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A High-Throughput Screening Platform to Evaluate Biostimulant Activity of Five Microalgae in *Arabidopsis thaliana*

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Abstract

Microalgae are increasingly recognized as promising biostimulants for sustainable agriculture, yet their potential remains underexplored due to the complexity of biostimulant activity and the vast diversity of species. Efficient standardized screening approaches are therefore needed. In this study, a high-throughput screening platform assessed the biostimulant activity of five microalgal species (*Limnospira platensis*, *Chlorella vulgaris*, *Dunaliella salina*, *Microchloropsis gaditana*, and *Isochrysis galbana*) in *Arabidopsis thaliana*. The system enabled full life-cycle assessment of *A. thaliana* under optimal and drought stress conditions, incorporating three application methods (soil amendment, irrigation, foliar spray) and a wide concentration range of 0.01–0.5 g/L. Biostimulant efficacy depended strongly on concentration and application method. Irrigation-based applications generally enhanced drought tolerance but delayed bolting and flowering. The highest concentration inhibited germination and root elongation, likely due to bioactive compound toxicity rather than salinity or pH. *L. platensis* exhibited broad activity across environmental conditions, while *I. galbana* likewise showed wide-ranging effects, including enhanced generative growth. In contrast, *D. salina* and *M. gaditana* primarily improved drought tolerance, and *C. vulgaris* acted mainly under optimal conditions. These findings highlight the value of *A. thaliana* to accommodate rapid biostimulant screening and identify both novel and established microalgae for further validation in crops.

Keywords: *Limnospira platensis*; *Arthrospira platensis*; *Chlorella vulgaris*; *Dunaliella salina*; *Microchloropsis gaditana*; *Nannochloropsis gaditana*; *Isochrysis galbana*; drought; sustainable agriculture

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1. Introduction

Agricultural systems face growing pressures to sustainably meet rising global food demand while minimizing environmental impacts [1,2]. This challenge is further exacerbated by the growing frequency and severity of abiotic stresses, among which drought represents one of the greatest threats to global crop production [3,4]. Among the proposed

solutions, biostimulants have emerged as a promising approach to enhance both crop productivity and sustainability. Biostimulants are substances that stimulate natural plant processes independently of their nutrient content. They function by enhancing nutrient availability and uptake, improving crop quality and yield, and increasing tolerance to abiotic stresses [5,6]. Within this field, microalgae have recently attracted considerable attention as a versatile and sustainable source of biostimulants. Compared to other sources, they offer several advantages, including a steerable chemical composition, the ability to be cultivated in diverse environments, and growth potential on waste streams [7–9]. Despite growing evidence for their biostimulant activity, the effects of microalgal applications are influenced by multiple factors, such as extraction method, application strategy, cultivation conditions, concentration, and timing of application within the plant life cycle [10,11]. This complexity allows for tailored application strategies but also necessitates extensive screening efforts.

The need for a standardized, high-throughput screening method is further underscored by the limited number of microalgal genera that have been investigated for biostimulant activity, most notably *Limnospira* sp. and *Chlorella* sp., leaving the broader biostimulant potential of an estimated 75,000 to 200,000 species largely unexplored [10–12]. The vast number of potential combinations of species, concentrations, application methods, and plant developmental stages presents a significant bottleneck in exploring biostimulant potential. While several screening approaches have been proposed using crop species such as spinach (*Spinacia oleracea*) [13] and tomato (*Solanum lycopersicum*) [14], these methods typically target specific growth stages and require extended cultivation periods, thereby limiting their scalability and comprehensiveness. In recent years, an alternative strategy has emerged that employs *Arabidopsis thaliana* as a screening species. Though considered an agricultural weed, *A. thaliana* is a cornerstone model organism in plant science. Its short life cycle, small size, and well-characterized genome make it particularly suitable for high-throughput biostimulant screening [15]. While *A. thaliana* has already been used in several biostimulant studies [16–18], its potential for standardized, full life-cycle biostimulant screening remains largely untapped.

