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Outcome of Microalgae Biomass Application on Seed Germination and Hormonal Activity in Winter Wheat Leaves

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Abstract: The present work aimed to test selected microalgae strains from the Mosonmagyaróvár Algae Culture Collection (MACC) on germination ability and certain physiological processes in winter wheat (*Triticum aestivum* L.) plants. Germination tests showed substantial differences between the strains, meaning that certain strains (such as MACC-430, MACC-612, MACC-922) improved the germination processes while others performed worse (MACC-438, MACC-755) than the control in a concentration-dependent manner. The germination index of seeds treated with MACC-430 @ 1 g L^{-1} concentrations was 87, while that of the control was 45. The mungbean rooting bioassay proves that microalgae biomass may exhibit auxin-like activity, especially in strain MACC-612 (*Nostoc* sp.), which was characterized by the highest endogenous level of plant growth regulator indole-3-acetic-acid among the selected strains. Foliar spray on the leaves of developed plants did not significantly alter the photosynthetic processes, but it influenced the secondary metabolite composition. After the application of microalgae biomass, there were also changes in plant hormones, including salicylic acid, abscisic acid, and jasmonic acid-leucine/isoleucine conjugate compositions, which play a role in plant stress signaling in plants. A decrease in indole-3-acetic acid was also observed in the Mv Nádor cultivar. These results suggest that the application of certain microalgae strains can be used effectively to improve the germination of wheat seeds, and as a foliar spray, they may also modify the acclimation processes in a genotype-dependent way.

Keywords: germination; microalgae biomass; secondary metabolites; indole-3-acetic acid; abscisic acid; *Triticum aestivum* L.

Citation: Mutum, L.; Janda, T.; Darkó, É.; Szalai, G.; Hamow, K.Á.; Molnár, Z. Outcome of Microalgae Biomass Application on Seed Germination and Hormonal Activity in Winter Wheat Leaves. *Agronomy* **2023**, *13*, 1088. https://doi.org/10.3390/agronomy13041088

Academic Editors: Manuel Ângelo Rosa Rodrigues, Carlos M. Correia, Paolo Carletti and Antonio Ferrante

Received: 6 March 2023
Revised: 6 April 2023
Accepted: 8 April 2023
Published: 10 April 2023

1. Introduction

Seed germination is controlled by several mechanisms, including plant hormones, interactions between plant hormones and secondary metabolites, genetic factors, external conditions, etc. Algae products may contain several phytohormones, including auxins, cytokinins, abscisic acid, gibberellins, etc. These hormones strongly affect seed germination and embryo development and can also influence the further development of plants. Most of the algae-related hormone measurements were carried out on the more complex macroscopic algae, which have been successfully utilized as seaweed extracts in agricultural practice for many years [1]. Besides phytohormones, free phenolic compounds were also detected in the acetone extract from *Ulva lacuna*. Vanillin and *p*-coumaric acid were recorded as among the most abundant compounds, while ferulic acid and salicylic acid existed in smaller amounts [2]. To date, most polyphenols isolated from marine sources and referenced in the literature have been of macroalgal origin [3]. However, in microalgae, hormone measurement has been mainly limited to endogenous gibberellins and brassinosteroids [4].

Although algae and higher plants belong to distinct evolutionary lineages, they dispose of similar biological and ecological functions, and their chemical defense responses

also show similarities [5]. The beneficial effects of microalgae extract on the growth and development of higher plants have already been established [6,7]. Recent results also indicate that certain microalgae can be efficiently used for the removal of certain drugs from wastewater, including salicylic acid [8,9]. The possible stimulating effects of certain microalgae extracts have been studied on wheat plants [10]. They were applied as foliar fertilizers, and they improved the uptake of nutrients, leading to enhanced growth of roots and shoots. Such properties have proven to be exhibited in winter wheat using MACC-612 in field conditions [11]. Furthermore, exogenous microalgae have also contributed to the reduction of membrane damage, proved by significantly decreased levels of malondialdehyde in the leaves of broccoli plants exposed to drought stress [7]. Phenolic or polyphenolic compounds are also products of the secondary metabolism of algae. The differences in phenolic content and composition among algae have taxonomic, ecological, and environmental aspects. In general, phenolic compounds tend to be more abundant in young, actively growing, and thus optimally productive seed portions [12].

The present work aimed to compare the effects of eight microalgae strains on the seed germination of wheat and to demonstrate changes in the metabolite composition of the leaf after the application of selected microalgae strains.

2. Materials and Methods

2.1. Growth Conditions of Algae Cultures and Preparation of Microalgae Biomass

The microalgae strains MACC-755 (*Chlorella vulgaris*), MACC-922 (*Chlorella vulgaris*), MACC-612 (*Nostoc linckia*), MACC-683 (*Nostoc* sp.), MACC-430 (*Chlamydopodium fusiforme*), MACC-677 (*Tetradesmus obliquus*), MACC-519 (*Chlorella sp.*), and MACC- 438 (*Chlorella sorokiniana*) were derived from Mosonmagyaróvár Algae Culture Collection (MACC), Hungary (Figure 1). The strains were incubated at 25 ± 2 °C, in a 12:12 light/dark cycle. The microalgae biomass was produced in laboratory culture units. It was illuminated from below with a light intensity of 130 µmol m^{-2} s^{-1} and grown in Tamiya nutrient solution [13], with a starting concentration of 10 mg L^{-1} dry algal weight (dwt). In total, 20 L h^{-1} of filtered compressed air enriched with 1.5% CO_2 during the light period was used for aerating the culture strains [14]. The cultures grown in these conditions for 10 days were then centrifuged for 15 min at 3000 rpm (Sigma 6 K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) and freeze-dried using Gamma 1–20 (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and stored at −18 °C. Biomass samples were re-suspended in distilled water and sonicated (VirTis, VirSonic 600 Ultrasonic Cell Disruptor-SP VirTis, Gardiner, MT, USA) 3 min just before plant treatments.

2.2. Germination Test

Factorial Complete Randomized Design was used for the experimental design (FCRD). For the bioassay, eight strains were taken with four different concentrations from Mosonmagyarovar Algae Culture Collection (MACC). A total of 4 replications were taken per treatment. The eight strains were selected as they are fast-growing species and produce high biomass in laboratory culture conditions among the strains in the culture collection (MACC).

