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The Effect of Macroalgal Extracts and Near Infrared Radiation on Germination of Soybean Seedlings: Preliminary Research Results

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Abstract: In the present study, synergistic effects between the application of near-infrared radiation (NIR) and macroalgal extracts on the germination of soybean seeds were searched for. NIR is captured by special photoreceptors (i.e. phytochromes, cryptochromes and phototropins) and next plants generate a wide range of specific physiological responses through these receptors. For the study, a special system of NIR was applied to irradiate soybean seeds. To our knowledge, this is the first time this kind of radiation was used for the biostimulation of soybean seeds. Previously, the effect of other ranges of light (e.g. green, red, blue) was analysed in terms of photosynthetic activity, growth and yield of different plants, except seeds. NIR for 3 and 5 minutes was also combined with the application of macroalgal extracts used for seeds soaking. They are known as a rich source of biologically active compounds that can stimulate plant growth. These preliminary studies show that the examined factors can stimulate plant's growth and their quality.

Keywords: germination; near-infrared radiation; seedling; macroalgal extracts; soybean seeds.

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1 Introduction

Soybean (*Glycine max*) is becoming the most rapidly growing source of protein in Europe. The profitability of the cultivation of this species is increasing every year. There is an upward trend in the price of soybeans, however, it is even more important that there is a rapidly growing demand for soybeans which are not GMO. There are more and more new companies in Europe buying soybeans directly from farmers. According to Lewandowska (2016) for many farmers the production of this plant is a “godsend” of defective rotation to which belongs cereals, maize and rapeseed. The inclusion of soybean cultivation in crop sequences is an important and valuable element and is associated with subsidies. The high quality and genetic diversity of modern varieties has been improving. Currently, soy is seen as an essential plant protein around the world, usable for both human food and valuable animal feed production [1]. According to Batista et al. the plant has an important feature from the viewpoint of quantitative culture of protein and fat in soybean seeds. At present there are numerous soybean products available commercially, the most common ones are: tofu, miso, edamame, natto, tonyu, soymilk, etc. These kinds of products are especially popular among vegetarians [2]. Hence, new natural products with plant growth-promoting/stimulating properties are being sought. Their additional function is to increase the resistance of plants to biotic (e.g., bacteria, viruses, fungi, parasites) and abiotic stresses (e.g., salinity, drought, low/high temperature) which are known to exert a negative effect on plant growth and productivity [3–5]. Among several categories of plant biostimulants are humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, chitosan and other biopolymers, inorganic compounds (Al, Co, Na, Se and Si), beneficial fungi and bacteria [6]. This paper focuses on macroalgal extracts, which are known

as a rich source of biologically active compounds that can stimulate plant growth [4,7].

Generally, seaweeds are categorized into three main groups according to the pigments: *Chlorophyta* – green seaweeds (G; mainly chlorophyll, β -carotene, xanthophylls), *Phaeophyceae* – brown seaweed (B; mainly fucoxanthin) and *Rhodophyta* – red seaweeds (R; mainly phycoerythrin, xanthophylls, phycocyanin) [8]. For the production of extracts tested on soybean – which was used as a model plant in the present study, the following seaweed species are the most frequently used: red algae – *Neorhodomela larix*, *Tichocarpus crinitus* [9], *Kappaphycus* sp., *Gracilaria* sp. [10], *Kappaphycus alvarezii* [11,12], *Grateloupia divaricata*, *Chondrus pinnulatus*, *Neorhodomela larix*, *Tichocarpus crinitus* [13]; brown algae – *Saccharina japonica*, *Sargassum pallidum* [9,13], *Stephanocystis crassipes*, *Coccophora langsdorfii*, *Sphaerotrichia divaricata*, *Chorda filum* [13] and finally green algae – *Ulva fenestrata* and *Codium fragile* [9,13]. Commercially available seaweed extracts, e.g. Acadian® produced from brown alga *Ascophyllum nodosum*, are also very often tested in plant cultivation, including soybean [5,14].

In the literature it is shown that seaweed extracts tested on soybean are mainly obtained by acid hydrolysis with sulphuric acid in an autoclave [12], grinder homogenization with further filtration [10,11] or heating with sterile distilled water at 60°C [9,13]. Seaweed products can be applied on soybean in the form of extract foliar spray [10,11] or they can be used for seed treatment [12].