To highlight the potential of *A. thaliana* as a screening platform and to address the microalgal diversity gap, the present study evaluates five microalgal species selected for their diverse biochemical profiles, morphological characteristics, and previously reported or hypothesized biostimulant activities. (I) *Limnospira platensis* is actually a filamentous cyanobacterium but it has historically been classified among microalgae because of its comparable characteristics and is still frequently included in microalgal literature. Its taxonomic classification was recently revised based on morphological, ecological, and genetic differences, prompting its transfer from the *Arthrospira* genus to the newly established *Limnospira* genus [19–21]. It is highly protein-rich (50–70% of dry weight), nutrient-dense, and known for its ease of production in open raceway ponds [22,23]. Though best known as a dietary supplement, *L. platensis* is also one of the most extensively studied microalgae in biostimulant research, with documented effects on both vegetative and generative growth under optimal and abiotic stress conditions across diverse crops, including grapevine (*Vitis vinifera*) [24], maize (*zea mays*) [25], leafy greens [26], and wheat (*Triticum aestivum*) [27]. (II) *Chlorella vulgaris* is a unicellular green alga with high levels of carbohydrates, lipids, and proteins. It has broad commercial applications, from cosmetics to nutraceuticals, and is among the most widely studied microalgae for its biostimulant effects [10,11,28]. (III) *Dunaliella salina* is a highly motile and halotolerant microalga, best known for producing high intracellular concentrations of β-carotene under extreme salt conditions. It is primarily used in the cosmetic and dietary supplement industries [23]. Although less commonly studied for biostimulant activity, it has shown potential for improving salt tolerance

in crops such as bell pepper (*Capsicum annuum*) [29], wheat [30], and tomato [31], particularly through its exopolysaccharide production. (IV) *Microchloropsis gaditana*, previously classified as *Nannochloropsis gaditana* before genetic analyses prompted reclassification [32], is the smallest species examined in this study and is characterized by a particularly robust cell wall. It is rich in polyunsaturated fatty acids and has been extensively researched for aquaculture feed and biofuel applications [23,33,34]. Biostimulant activity has only scarcely been reported, with two studies noting improved germination in bean (*Phaseolus vulgaris*) [35] and garden cress (*Lepidium sativum*) [36]. (V) *Isochrysis galbana* is a brown microalga characterized by rapid growth, low cultivation demands, and a high content of lipids and fucoxanthin. Though not widely produced on an industrial scale, it is commonly used in aquaculture feed [23,37]. Biostimulant activity of *I. galbana* remains largely uncharacterized, although it has been reported to improve garden cress germination [36].

In the present study, a multi-assay *Arabidopsis*-based screening method is explored to assess the biostimulant activity of these five microalgal species in *A. thaliana* across the full plant life cycle, from germination through vegetative development to generative growth. In addition to optimal growth conditions, biostimulant effects under drought stress are evaluated. To comprehensively assess the potential of each species, multiple application methods (soil amendment, irrigation-based, foliar spray) and concentrations (0.01–0.5 g/L) are tested. This study aims to provide a systematic overview of the biostimulant activity of diverse microalgal species in *A. thaliana* and to identify the specific conditions, application strategies, and plant developmental stages at which they are most effective. These findings enable relatively fast and standardized comparison of microalgal biostimulant activities and serve as a foundation for further mechanistic and economic validation studies.

2. Materials and Methods

2.1. Preparation of Microalgal Treatments

Five microalgae species were included in the study: Limnospira platensis (formerly Arthrospira platensis), Chlorella vulgaris, Dunaliella salina, Microchloropsis gaditana (formerly Nannochloropsis gaditana), and Isochrysis galbana. Spray-dried powders of each species were obtained from a commercial supplier (Algikey, Lisbon, Portugal).

To enhance the release of intracellular components, cell disruption was performed prior to their use in the *Arabidopsis*-based bioassays. Cells of *C. vulgaris*, *D. salina*, *M. gaditana* and *I. galbana* were disrupted using bead milling. For each species, a 10% (*w*/*w*) suspension of the powder was prepared in demineralized water and stirred for 1 h at room temperature with a magnetic stirrer. The suspensions were then processed in a bead mill (MKII–M250 wet bead mill, Eiger Torrance Ltd., Warrington, UK) using 1 mm yttria-stabilized zirconia ceramic beads (TOSOH YTZ, Tosoh corporation, Tokyo, Japan) and a bead loading percentage of 65% (*v*/*v*). A tip speed of 15 m/s was used. To guarantee full disruption, a bead milling time of 60 min was chosen for all species except *C. vulgaris*, for which an optimal milling time of 35 min was selected which had been optimized in previous experiments by monitoring release of soluble proteins and pigments. The milling chamber was water-cooled to ensure that the sample temperature remained below 40 °C. Following bead milling, suspensions were frozen overnight using a blast chiller (Coldline, Torreglia, Italy; −38 °C for 4 h, then held at −18 °C) and subsequently freeze-dried (Lyovapor™ L-200, BÜCHI Labortechnik GmbH, Flawil, Switzerland) for 48-72 h using a manual freeze-drying program. The dried biomass was ground with a mortar for 5 min to obtain a fine powder. In case of *L. platensis*, no bead milling was performed as the cells were shown to be easily disrupted by drying and subsequent resuspension. All microalgal powders were stored at 4 °C until use for soil-mixing applications or preparation of microalgal stock suspensions.