The freeze-dried biomass of microalgae strains MACC-922, MACC-612, MACC-683, MACC-755, MACC-430, MACC-677, MACC-519, and MACC-438 was taken as the main treatment at four different concentrations, viz., 0.1 g L^{-1}, 0.3 g L^{-1}, 0.5 g L^{-1}, and 1 g L^{-1} taken as the sub-treatments. The concentrations were selected as per the logarithmic scale. The highest concentration was determined as per findings in previous experiments conducted in the Department of Plant Science in Mosonmagyarovar [4,11]. The germination test was for the selection of strain, and we felt comparison with standards could be neglected as it was not needed to compare the potentiality with an already standardized value. The control was treated with distilled water.

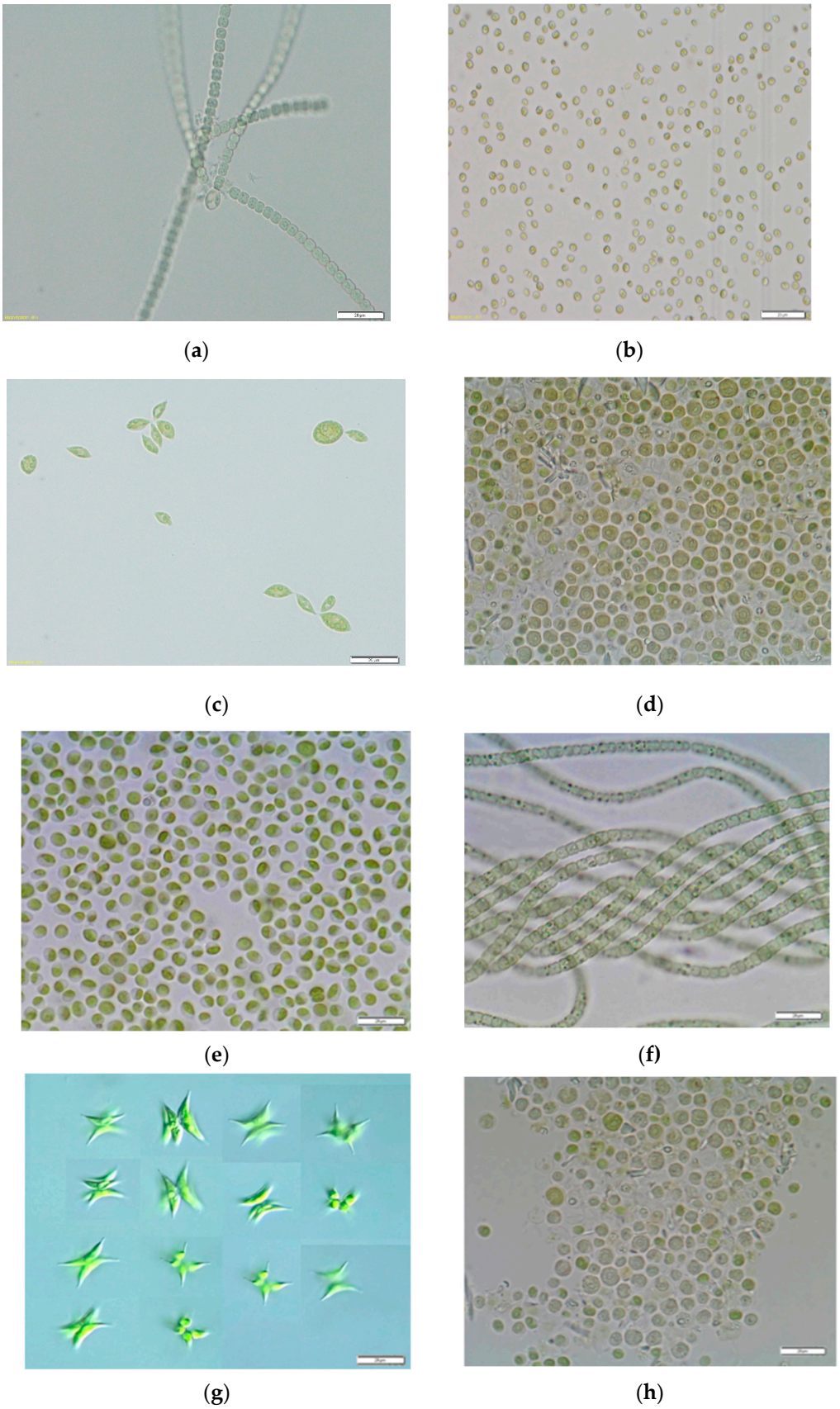

Figure 1. Images of the eight strains involved in the initial germination test (**a**) MACC-683, (**b**) MACC-755, (**c**) MACC-430, (**d**) MACC-922, (**e**) MACC-519, (**f**) MACC-612, (**g**) MACC-677, (**h**) MACC-438. The bar in the figure indicates 20 μm.

Ten seeds of winter wheat (*Triticum aestivum* L. Mv Nádor), laid between two filter papers, were placed in a Petri dish of 9 cm. Each was replicated three times. Microalgae strain biomass of different concentrations was used in each treatment. The solutions were used only once at the initial stage of seeding; later, the moisture requirement was supplied by ionized water. The germination recordings were carried out every morning at 9 am. The Petri dishes were kept at 8 °C for 7 days (a total of 56-degree days). The radicle length was taken by the traditional method using thread and a scale. The number of germinated seeds with emerged coleoptile was also recorded. The germination index, the speed of germination, and the germination rate index were calculated using the recorded data. The formulas were as follows:

$$\text{Germination index (GI)} = (7 \times N1) + (6 \times N2) + \ldots\ldots\ldots\ldots\ldots + (1 \times N7)$$

where N1, N2 … … N7 is the number of germinated seeds on the first, second, and subsequent days until the 7th day, and the multipliers are the weights given to the days of germination.

$$\text{Speed of germination (SG)} = N1/D1 + N2/D2 + N3/D3\ldots\ldots\ldots\ldots + N7/D7$$

where N = number of germinated seeds and D = days of germination.