Macroalgal extracts applied to soybean (1) alleviate drought stress which negatively impacts plant physiology and crop productivity [5,14], (2) increase growth parameters: plant height [10–12]; dry matter accumulation, pod setting index, 100 seed weight [10]; number of pods per plant [10,11]; number of plants per square meter, number of grains per pod, number of branches per plant, test weight [11], (3) increase the yield [10,11], (4) improve nutrient uptake – e.g., N, P, K and S [11], (5) increase the length of soybean seedling roots [9,12,13], (6) improve flowering behaviour – decrease the number of flower drops per plant [10]. Additionally, the main advantages of extracts derived from macroalgae are their biodegradability, lack of toxicity and also the fact that they are non-polluting and non-hazardous to humans, animals and birds [4,15].

Apart from macroalgal extracts, the growth of plants (germination of seeds) can be influenced by near infrared radiation (NIR). In general terms, it acts at the quantum level (by influencing the atomic and molecular level), however, it also has impact at the level of plant cells and tissues. It has been known for a long time that the use of near infrared radiation improves seed germination

(although the mechanism of this process is not completely recognized yet). The British patent GB 2 303 533 shows the possibilities of stimulating seed using near infrared radiation, also when it is combined with red light [16]. Usually the stimulation of seed using radiation in the wavelength range between 800 and 1000 nm improved the germination of various garden plants [17–21]. Moreover, seedling vitality was enhanced when NIR light was used. Typically the lighting time was in the range from 1 to 10 minutes [22,23].

NIR light does not have significant influence on the temperature of plant tissues and, as a result, there is no direct relation between temperature and the influence of NIR on plants [24,25]. Consequently, the role of infrared radiation in plant development is not clear even if there are evident symptoms indicating that NIR light may have influence on plant growth and development [17]. According to numerous scholars, NIR may inhibit plant growth, which contradicts many documented results. Most probably the impact of NIR on plants is strongly correlated with wavelength and exposure time. Due to this complex problem, currently infrared radiation light is not used in commercial lighting systems for plant germination and growth. In addition to this, the connection between visible light and near infrared radiation has not been verified yet. This article is yet another attempt to fill the gap in the knowledge on NIR impact on plant germination to a certain extent.

Numerous authors build the mechanism of NIR impact on plants on the basis of the absorption level of this radiation. Healthy plants can be identified using the infrared radiation spectrum, because they reflect most of this radiation (about 80%), while unhealthy plants reflect much less NIR. Hence, plant stress (the physiological reaction of plants to unfavourable, high intensity environmental and cultivation factors; as a result of this stress plant growth and development are limited, which produces a negative effect on yield; in extreme conditions such stress may even lead to complete yield loss or plant destruction) is identified on the basis of a progressive decrease in the NIR reflection coefficient. Such information seems to indicate that green plants need NIR light for some physiological and biochemical processes connected with their growth, development and defective tissue regeneration. This is why disease affected plants need more near infrared radiation as it activates plant metabolism in the defected tissues, probably in a way which is similar to the same process in animal and human tissues [18,24]. One of the NIR mechanisms of actions is related to the cell breathing system located in the mitochondrion [18,26–29]. According to the first law of

photobiology, low power visible light can generate some effect on a biological tissue on condition that electron absorption bands related to molecular photo-acceptors in a tissue absorb photons. Thus, photobiomodulation is based on the following rule; when light is cast on certain chromophore particles, photon energy causes electrons to move from low energy levels to higher energy levels. In fact, the energy which is stored in this way at higher energy levels in chromophore particles can be used by the biological system for various cell tasks, such as photosynthesis and photomorphogenesis (light-regulated control of plant development). Chromophores encompass chlorophyll in plants, bacteriochlorophyll in blue-green algae, flavoproteins or respiratory enzymes in cells and haemoglobin in erythrocytes. Due to the fact that mitochondria play an important role in energy generation and metabolism, each of the proposed photobiomodulation (biostimulation) mechanisms requires a certain type of reaction between light and mitochondria. It was observed that mitochondria components absorb light in the red and near infrared wavelength range transforming light energy into metabolic energy, which results in the modulation of cell biological functions [28,30].