On the day of application, stock suspensions (10 g/L) were prepared in demineralized water for germination medium supplementation, irrigation, and foliar spray treatments. After brief stirring, suspensions were sonicated in a sonication bath at 10 °C to disrupt aggregates and further promote the release of intracellular components. Sonication duration was species-specific: 30 min for *L. platensis* and *C. vulgaris*, 1 h for *D. salina* and *M. gaditana*, and 1.5 h for *I. galbana*. Optimization was based on the time point at which absorbance at 470, 646, and 663 nm reached a maximum [38], coinciding with microscopic confirmation of aggregate disruption.

2.2. Arabidopsis-Based Screening Platform

The *Arabidopsis*-based screening platform comprises three assays (a germination assay, a vegetative potting assay, and a generative potting assay) that together provide a comprehensive, full life-cycle assessment of microalgal biostimulant potential on *A. thaliana*. Each assay can also be applied independently to address specific research questions. In the present study, biostimulant activity of the microalgae was evaluated across the full life cycle of *A. thaliana* under optimal conditions, while drought stress was specifically investigated using the germination and vegetative potting assays.

2.2.1. Germination Assay

Square Petri plates were prepared using the 'Arabidopsis standard' nutrient medium (Table S1; [39]), supplemented with 1% (*w*/*v*) plant agar (Carl Roth GmbH, Karlsruhe, Germany). After autoclaving and cooling to approximately 40 °C, the medium was supplemented with the algal stock suspension (10 g/L) to achieve final concentrations of 0.01, 0.1, or 0.5 g/L. The concentration range used in all experiments (0.01–0.5 g/L) was selected in accordance with the biostimulant definition, which emphasizes activity at low concentrations, and was further guided by existing literature [9,31,40]. For drought stress simulation, 5% (*w*/*v*) polyethylene glycol 6000 (PEG6000; Gerbu Biotechnik GmbH, Heidelberg, Germany) was added to the medium after autoclaving. Control treatments included mock-treated plates without algal extract (0 g/L) and unstressed control (UC) plates without PEG6000, to evaluate the baseline effect of drought stress. A subset of suspensions without agar was used to determine electrical conductivity (EC), pH, and water potential, measured with an EC8500 meter (Apera Instruments, Columbus, OH, USA), PH8500 meter (Apera Instruments) and a WP4C Dewpoint Potentiameter (METER Group, Pullman, WA, USA), respectively. An overview of the measured values is provided in Supplemental Table S2.

Arabidopsis thaliana (Col-0) seeds were obtained from the Nottingham Arabidopsis Stock Centre (NASC, Nottingham, UK) and stored at 4 °C. Seeds were surface-sterilized by immersion in 70% (*v*/*v*) ethanol for 2 min, followed by 5% (*v*/*v*) bleach (sodium hypochlorite, NaOCl) for 5 min, and subsequently rinsed five times with sterile demineralized water. Sterilized seeds were sown in five rows of ten seeds per square plate. Plates were positioned vertically in a growth chamber under a 16 h light/8 h dark photoperiod, with day/night temperatures of 23 °C/18 °C, relative humidity of 70%, and a light intensity of 130 µmol/m^2·s. Treatments were arranged in a randomized block design with three replicates per treatment, each block consisting of 50 seeds.

Germination was monitored twice daily based on radicle emergence to assess germination rate. Germination is expressed as the percentage of germinated seeds at 44 h after sowing, which represented the critical time point at which germination increased exponentially. Five days after sowing, plates were photographed, and root and hypocotyl lengths were measured using ImageJ (version 1.53k, National Institutes of Health, Bethesda,

MD, USA) with the NeuronJ plugin (version 1.4.3) [41,42]. For each treatment, 20 randomly selected seedlings were analyzed.