$$\text{Germination rate index (GRI), \%/day} = G1/1 + G2/2 + \ldots\ldots\ldots + G7/7$$

where G1, G2 … … G7 = Germination percentage on each day × 100

2.3. Mungbean Rooting Bioassay

One of the characteristic effects of auxins is their role in the induction of adventitious roots on stem cuttings. This effect was utilized in a bioassay developed by Hess [15]. The bioassay is simple to perform and largely insensitive to the presence of inhibitors. The bioassay was conducted with a control, three concentrations of indole butyric acid (IBA), MACC-430, MACC-612, MACC-922, and MACC-438, replicated 4 times. The concentrations of the positive control, IBA, were 0.3, 0.5, and 0.7 mg L^{-1}, and that of the microalgae biomass was 1g L^{-1}. Out of the 8 strains based on radicle length and germination parameters, the 3 better strains from different genera, MACC-612, MACC-430, and MACC-922, were selected; additionally, MACC-438 was used in the bioassay for better comparison because its performance was the worst of all treatments. The concentration 1 g L^{-1} was used for the strains in the bioassay as there was no huge difference between the concentrations in the germination test and to make it feasible for higher research. The mung bean (*Vigna radiata* (L.) Wilczek) seeds were soaked for 4 min in a 0.33% sodium hypochlorite solution, then removed and rinsed under running tap water for 24 h. The seeds were planted at a depth of 1 cm in moistened perlite in plastic trays. The trays were placed in the growth chamber maintained at 27 °C and relative humidity of about 60 to 65%, illuminated with fluorescent lamps for 7 days. The seedlings should have fully expanded unifoliate and unexpanded (rolled) trifoliate leaves in the bud. The seedlings were then cut with a clean razor 3 cm below the cotyledons. Uniform seedling cuttings were selected for further use. They consisted of a 3 cm hypocotyl, the epicotyl, the unifoliate leaves, and the trifoliate leaf bud. The cotyledons were carefully removed.

Five seedling cuttings were placed in vials of 25 × 90 mm (three vials per treatment) containing 10 mL of distilled water, algal nutrient solution as controls, and algal suspension (generally 2 g L^{-1} dry matter) as a treatment for 6 h of soaking. For the comparison of the treatments, a specific auxin, such as indole-3-butyric acid (IBA), can be used in concentrations of 0.3, 0.5, and 0.7 mg L^{-1} as a positive control. After the soaking, seedling cuttings were rinsed with distilled water and placed back into the vials with 10 mL of distilled water. They were placed back in the original growth conditions for 7 days. The solution level (lost by transpiration) was restored to its original state with distilled water

daily. After the incubation period, the number of roots (longer than 1 mm) was counted on each hypocotyl. The number was directly proportional to the auxin concentration within the assay range. The mean number of roots derived from each vial must be compared to the controls; then, it could be analyzed using the comparison concentrations made by a specific auxin (IBA).

2.4. Growth Conditions of Wheat Plants and Microalgal Treatments

Winter wheat plants (Mv Béres and Mv Nádor cultivars) were sown in plastic pots (20/pot) and were grown in loamy soil under greenhouse conditions at 22/20 °C under natural light supplemented with artificial metal halide lamp illumination when the light intensity was lower than 250 μmol m^{-2} s^{-1}. A complete randomized design was used for these experiments with 5 treatments and 5 replications. Plants were watered according to plant requirements, usually 2–3 times a week. Specifically, 15–17-day-old wheat plants were foliar-sprayed with different microalgae (MACC-612, MACC-430, MACC-922, MACC-438) biomass at 1 g L^{-1} concentration. Control plants were sprayed with tap water. Measurements and sample collections were carried out as indicated in the experiments.

2.5. Photosynthesis Measurements

Gas exchange parameters were determined using a Ciras 3 Portable Photosynthesis System with a narrow (2.5 cm^2) leaf cuvette holder (PP, Systems Company, Amesbury, MA, USA) as it was previously described [16]. Briefly, net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), and intracellular CO_2 concentration (Ci) were determined at the steady-state level of photosynthesis using a CO_2 level of 390 μL L^{-1} and photosynthetic active photon flux density (PPFD) of 450 μmol m^{-2} s^{-1}. Five leaves were measured for each treatment.

Chlorophyll-*a* fluorescence induction measurements were carried out using a pulse amplitude-modulated fluorometer with a blue LED-Array Illumination Unit (Imaging-PAM M Series, Walz, Effeltrich, Germany), as described earlier [17]. Briefly, leaf samples were dark-adapted at least 30 min before the measurements. After the determination of the maximum quantum efficiency (Fv/Fm) using a saturation pulse, during the quenching period, a continuous PPFD = 270 μmol m^{-2} s^{-1} actinic light was applied until it reached the steady-state level. The fluorescence induction parameters were calculated as described by Klughammer and Schreiber [18].

2.6. Metabolomics Analyses

An Acquity I/Class UPLC system—Xevo TQ/XS (Waters, Milford, MA, USA) tandem mass spectrometer was used for the quantification of the four strains, viz., MACC-612, MACC-430, MACC-922, and MACC-438. The method was explained in [19]. The freeze-dried samples were diluted with water in ratios of 1:250, 1:500, 1:1000, 1:1500, or 1:2000 (*v/v*), depending on the plant species, to maximize their stimulatory biological activity. Combinations of tandem mass spectrometry and gas or liquid chromatography are popular analytical techniques for the analysis of plant hormones and related compounds, providing the ultra-high sensitivity and selectivity of mass analyzers with excellent separation of analytes in samples with complex biological matrices [20]. The same method was used for the quantification of leaf samples. Sample chromatograms are presented in the Supplementary Material. The list of target compounds monitored during LC/MS/MS analysis, sample chromatograms of compounds found in wheat leaves and algae, standard chromatograms, and examples for the limit of quantitation (LoQ) for various compounds are presented in the Supplementary Material.

2.7. Statistical Analyses

Correlation analysis was conducted using the Pearson correlation coefficient (SAS 9.2) to find the relation between germination and secondary metabolites. Significant differences

between the treatments and the genotypes were probed using the *t*-test method and ANOVA table (Microsoft 365 Apps for enterprise, version 2112– Microsoft Co., Seattle, DC, USA).