Soybean was used as a model plant because nowadays it is an important crop cultivated worldwide due to its high protein and fat content and also other nutritional values. What is more, the world population is increasing and a sufficient supply of food is a fundamental issue for both the world and each individual country. Hence, a sufficient supply of sustainable food is essential.

The aim of the present paper was to study the influence of macroalgal extracts used for seeds soaking and near infrared radiation on the soybean germination ability, uniform emergence and composition.

2 Materials and methods

2.1 Soybean seeds

Soybean seeds (*Glycine max*), cv. Merlin, (not genetically modified) were used in the present study. Soybean seedlings were extracted from a paper towel and converted into various digital representations. These representations were used to analyse the seedlings and segment them into normal and abnormal categories. The normal seedlings were further processed so that a one-pixel-wide summary structure of the shape of the seedling was produced. From this summary structure, the software classified the seedlings into six type categories based on their shape.

2.2 Freshwater macroalgae

The biomass of freshwater macroalgae – *Cladophora glomerata*, was collected from the surface of the pond in Tomaszówek, Łódź Province, Poland (51°27'21"N 20°07'43"E) in October 2016. Then it was air-dried. Before extraction it was fine milled using grinding mills (Retsch GM 300, Germany).

2.3 Production of the macroalgal extract

One gram of a crushed algal weight was diluted with 100 mL of distilled water and shaken (200 rpm) at room temperature for 60 min. The obtained extract was passed through a paper filter and preserved at 4°C for subsequent studies. The final macroalgal extract was treated as 100% (E100). This concentration was also used to prepare 50% (E50) [13]. Water was chosen as a solvent since it is safe for plants.

2.4 Seed soaking

One hundred seeds of soybean were soaked in each selected concentration (50 and 100 %) of aqueous macroalgal extracts for 1 h according to the methodology described by Pise and Sabale (2010) [31] and Michalak et al. (2017) [32]. For this reason, Petri dishes (9 cm diameter) were chosen. The amount of 40 mL of macroalgal extract was added to each dish, as shown in Figure 1. After one hour they were dried with filter paper.

2.5 Infrared radiation of soybean seeds

The stimulation with near infrared radiation (NIR) was conducted using a halogen lamp (Figure 2A) equipped with a 700-2000 nm filter. Infrared beams were focused on a glass container with limited access to other types of light using a flat, glass pipe. During the irradiation of samples in this range, no sample overheating occurred because the system was equipped with a cooling device (Figure 2B). The temperature was maintained at a stable level, i.e. 21±1°C [17]. The power density of the applied radiation was 6.9 mWcm⁻². Soybean seeds were treated with infrared radiation separately for 3 (NIR3) and 5 minutes (NIR5).

2.6 Germination tests

The germination test of soybean seeds was assessed according to the International Seed Testing Association (ISTA). A sand substrate was used to conduct the germination test which was carried out in cuvettes with 4 replications. One replication consisted of 100 soybean seeds. The first counting of seedlings was conducted after 5 days and the second one after 8 days. The seedlings were stored in a growth chamber at a constant temperature 25°C.

Figure 1: Soaking of soybean seeds with macroalgal extracts.

The germination ability was assessed and given a percentage. The minimum germination requirement is 80%. After the final counting, the seedlings were divided into normal and abnormal groups. Soybean germination is referred to as epigeal because food storage structures (cotyledons) are pulled above the soil surface. Usually the differences between well-developed seedlings relate to a lack of chlorophyll (albinotic seedling), dwarfism, or an irregular hypocotyl shape.

In the present research we tested following groups.

1. Control group – C ($N=4$)
2. NIR 1 (3 minutes) + E1 (50%) – NIR3 E50 ($N=4$)
3. NIR 1 (3 minutes) + E2 (100%) – NIR3 E100 ($N=4$)
4. NIR 2 (5 minutes) + E1 (50%) – NIR5 E50 ($N=4$)
5. NIR 2 (5 minutes) + E2 (100%) – NIR5 E100 ($N=4$)
6. NIR 1 (3 minutes) – NIR3 ($N=4$)
7. NIR 2 (5 minutes) – NIR5 ($N=4$)
8. E1 (50%) – E50 ($N=4$)
9. E2 (100%) – E100 ($N=4$)

In order to check the effect of the combination of infrared radiation and the macroalgal extract on the germination of seeds, first the seeds were treated with NIR (3 and 5 minutes separately) and then soaked in the extract for one hour. After germination tests, the seedlings (normal separately from abnormal ones) were stored in a freezer at a temperature -21°C.

