalak, I.; Messyasz, B. Concise review of *Cladophora* spp.: Macroalgae of commercial interest. *J. Appl. Phycol.* **2021**, *33*, 133–166. [[CrossRef](#)]
42. Fraser, J.; Eaton, G.W. Applications of yield component analysis to crop research. *Field Crop. Abstr.* **1983**, *36*, 787–796.
43. Mohammadi, S.A.; Prasanna, S.A.; Singh, N.N. Sequential path model for determining interrelationships among grain yield and related characters in maize. *Crop Sci.* **2003**, *43*, 1690–1697. [[CrossRef](#)]
44. Rasmusson, D.C.; Cannell, R.Q. Selection for grain yield and components of yield in barley. *Crop Sci.* **1970**, *10*, 51–54. [[CrossRef](#)]
45. Egli, D.B.; Guffy, R.D.; Heitholt, J.J. Factors associated with reduced yields of delayed plantings of soybean. *J. Agron. Crop Sci.* **1987**, *159*, 176–185. [[CrossRef](#)]