3. Results

3.1. Germination Bioassay

In the first experiment, the concentration-dependent effects of various types of algal strain biomass on the germination of a winter wheat Mv Nádor were tested. All parameters of germination are shown in Table 1. MACC-430 at 1 g L^{-1} concentration presented the highest germination index (GI), while the lowest value occurred with MACC-438 at 1 g L^{-1}. Overall, every treatment was better compared to the control except for MACC-438 of all four concentrations and MACC-755 at 1 g L^{-1}. In some strains, the lower the concentration, the higher the GI observed, while some strains, such as MACC-430 and MACC-683, performed better at higher concentrations (Table 1). As for the speed of germination, the concentration of 0.3 g L^{-1} was average in all the strains. In MACC-922, MACC-755, and MACC-612, the seeds germinated fastest in the 0.3 g L^{-1} concentration.

Table 1. Different germination parameters of Mv Nádor winter wheat under the influence of various concentrations and strains of microalgae. The critical difference (CD) with a *p*-value of 0.01 (1%) and standard error (SEd) was calculated.

Treatments	Germination Index	Germination Rate Index	Speed of Germination	Radicle Length on 7th Day (cm)
MACC-430@ 0.1 gL^{-1}	53	45.79	4.58	1.1
MACC-430@ 0.3gL^{-1}	78	62.29	6.23	1.1
MACC-430@ 0.5 gL^{-1}	65	53.79	5.38	1.2
MACC-430@ 1 gL^{-1}	87	68.12	6.81	1.2
MACC-683@ 0.1 gL^{-1}	48	42.95	4.30	1.0
MACC-683@ 0.3 gL^{-1}	61	51.45	5.15	0.9
MACC-683@ 0.5 gL^{-1}	74	59.62	5.96	1.2
MACC-683@ 1 gL^{-1}	77	61.79	6.18	1.2
MACC-922@ 0.1 gL^{-1}	68	55.79	5.58	1.2
MACC-922@ 0.3 gL^{-1}	74	59.62	5.96	0.9
MACC-922@ 0.5 gL^{-1}	62	51.95	5.20	0.9
MACC-922@ 1 gL^{-1}	70	57.29	5.73	0.8
MACC-519@ 0.1 gL^{-1}	66	54.12	5.41	0.8
MACC-519@ 0.3 gL^{-1}	62	51.95	5.20	0.8
MACC-519@ 0.5 gL^{-1}	54	46.95	4.70	0.6
MACC-519@ 1 gL^{-1}	57	48.95	4.90	0.7
MACC-612@ 0.1 gL^{-1}	75	60.29	6.03	1.2
MACC-612@ 0.3 gL^{-1}	78	62.29	6.23	1.1
MACC-612@ 0.5 gL^{-1}	77	61.45	6.15	1.0
MACC-612@ 1 gL^{-1}	62	51.95	5.20	1.1
MACC-677@ 0.1 gL^{-1}	57	48.29	4.83	0.7
MACC-677@ 0.3 gL^{-1}	61	51.12	5.11	0.7
MACC-677@ 0.5 gL^{-1}	56	47.79	4.78	0.6
MACC-677@ 1 gL^{-1}	55	47.45	4.75	0.7
MACC-755@ 0.1 gL^{-1}	61	51.45	5.15	0.5
MACC-755@ 0.3 gL^{-1}	58	49.45	4.95	0.5
MACC-755@ 0.5 gL^{-1}	64	53.45	5.35	0.6
MACC-755@ 1 gL^{-1}	39	36.95	3.70	0.5
MACC-438@ 0.1 gL^{-1}	36	34.95	3.50	0.3
MACC-438@ 0.3 gL^{-1}	34	33.29	3.33	0.2
MACC-438@ 0.5 gL^{-1}	32	31.62	3.16	0.2
MACC-438@ 1 gL^{-1}	29	29.62	2.96	0.3
CONTROL	45	40.12	4.01	0.8
CD @1%	4.5396	3.926	0.9246	0.1444
SE d	1.7115	0.987	0.3486	0.0544

The radicle lengths on the 7th day were the highest in the case of MACC-612, MACC-430, and MACC-683 strains. For MACC-612, the lowest concentration, 0.1 g L^{-1}, had the longest radicle length, while in MACC-430, 1 g L^{-1} presented the longest radicle length. In addition, in MACC-683, only the higher concentrations (0.5 and 1 g L^{-1}) showed longer radicle lengths than the control (Table 1). The strain MACC-438 appeared to have a consistently lower value when compared with the control in all the parameters. It seemed to affect germination, and in parameters such as the speed of germination and the germination index, a considerable decline in the values was experienced with increasing germination. On the other hand, MACC-677 and MACC-755 also performed worse than the control, depending on the concentration.

After the germination test, the strains with overall better performance than the control, viz., MACC-612, MACC-430, and MACC-922, and the strain that showed a negative effect on the germination of wheat, i.e., MACC-438, were used for further experiments with 1 g L^{-1} concentration.

3.2. Mungbean Rooting Bioassay

Mungbean rooting bioassay was conducted for the confirmation of the auxin-like activity of the selected strains at 1 g L^{-1} (Figure 2a,b).

Mungbean plants treated with microalgae exhibited strain-dependent auxin-like activity, and the number of their rootlets was correlated to the concentration equivalent of IBA. Figure 2 shows that MACC-612 produced the highest level of root development in mungbean plants, while MACC-430 caused no significant effect.

3.3. Metabolite Characterization of the Microalgae Strains

Metabolomics analyses focusing mainly on phenolic compounds in the algae strains were also carried out (Table 2). The secondary compounds detected are indole-3-acetic acid, salicylic acid, para-hydroxybenzoic acid, benzoic acid, and trans-cinnamic acid. All strains contained salicylic acid, para-hydroxybenzoic acid, and benzoic acid, while strain MACC-612 had additional polyphenols such as trans-cinnamic acid. The amount of all the polyphenols was higher in MACC-612 than in the others, while the differences between the other three strains were negligible. Interestingly, MACC-612 also showed a relatively high amount of IAA content, while in the other strains, this hormone was under the detection limit.