Figure 2: Experimental setup for NIR stimulation (A) and the cooling device (B); on the basis of Niemczyk (2017) [17].

2.7 Measurement techniques

The multielemental composition of macroalgal extracts was measured by ICP-OES (Vista-MPX, VARIAN, Australia) in the Chemical Laboratory of Multielemental Analyses at Wrocław University of Science and Technology (Wrocław, Poland), which is accredited by ILAC-MRA and Polish Centre for Accreditation (No. AB696). The quality assurance of the test results was achieved by the Combined Quality Control Standard from ULTRA Scientific (LGC STANDARDS SP. Z O.O., Łomianki, Poland). The samples were analysed three times (the reported results of the analyses were the arithmetic mean and the relative standard deviation was <5%) within the quality management system according to PN-EN ISO/IEC 17025:2005.

A hand-held SPAD-502 Chlorophyll Meter (Konica Minolta, Japan) (SPAD: Soil-Plant Analyses Development) was used to provide a relative index of chlorophyll concentrations in soybean seedlings. Before the measurements, the leaves of soybean seedlings were defrosted. In each replication (N=4) in each tested group (N=9), one SPAD reading from each of ten randomly selected seedlings (from the normal and abnormal group) was taken and averaged for each replication [33].

Every single soybean seedling was assessed according to the ISTA regulations and demands. Finally, the germination ability was assessed and given a percentage. The minimum germination requirement in soybean is 80%. The germination ability is an important indicator of seed quality.

2.8 Statistical analysis

The obtained results were elaborated statistically by *Statistica* ver. 12.0 (StatSoft, Cracow, Poland). Descriptive statistics (average, standard deviations) for all experimental groups was performed. The normality of the distribution of experimental results was assessed by the Shapiro–Wilk test and the homogeneity of variances by the Brown & Forsythe's test. On this basis, the statistical test used to investigate the significance of differences between the tested groups was selected. The differences between the two groups were investigated with a *t*-test and between several groups with the one-way analysis of variance (ANOVA) using the Tukey multiple comparison test (for normal distribution and the homogeneity of variances). In the case of the lack of the normal distribution, the Mann-Whitney test was used (for two groups) and the Kruskal–Wallis test (for more than two groups). The results were considered significantly different when $p < 0.05$.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

In the present study we examined the effect of NIR, macroalgal extracts and their combination on the germination of soybean seeds as well as quality by measuring the relative index of chlorophyll content in the soybean seedling leaves. We also examined the multielemental composition of macroalgal extracts because it can be relevant to plants (their vital processes, growth and quality).

3.1 Multielemental composition of macroalgal extracts

In Table 1, the multielemental composition of macroalgal extracts obtained with water by different extraction techniques is presented. In this study, water was chosen as an extract due to the application of algal extracts in plant cultivation. As it is shown in Table 1, different extraction techniques influenced the elemental composition of macroalgal extracts. The content of micro- and macroelements in an extract from freshwater *Cladophora glomerata* was much lower than for marine macroalgae – for example for the mixture of Baltic seaweeds: *Polysiphonia*, *Ulva* and *Cladophora*. It is not only the algal species, but also the location of their collection that determines the composition of the obtained extracts.

3.2 Germination tests on soybean (*Glycine max*)

3.2.1 The effect of examined factors on seed germination

Table 2 presents the results concerning the average number of germinated seeds in each tested group which were classified as normal, abnormal and dead. The highest average of germinated normal seedlings was obtained for NIR5, then NIR3 which was comparable to the control group (Figure 3). The application of macroalgal extracts in both concentrations significantly decreased the number of germinated seedlings. The simultaneous application of the macroalgal extract with NIR increased the number of germinated seedlings (differences were not statistically significant) when compared to the groups where only the macroalgal extract was used, but these values were much

Table 1: Multielemental composition (mg/L) of aqueous macroalgal extracts.