3.4. Effects of Algae Strains on Photosynthesis and Metabolite Contents of Wheat

Based on the above-mentioned results, a pot experiment was designed to investigate the long-term effects of the physiological processes of microalgae treatments in the vegetative state of wheat plants. First, 15-day-old non-vernalized Mv Béres cultivar wheat plants were sprayed with the biomass of different algae strains, then 20 days after the spraying, photosynthetic parameters were measured, and leaf samples were collected for metabolome analyses. Previous studies indicated that salicylic acid may also influence the photosynthetic efficiency in plants [21–23]. This can be due to the modification of stress acclimation processes, leading to crosstalk between various signaling pathways [24–30]. Since all the algae strains contained salicylic acid together with its putative precursors, benzoic acid and trans-cinnamic acid, we mainly focused on the photosynthesis-related processes. Different gas exchange parameters, such as net photosynthesis (Pn), stomatal conductivity (gs), or intercellular CO_2 concentration (Ci) and chlorophyll-a fluorescence induction values, such as Fv/Fm and $\Delta F/Fm'$, indicating the maximum and actual photochemical efficiency of Photosystem 2, respectively, or the Y(NPQ) and Y(NO), indicating the regulated and non-regulated non-photochemical quenching processes, respectively, suggested that neither the primary carbon assimilation nor the photosynthetic electron transport process was significantly affected by the treatments with algae strains used in the present experiment (Table 3).

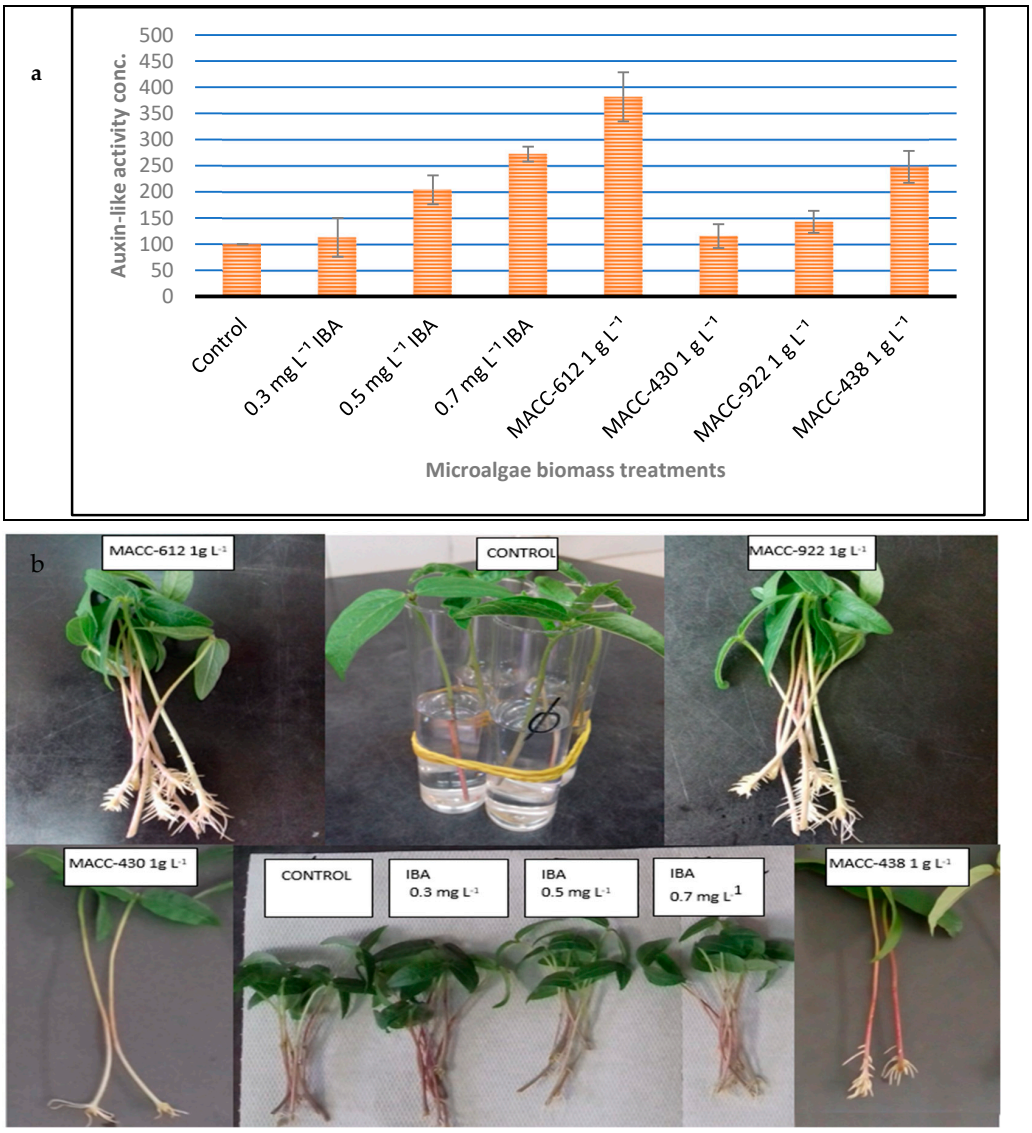

Figure 2. (**a**) Mungbean hormonal activity bioassay for 4 selected microalgae strains, compared with the physiological effect of different concentrations of IBA. Number of rootlets calculated as concentrations of auxin-like substance under the influence of auxin-like hormone present in the treatments. (**b**) An image of mungbean bioassay with IBA (indole butyric acid) as a positive control to compare with the 4 microalgae strains biomass.

Table 2. Determination of indole-3-acetic-acid (IAA), salicylic acid (SA), p-hydroxybenzoic acid (pHBA), benzoic acid (BA), and t-cinnamic acid (tCA) from freeze-dried algae biomass samples using an Acquity I/Class UPLC system - Xevo TQ/XS tandem mass spectrometer. Data represent mean ng g^{-1} DW ± SD; n = 3. n.d.: not determined, under the detection limit. Different letters indicate significant differences at $p < 0.05$.

	IAA	SA	pHBA	BA	tCA
MACC-438	n.d.	21.1 ± 1.3 bc	86.3 ± 6.6 b	798.3 ± 84.9 b	n.d.
MACC-430	n.d.	22.5 ± 0.7 b	65.8 ± 4.2 c	971.7 ± 55.4 b	n.d.
MACC-612	59.3 ± 2.6	35.1 ± 1.9 a	698.3 ± 27.8 a	2210.0 ± 63.8 a	38.2 ± 1.0
MACC-922	n.d.	14.6 ± 3.5 c	80.7 ± 3.7 b	883.3 ± 48.7 b	n.d.

Table 3. Chlorophyll-*a* fluorescence induction and gas exchange parameters in the control and the alga-treated Mv Béres wheat plants 28 days after spraying. Pn: net photosynthesis (μmol m^{-2} s^{-1}); gs: stomatal conductivity (mmol m^{-2} s^{-1}); Ci: intercellular CO_2 concentration (μmol CO_2 mol^{-1}); E: transpiration (mmol m^{-2} s^{-1}), WUE: water use efficiency (Pn/E). Data are mean ± SD values.

	Control	MACC-922	MACC-430	MACC-612	MACC-438
Fv/Fm	0.784 ± 0.01	0.788 ± 0.006	0.786 ± 0.004	0.780 ± 0.011	0.778 ± 0.013
Y (II)	0.541 ± 0.04	0.556 ± 0.022	0.550 ± 0.020	0.530 ± 0.029	0.535 ± 0.039
Y(NO)	0.228 ± 0.03	0.219 ± 0.041	0.242 ± 0.025	0.250 ± 0.033	0.253 ± 0.028
Pn	12.8 ± 2.4	14.9 ± 3.9	12.6 ± 2.2	14.4 ± 1.2	9.3 ± 1.2
gs	337 ± 157	525 ± 183	358 ± 98	438 ± 54	246 ± 51
Ci	195 ± 33	210 ± 17	202 ± 16	200 ± 17	197 ± 8
E	3.85 ± 1.09	5.05 ± 0.90	4.05 ± 0.67	4.68 ± 0.37	3.20 ± 0.49
WUE	3.42 ± 0.49	2.95 ± 0.64	3.11 ± 0.21	3.09 ± 0.29	3.20 ± 0.49

In contrast to the photosynthetic parameters, certain secondary metabolites showed significant differences between the control and the sprayed plants (Table 4; Béres1). While none of the algal treatments caused significant changes in indole-3-acetic acid, p-coumaric acid, abscisic acid, neochlorogenic acid, rutin, and naringenin, a slight but statistically significant increase was discovered in the salicylic acid content after the treatment with MACC-922 or in p-hydroxybenzoic acid after the treatment with MACC-430 or MACC-438. All the algae treatments significantly reduced the jasmonic acid and the jasmonic acid/isoleucine conjugate contents.

Table 4. Metabolome analysis of the leaves of wheat Mv Béres and Mv Nádor treated with different algal strains. Béres1 refers to data from the 1st experiment with Mv Béres; Béres2 and Nádor concern data from the 2nd experiments with Mv Béres and Mv Nádor, respectively. Data are in ng g^{-1} Fresh weight, mean ± SD, n = 5. * represents significant differences from the control at $p < 0.05$.

	Indole-3-acetic-Acid	Salicylic Acid	P-Hydroxybenzoic Acid	p-Coumaric Acid	Jamonic Acid	Abscisic Acid	Neochlorogenic Acid	Rutin	Naringenin	Jasmonic Acid-Leucine/ Isoleucine Conjugate
Béres1										
Control	1.03 ±0.21	47.2 ±6.5	25.9 ±1.0	19.7 ±5.0	106.4 ±11.5	3.92 ±0.26	118 ±65	27.7 ±3.7	1.28 ±0.20	50,194 ±7023
430	0.84 ±0.13	50.3 ±11.9	29.2 ±1.5 *	16.4 ±4.1	62.8 ±12.8 *	4.36 ±0.30	178 ±76	30.3 ±2.6	1.27 ±0.19	21,850 ±3644 *
438	1.02 ±0.22	49.1 ±10.1	29.3 ±2.5 *	29.0 ±11.4	72.8 ±15.4 *	3.83 ±0.29	105 ±17	31.9 ±4.2	1.61 ±0.36	27,942 ±5796 *
612	0.99 ±0.20	45.8 ±12.8	27.6 ±3.2	22.9 ±5.8	40.4 ±23.7 *	4.05 ±0.81	191 ±57	28.4 ±2.2	1.97 ±1.28	15,112 ±12,476 *
922	1.05 ±0.21	64.2 ±12.3 *	26.6 ±2.7	18.8 ±3.5	57.1 ±11.3 *	4.20 ±0.50	129 ±80	31.5 ±3.5	1.71 ±0.31 *	19,417 ±5716 *
Nádor										
Control	1.04 ±0.20	55.8 ±8.7	20.5 ±5.4	32.7 ±5.1	18.1 ±2.9	15.24 ±0.73	1271 ±405	3.4 ±0.8	3.94 ±1.40	9078 ±1267
430	0.78 ±0.13	44.8 ±5.0	18.1 ±2.5	27.4 ±8.2	22.9 ±4.9	8.61 ±2.31 *	1064 ±42	3.3 ±0.8	3.13 ±0.33	7405 ±2070
438	0.75 ±0.04 *	115.4 ±29.3 *	15.3 ±2.0	25.8 ±1.5 *	59.5 ±20.1 *	14.12 ±1.05	476 ±157 *	3.4 ±0.5	2.38 ±0.39	17,980 ±5702 *
612	0.78 ±0.06 *	50.8 ±9.1	19.6 ±0.4	29.2 ±5.1	41.4 ±7.8 *	9.56 ±0.85 *	522 ±277 *	3.4 ±0.3	3.09 ±0.37	11,842 ±2164
922	0.69 ±0.06 *	67.0 ±6.3	19.1 ±3.1	26.4 ±3.6	20.9 ±3.2	7.34 ±0.30 *	760 ±155 *	3.5 ±0.5	2.82 ±0.35	9934 ±1857