Element	<i>Cladophora glomerata</i> (G) ¹		<i>Kappaphycus alvarezii</i> (R) ²	Algal mixture (<i>Polysiphonia</i> (R), <i>Ulva</i> (G), <i>Cladophora</i> (G))			
	100%	50%		3	4	5	6
B	0.497±0.075	0.466±0.070	n.a.	4.74±0.71	6.50±0.97	2.62±0.39	2.86±0.43
Ca	31.6±4.7	25.1±3.8	460	365±54	333±50	410±61	305±46
Cd	0.0237±0.0047	< LOD	n.a.	0.001±0.000	<LLD	<LLD	< LOD
Co	0.122±0.018	0.0753±0.0188	n.a.	0.0135±0.0034	0.0100±0.0025	0.0100±0.0025	n.a.
Cu	0.0453±0.0113	0.0355±0.0089	0.30	0.108±0.016	0.140±0.021	0.0200±0.005	0.310±0.047
Fe	0.388±0.058	0.148±0.022	10.6	4.47±0.70	2.53±0.38	17.6±2.6	6.11±0.92
K	240±36	120±18	19700	951±142	969±145	978±147	1027±205
Mg	5.99±0.90	3.17±0.48	581	322±48	300±45	357±54	308±46
Mn	0.194±0.029	0.108±0.016	2.50	3.07±0.46	2.43±0.36	3.71±0.56	2.32±0.35
Mo	0.203±0.030	< LOD	n.a.	0.0108±0.0027	0.0200±0.005	0.000	0.0100±0.0025
Na	7.07±1.06	6.84±1.03	5100	1250±250	1239±248	1302±260	1286±257
Ni	0.277±0.042	0.186±0.028	n.a.	0.132±0.019	0.130±0.019	0.120±0.018	n.a.
P	6.51±0.98	3.71±0.56	n.a.	32.9±4.9	34.7±5.2	5.22±0.78	27.3±4.1
Pb	< LOD	< LOD	n.a.	0.032±0.006	0.0400±0.008	0.0100±0.0025	0.020±0.005
S	49.6±7.4	27.9±3.7	600	702±105	670±100	599±90	547±82
Si	0.821±0.123	0.595±0.089	n.a.	11.9±1.8	9.10±1.36	9.73±1.46	9.64±1.45
Zn	0.876±0.131	0.237±0.036	0.62	0.169±0.025	0.240±0.036	0.100±0.015	0.210±0.032

¹ shaking/filtration (present study); ² homogenization/filtration [11]; ³ microwave assisted extraction, 60°C [49]; ⁴ boiling for 30 minutes/filtration [50]; ⁵ maceration for 2 days/filtration [50]; ⁶ shaking with deionised water with pH 7 < LOD – below limit of detection; n.a. – not available

Table 2: The number of normal, abnormal and dead seedlings in the tested groups.

Group		Normal seedlings **		Abnormal seedlings ***		Dead seedlings ***	
		Average*	SD	Average*	SD	Average*	SD
1	C	84.5 ^{abcdef}	3.1	14.5 ^a	3.1	1.0	0.0
2	NIR3 E50	52.5 ^{agh}	4.8	28.3	3.0	19.3	2.1
3	NIR3 E100	55.5 ^{bijk}	2.9	24.8	3.5	19.8	4.8
4	NIR5 E50	47.3 ^{clm}	8.1	31.0	7.0	21.8	3.0
5	NIR5 E100	49.0 ^{dno}	2.4	26.0	1.8	25.0 ^a	3.5
6	NIR3	85.0 ^{gilmpr}	2.2	14.8 ^b	1.7	0.3 ^a	0.5
7	NIR5	85.3 ^{hjmst}	3.0	14.0 ^c	2.9	0.8	1.0
8	E50	44.5 ^{ekps}	4.2	33.3 ^{abc}	3.1	22.3	6.1
9	E100	47.8 ^{frt}	3.8	29.0	5.4	23.3	2.4

* – average from 4 replications in each group; SD – standard deviation

** – normal distribution, ANOVA test

*** – abnormal distribution, Kruskal-Wallis test

a, b... – statistically significant differences for $p < 0.05$

smaller than for NIR3, NIR5 used alone and the control group (differences were statistically significant). The results were by 13.9% and 2.4% higher in NIR3 E100 and NIR5 E100 than in E100, respectively, and by 15.2% and 5.9% higher in NIR3 E50 and NIR5 E50 than in E50. The

treatment of soybean seeds with near infrared radiation for 3 minutes and then soaking them in macroalgal extracts gave better results than when the same treatment lasted 5 minutes.