Table 4. *Cont.*

	Indole-3-acetic-Acid	Salicylic Acid	P-Hydroxybenzoic Acid	p-Coumaric Acid	Jamonic Acid	Abscisic Acid	Neochlorogenic Acid	Rutin	Naringenin	Jasmonic Acid-Leucine/ Isoleucine Conjugate
Béres2										
Control	0.98 ±0.12	142.2 ±8.7	19.8 ±1.1	14.0 ±1.4	17.8 ±3.2	6.51 ±0.42	302 ±101	22.7 ±5.3	2.26 ±0.25	5709 ±1081
430	1.05 ±0.11	114.3 ±29.3	25.1 ±5.4	16.8 ±2.6	16.7 ±4.3	7.28 ±0.22 *	481 ±285	24.4 ±8.6	3.12 ±0.83	4873 ±1436
438	0.99 ±0.03	98.7 ±14.6 *	19.4 ±1.2	14.6 ±5.1	12.4 ±3.2 *	6.75 ±0.36	605 ±319	19.9 ±6.8	2.23 ±0.21	3782 ±829 *
612	0.94 ±0.12	91.1 ±18.2	23.5 ±2.6	15.0 ±3.1	16.5 ±5.2	5.97 ±0.18	115 ±32 *	16.7 ±2.8	2.60 ±0.33	4202 ±1266
922	0.85 ±0.11	93.8 ±18.7 *	18.3 ±1.1 *	12.3 ±1.8	17.1 ±5.9	6.56 ±0.30	399 ±107	21.4 ±2.6	2.02 ±0.11	7795 ±4907

However, since the experiment was carried out under greenhouse conditions, where the environmental factors were only partly controlled, and most of the above-mentioned compounds are key components of stress signaling processes, we repeated the experiments with the same genotype Mv Béres and with another winter wheat cultivar, named Mv Nádor. The growth conditions were similar but not the same. Plants were treated with algae 17 days after sowing, and samples were taken 28 days after the spray. Since the first sampling was carried out on 13 November 2020 and the second sampling on 11 February 2021, light conditions could also slightly differ. Data from the second set of experiments also showed that certain algae treatments significantly affected the hormonal contents of wheat plants. However, a long-term field trial is needed to validate the noted effect.

3.5. Correlations

For both correlations, no conclusive information could be drawn; however, we see a constant in Table 5. Even if non-significant, all variables were positively related. The metabolites in the strains had weak to no relationship except the relationship between radicle length and the three observed metabolites, which were moderately related. Transpiration was moderately correlated to p-hydroxybenzoic acid and benzoic acid.

Table 5. Correlation between the three metabolites detected in all four selected strains and various parameters. The germination index and radicle length taken were for seeds treated with microalgae biomass at 1 g L^{-1} concentration. NS represents significant differences from the control at $p < 0.05$.

	Salicylic Acid	p-Hydroxybenzoic Acid	Benzoic Acid
Germination index	0.262 ^{NS}	0.101 ^{NS}	0.303 ^{NS}
Radicle length	0.415 ^{NS}	0.388 ^{NS}	0.412 ^{NS}
Net photosynthesis	0.045 ^{NS}	0.384 ^{NS}	0.345 ^{NS}
Stomatal conductivity	0.140 ^{NS}	0.315 ^{NS}	0.370 ^{NS}
Intercellular CO_2	0.178 ^{NS}	−0.010 ^{NS}	0.244 ^{NS}
Transpiration	0.215 ^{NS}	0.407 ^{NS}	0.450 ^{NS}

When the correlation was analyzed between the hormonal activity in the treated plant samples and the three major metabolites detected from the microalgae biomass, as shown in Table 6, an interestingly strong negative relationship was established between all three independent variables and the dependent variable, jasmonic acid. In the Béres 2 trial, a non-significant but observable relation was of rutin, and with the three metabolites, a strong negative relationship.

Table 6. Correlation between the metabolites present in the microalgae biomass and the metabolites extracted from microalgae biomass-applied leaves. * represents significant differences from the control at $p < 0.05$ and NS represents significant differences from the control at $p < 0.05$.

	Salicylic Acid	p-Hydroxybenzoic Acid	Benzoic Acid
Indole-3-acetic acid			
Béres1	−0.345 ^{NS}	0.013 ^{NS}	−0.184 ^{NS}
Nádor	−0.660 ^{NS}	−0.231 ^{NS}	−0.560 ^{NS}
Béres2	0.043 ^{NS}	−0.190 ^{NS}	−0.169 ^{NS}
Abscisic acid			
Béres1	0.226 ^{NS}	−0.028 ^{NS}	0.224 ^{NS}
Nádor	−0.500 ^{NS}	−0.289 ^{NS}	−0.552 ^{NS}
Béres2	−0.229 ^{NS}	−0.732 ^{NS}	−0.478 ^{NS}
Jasmonic acid			
Béres1	−0.903 *	−0.707 ^{NS}	−0.917 *
Nádor	0.544 ^{NS}	0.341 ^{NS}	0.382 ^{NS}
Béres2	−0.330 ^{NS}	0.033 ^{NS}	0.089 ^{NS}
Rutin			
Béres1	0.172 ^{NS}	−0.362 ^{NS}	−0.035 ^{NS}
Nádor	−0.218 ^{NS}	−0.018 ^{NS}	−0.039 ^{NS}
Béres2	−0.625 ^{NS}	−0.847 ^{NS}	−0.734 ^{NS}

4. Discussion

The comparison of different algal strains in germination tests with winter wheat showed that while certain strains positively affected the germination processes, others had no or even negative effects. To better understand the molecular mechanisms behind the effects, further physiological and biochemical experiments were carried out.

In the germination test, we found the radicle length was also influenced by the microalgae strain used. Before the quantification of metabolites in the strains, we selected four strains, and the mungbean rooting bioassays were conducted to evaluate their auxin content. A similar bioassay has been conducted in *Chlorella vulgaris* [31] and Scenedesmus obliquus [32] and was proven to have auxin-like activity. These bioassays proved the presence of auxin without quantification. Ulva extract could also stimulate root growth [2]. This effect of the Ulva extract resembles the effect of abscisic acid (ABA), which is a negative regulator of germination [33], but it shows a biphasic effect on primary root growth [34,35] and inhibits lateral root growth in concentrations that do not affect the primary root growth [36].

There are some important hormone-like and growth-regulation substances that may be involved in various physiological activities, such as brassinosteroids, jasmonic acid, polyamines, salicylates, and signal peptides [37]. We detected some of the substances in the strains, which could explain why even though no IAA was detected in some strains, we found auxin-like activity. Apart from MACC-612, IAA was under the detection limit in the other strains, which showed the differences in the composition of one strain from another, and that not all strains showing positive results in mungbean bioassay need to have IAA content.

According to Reigosa and P-Malvido [38], trans-cinnamic acid inhibited A. thaliana's total germination above 500 µM. Although this compound could also be detected in MACC-612, it seemed to have a negligible effect on germination, as the germination index was 62 at 1 g L^{-1} concentration, still higher than the germination index of the control, i.e., 45. This could be due to the fact that the concentration of trans-cinnamic acid in the solution containing algae biomass was lower by several orders of magnitude (Table 2) than what was found critical in the case of Arabidopsis. Similarly, the phytotoxic concentration of the p-hydroxybenzoic acid was 750 µM, but in the strains used in the present study, the concentrations were well below this critical value. In support of this result, no action of

p-hydroxybenzoic acid on Poa annua L. germination was reported [39]. In eight strains belonging to *Nostoc sp.*, *Chlorella sp.*, and *Leptolyngbya* sp., the salicylic acid (SA) content was in the range between 5 and 7 µg mL^{-1} [40]. Whereas in the quantification we conducted, salicylic acid content was between 14 and 35 ng g^{-1}. Thus, with the above-compared studies, the secondary metabolites in the algae were in a safe range for the plants.

To demonstrate the effects of algal treatment on physiological and biochemical processes in developed plants, in the following steps of the experiments, the algal strain was also used as a foliar spray on winter wheat varieties. For comparing different genotypes, Mv Nádor, the same variety used for germination tests, and another winter wheat variety, Mv Béres, were also used.

All the algal strains contained salicylic acid. Earlier findings showed that salicylic acid and related compounds may have indirect or direct effects on photosynthetic processes [21,29]. For example, a recent study also showed that salicylic acid could provide protection against the negative effects of drought stress, resulting in an increase in net photosynthesis in winter wheat [41]. Furthermore, the application of salicylic acid also influences the metabolite compositions of plants, affecting their stress tolerance, too [42,43]. In our experiments, none of the photosynthetic parameters was significantly affected by the algal spray. Its main reason is that the dose of salicylic acid was lower than the effective dose in other experiments. However, the metabolite compositions were significantly affected. Salicylic acid was detected in the freeze-dried strain, and it increased tremendously in the plants. The effect was seen clearly in experiments Béres1 and Nádor. This could mean a direct effect on the salicylic content of the plant, thereby benefitting the plant with its various roles in stress-related signaling processes, too. Another notable observation made was the increased concentration of p-hydroxybenzoic acid in the applied plants. These results support the earlier view that the exogenous application of salicylic acid may also induce its de novo synthesis in plants [44].

In stress conditions such as drought, extreme temperature, and high salinity, the ABA content in plants increases considerably, inspiring stress-tolerance effects that help plants to adapt and survive under these stressful situations [45]. An improved stress tolerance capacity of microalgae-applied plants may occur because of increased ABA accumulation. Under the present experimental conditions, in the first experiment, none of the treatments resulted in a long-term change in the ABA level. However, in the second experiment, some of the strains caused genotype-dependent changes: in Mv Nádor, strains MACC-430 and MACC-612 decreased the ABA level, but in Mv Béres, MACC-430 induced a slight but statistically insignificant increase.

While spraying with microalgae only caused no or slight modifications in neochlorogenic acid, rutin, or naringenin contents in the wheat leaves, in several cases, substantial changes occurred in the amount of jasmonic acid and jasmonic acid-leucine/isoleucine conjugate contents. Jasmonic acid is known as a key signaling compound involved in the suppression of necrotrophic pathogens and herbivorous insects, and it also plays a role in the responses to abiotic stressors [46,47]. In addition, recent results showed that jasmonic acid may also modify the diversity and functioning of the microbiome in wheat roots [48]. In the first experiment, all the strains reduced their levels in the leaves; in the second experiment, these decreases were only significant in the case of MACC-438. However, in Mv Nádor, algae biomass treatments usually increased the jasmonic acid level, suggesting that although the physiological parameters indicated that plants are not exposed to severe stress factors, algae biomass treatments may modify their stress-related signaling processes.

If quantification of the composition of all microalgae can be added to a database (a common worldwide database added by researchers in the field) along with the bioassay results, then it will be an easier pick for a suitable strain to be applied as a biostimulant as per the composition. In addition, the studies on physiological parameters give insight into the possible changes in plants depending on the type of strain used. Such studies identifying the suitable strain enable the producers to focus on a particular strain for mass production.

5. Conclusions

While certain algae strains improved, others inhibited the germination processes. However, the way they affected germination may not work the same way when they were used as foliar spray. What inhibits germination does not necessarily have an inhibitory effect on adult plants. A significant proportion of the strains were characterized by auxin-like activity. However, the auxin-like effect was not necessarily directly related to the auxin content but to their ability to influence secondary metabolism. Spray application did not significantly alter photosynthetic processes but influenced the secondary metabolite composition. However, this effect is also influenced by the effect of genotype and environmental factors. The biomass of algae strains such as MACC-612, MACC-430, and MACC-922 showed significant differences with the control compared to other strains, so these three strains can be upgraded for the field experiment. However, the differences are unstable and need more trials involving different genotypes for confirmation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13041088/s1, Chromatograms S1: chromatograms from algae and wheat sample.

Author Contributions: L.M. was involved in the compilation of literature, conducting the research, and drafting into a research paper; T.J. participated in the supervision and incorporation of scientific views; É.D., G.S. and K.Á.H. aided in data validation, reviewing, and editing; Z.M. was involved in the supervision and rectification of the research paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the project No. TKP2021-NKTA-06 funded by the Ministry of Innovation and Technology from the National Research Development and Innovation Fund, in the frame of the Thematic Excellence Program 2021.

Data Availability Statement: Not applicable.

Acknowledgments: The technical help of Ildikó Lobik and Péter Bálint related to the experiments is highly appreciated.

Conflicts of Interest: The authors declare no conflict of interest.

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