Figure 3: Number of normal, abnormal and dead seedlings in the tested groups.

The soaking of seeds caused many of the germinated seedlings to be classified as abnormal and dead (the highest percentage). Additionally, it was found that the concentration of the macroalgal extract had no effect on the seed germination.

3.2.2 The effect of the examined factors on the chlorophyll content in the cultivated soybean seedlings

Chlorophyll meter (SPAD-502) is a hand-held, self-calibrating, convenient lightweight device used to calculate the amount of chlorophyll present in plant leaves [33]. It measures the absorbance of the leaf of two different wavelengths in the spectral domain of red (650 nm) and near-infrared (940 nm). As an output, it calculates index-values (e.g. SPAD-value) that specify leaf chlorophyll content [34]. Another advantage is that it is non-destructive [33,35]. The proper interpretation of SPAD values could be a useful tool in developing N fertilizer programs, because the content of chlorophyll in plants is closely related to the nutritional status of plants (it increases with the increase of nitrogen – an important plant nutrient in leaves) [33].

The objective of this study was to evaluate the relative index of chlorophyll concentrations in soybean seedling leaves using a chlorophyll meter. The SPAD values indicated by the meter are proportional to the chlorophyll content in the leaf [36]. The obtained results are presented in Table 3 – for normal and Table 4 – abnormal soybean seedlings. The content of chlorophyll was comparable in the groups treated only with extracts (E50 and E100) and infrared radiation (NIR3 and NIR5). The increase was observed in the groups where the combination of an

extract and radiation was applied – especially in NIR3 E50, NIR3 E100 and NIR5 E50.

In the present study, the concentration of the macroalgal extract (50 or 100%) had no effect on the chlorophyll content, both in normal and abnormal seedlings. In the case of near infrared radiation, the content of chlorophyll in seedlings whose seeds were radiated for 3 minutes was 18.5% higher than in those which underwent 5-minute long radiation.

Table 5 presents the comparison of chlorophyll content between normal and abnormal soybean seedlings in each group. The highest differences in the SPAD value between normal and abnormal seeds were observed for the group treated with NIR for 3 minutes – for normal seedlings the average SPAD value was 38% higher than for the abnormal ones, for NIR 5 it was 31% higher. The differences in the groups treated with E50 (12.5% higher) and E100 (13.7% higher) were comparable to the control group (13.4% higher) and were statistically significant. It was also shown that the treatment of the seeds with NIR3 E50 and NIR5 E50 resulted in the smallest difference in the SPAD value between normal and abnormal seeds, it was 5.5% and 3.0%, respectively.

4 Discussion

In the present study the effect of different factors – NIR, macroalgal extracts and their combination on the germination of soybean seeds was examined. Light is one of the most important environmental factors exerting a strong influence on the vital processes of biological samples, i.e. plants. It is captured by special photoreceptors (i.e. phytochromes, cryptochromes and phototropins) and next plants generate a wide range of specific physiological responses through these receptors. Light can influence, for example, plant morphology and composition [37]. Light radiation is used by plants as a source of energy during photosynthesis as well [38]. It also affects a number of other photosynthesis-independent processes related to plant growth and development, this is known as photomorphogenesis [39]. These processes take place throughout the life cycle of the plant, from germination through vegetative and generative development to the aging of plants. Generally, light can affect plant growth and biomass production. In many works, there was a strong dependence between the quantity and quality of available light and plant productivity [40]. Insufficient quantity and/or inadequate spectral composition of light may cause not only limitation of the yield value obtained, but also deterioration of its quality [41]. For example, green light has reduced photosynthesis [42].

Table 3: Descriptive statistics for the measurements of chlorophyll by SPAD method in the normal seedlings of soybean.

Group	Average*	SD	Chl-Q25	Chl-Median	Chl-Q75	Percentyl-10	Percentyl-90
1 C	53.07	0.88	52.40	53.26	53.74	51.89	53.87
2 NIR3 E50	51.28	3.38	49.16	50.29	53.39	48.37	56.15
3 NIR3 E100	55.79 ^a	1.12	55.04	55.93	56.53	54.30	57.00
4 NIR5 E50	55.17 ^b	1.42	54.12	55.58	56.22	53.22	56.31
5 NIR5 E100	49.32	1.59	48.16	49.85	50.48	47.11	50.49
6 NIR3	52.65	0.35	52.42	52.70	52.87	52.17	53.01
7 NIR5	42.92 ^{ab}	5.98	37.91	42.41	47.92	37.21	49.64
8 E50	48.52	1.90	47.24	48.85	49.81	45.96	50.44
9 E100	48.07	1.00	47.24	47.94	48.89	47.15	49.24

* average from 4 replications (in each replication – 10 measurements of chlorophyll)

a. b – statistically significant differences for $p < 0.05$ (Kruskal-Wallis test; abnormal distribution)

Table 4: Descriptive statistics for the measurements of chlorophyll by SPAD method in abnormal soybean seeds.

Group	Average*	SD	Chl-Q25	Chl-Median	Chl-Q75	Percentyl-10	Percentyl-90
1 C	45.96 ^{ab}	3.93	43.28	45.93	48.64	41.21	50.78
2 NIR3 E50	48.48 ^{cd}	7.15	43.24	50.18	53.71	38.70	54.86
3 NIR3 E100	45.89 ^{ef}	2.88	43.95	46.75	47.82	41.77	48.29
4 NIR5 E50	53.53 ^{ghij}	2.21	52.02	53.99	55.04	50.49	55.67
5 NIR5 E100	44.12 ^{kl}	3.78	40.90	43.87	47.33	40.58	48.15
6 NIR3	32.46 ^{acegkm}	4.41	28.66	32.29	36.25	28.49	36.75
7 NIR5	29.48 ^{bdfhino}	2.30	27.79	28.74	31.18	27.78	32.67
8 E50	42.46 ^{imn}	4.32	39.79	43.94	45.13	36.14	45.81
9 E100	41.47 ^{jo}	3.02	39.43	40.71	43.51	38.74	45.72

* average from 4 replications (in each replication – 10 measurements of chlorophyll)

a. b... – statistically significant differences for $p < 0.05$ (ANOVA test; normal distribution)

Near-infrared radiation is a part of IR portion of the electromagnetic spectrum extending from visible red light to the microwave region. More than 50% of NIR light is in the spectrum of solar radiation. Generally, near-infrared light-tissue interaction concerns both thermal and photochemical effects. The NIR radiation is absorbed by the main components of tissue and transformed into internal energy that produces a local temperature increment. After photon absorption the photochemical reactions can also occur in tissues or their components [43,44]. NIR can penetrate deep into human tissue where it can cause photochemical changes [45]. The kind of observed reaction depends on different conditions, i.e. optical tissue properties and light parameters.

One of the main human, animal and plant tissue components is water. Tissue area which has a rich water content will strongly absorb NIR photons, especially from

600–1200 nm, the so-called “biological” or “therapeutical window” [46,47]. NIR radiation is weakly scattered by spatial structures, which allows the penetration of light in tissues. A specific NIR spectrum can stimulate different morphological and physiological responses. To our knowledge, this is the first time this kind of radiation has been used for biostimulation of soybean seeds.

Additionally, near infrared radiation was combined with macroalgal extracts, which are used in agriculture as foliar applications and/or seed treatment, specifically as the biostimulants of plant growth or bioregulators [12]. It is well known that macroalgal extracts have beneficial effects on plants, probably due to the synergistic influence of many factors. However, their mode of action still remains unknown [4,11]. The chemical composition of algal extracts can be relevant to plants. It is hypothesised that the growth enhancing potential of macroalgal extracts

Table 5: Comparison of chlorophyll content between normal (N) and abnormal (A) soybean seedlings in each group (SPAD measurements).

Group	Average*	SD	<i>p</i> (<0.05)**
C N	53.07	0.88	0.0124
C A	45.96	3.93	
NIR3 E50 N	51.28	3.38	0.506
NIR3 E50 A	48.48	7.15	
NIR3 E100 N	55.79	1.12	0.000686
NIR3 E100 A	45.89	2.88	
NIR5 E50 N	55.17	1.42	0.258
NIR5 E50 A	53.53	2.21	
NIR5 E100 N	49.32	1.59	0.0442
NIR5 E100 A	44.12	3.78	
NIR3 N	52.65	0.35	0.0000970
NIR3 A	32.46	4.41	
NIR5 N	42.92	5.98	0.00572
NIR5 A	29.48	5.98	
E50 N	48.52	1.89	0.0423
E50 A	42.46	4.32	
E100 N	48.07	1.00	0.00603
E100 A	41.47	3.02	

* average from 4 replications (in each replication – 10 measurements of chlorophyll)

** statistically significant differences between two groups (*in italics*) for $p < 0.05$ (test *t*; normal distribution)

can result from the presence of micro and macroelements [11]. Moreover, they can increase/stimulate the nutrient uptake by plants [3,10,11].

In the present study it was shown that the application of NIR affected germination and seedling growth. The average number of germinated seeds in both groups treated with NIR (for 3 and 5 minutes) was higher than in the control group, but this difference was not statistically significant. The application of the combination of NIR with macroalgal extracts did not enhance the germination of seeds. Soaking of seeds aims at a more rapid imbibition, resulting in more rapid seed germination [48]. However, in the present study the soaking of soybean seeds in extract turned out to be an ineffective method, due to swelling of seeds. Almost half of them were classified as abnormal seeds. Soaking of seeds can be recommended to seeds that are hard to start or have trouble with germinating. Therefore, in future experiments, other methods of algal extract applications should be tested – for example: foliar spray or soil application.

On the other hand, the measurements of the relative index of chlorophyll concentrations in soybean seedling leaves showed that it was the highest in the groups treated with the combination of NIR and the macroalgal extract (when comparing to other experimental groups, as well as control group). The highest relative index of chlorophyll content in the normal seedlings was observed in the group – NIR3 E100. It can be supposed that NIR stimulated seed germination, whereas algal extracts being a rich source of biologically active compounds affected cellular metabolism in treated plants leading to enhanced growth and crop yield [4]. Seaweed products are also known to enhance plant chlorophyll content due to increased photosynthetic efficiency [4,49,50]. The results of the experiments also showed that the concentration of the macroalgal extract influences both – the average number of germinated seeds, as well as the relative index of chlorophyll content in soybean seedling leaves. Generally, lower concentrations of extracts are recommended. According to the literature data, seaweed extracts are bioactive at low concentrations – diluted as 1:1000 or more [51]. Sivasankari, et al. (2006) indicated that seeds soaked with lower concentrations of seaweed extracts (5, 10 and 20%) (from *Sargassum wightii* and *Caulerpa chemnitzia*) showed higher rates of *Vigna sinensis* germination, while higher concentrations (30, 40, 50 and 100%) inhibited the germination [52]. It was also found that among different concentrations (0, 2.5, 5.0, 7.5, 10 and 15%) of seaweed extracts (*Kappaphycus* and *Gracilaria*) used as a foliar spray on soybean, the best results concerning growth, flowering behaviour and yield were obtained by a 15% extract from *Kappaphycus* [10]. In the future, we plan to test macroalgal extracts at concentrations lower than 50%.

5 Conclusion

In conclusion, soybean has become more and more popular in Europe because of high protein and fat content, hence there is a great need to increase the production of this crop. So, it is important to test new, innovative factors (macroalgal extracts and near infrared radiation) and implement them to practice in order to speed up the germination ability and final seed yield. A huge part of the feed industry sector indicates the need to increase soy production.

The preliminary results, presented in this study show that the combination of macroalgal extracts and near infrared radiation can influence the seed germination, as well as the quality of the cultivated plants. The highest

percentage of germinated seeds was observed for NIR5, whereas the highest relative index of chlorophyll content in the group NIR3 E100, indicating that macroalgal extracts can stimulate physiological processes in plants. However, further experiments with an increased number of stimulating factors are required. They will concern the method of application of algal extracts, their concentration, frequency of application, as well as the stimulation of seeds not only with near infrared radiation but also with a magnetic field at different doses and exposure time.

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