2.2.2. Potting Soil Assay

Arabidopsis thaliana (Col-0) seeds, obtained from the NASC, were stored at 4 °C and surface-sterilized as described previously. Sterilized seeds were sown on square Petri plates containing autoclaved half-strength Murashige and Skoog (½ MS) medium (Duchefa Biochemie B.V., Haarlem, The Netherlands) supplemented with 1% (*w*/*v*) plant agar (Carl Roth), 1% (*w*/*v*) sucrose, and 10 mM MES buffer (Acros Organics, Geel, Belgium) [43]. The pH was adjusted to 5.7 using 1 M Tris (hydroxymethyl)aminomethane (Tris base; Sigma-Aldrich, St. Louis, MO, USA). Plates were placed vertically in a growth chamber under a 16 h light/8 h dark photoperiod, with day/night temperatures of 23 °C/18 °C, 70% relative humidity, and a light intensity of 130 µmol/m^2·s. After 10 days, seedlings of uniform size were selected and transplanted into commercial potting soil ("Kwekerpotgrond", DCM, Sint-Katelijne-Waver, Belgium) using 12 pots (8 cm diameter, 200 mL soil/pot) per treatment. Plants were maintained under the same growth conditions, and irrigated twice weekly with 17 mL of water per pot. Drought stress was simulated by reducing the irrigation volume from 17 mL to 5 mL per pot. This irrigation regime was optimized in preliminary experiments designed to establish mild drought stress conditions [44].

Microalgae treatments began at transplantation and included three application methods (soil, irrigation-based and foliar) at four concentrations (0.01, 0.05, 0.1 and 0.5 g/L). A schematic overview of the treatment combinations is provided in Figure 1. For soil treatments, microalgal powder was mixed directly into the potting soil (g/L soil). For irrigation and foliar applications, microalgal powder was suspended in demineralized water at 10 g/L, stirred, and sonicated as described previously, then diluted to the desired concentrations (g/L water). Irrigation-based treatments were performed by adding the suspension to the water supply, while foliar treatments were applied as a fine spray at 5 µL/cm^2, twice weekly. Mock-treated controls (0 g/L) were included for each application method: algae-free potting soil for soil treatments, and algae-free demineralized water for both irrigation and foliar spray applications. In addition, unstressed controls (UC) were included to observe the effects of drought stress. The unstressed controls consisted of full (non-reduced) irrigation volumes for soil and irrigation-based applications, and full irrigation combined with a water spray for foliar applications.

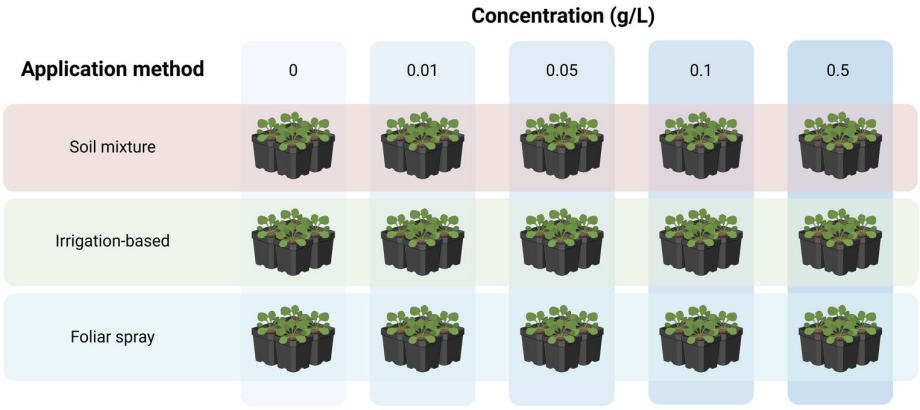

Figure 1. Schematic overview of treatment combinations in potting soil assays, considering different concentrations of all five microalgal species, under optimal and drought conditions, and during vegetative and generative growth. (Created with BioRender).

Vegetative parameters were assessed 14 days after transplantation (24 days after sowing), just prior to bolting (emergence of the bolting stem). Leaf relative water content

(RWC) was determined by selecting one fully expanded leaf per plant and recording fresh weight (FW_1) immediately after harvest. Leaves were then incubated overnight in demineralized water at 4 °C in the dark to obtain the turgid weight (TW_1), followed by drying at 70 °C for three days to determine dry weight (DW_1). RWC was calculated using the following formula [45]:

$$\text{RWC } (\%) = \frac{(FW_1 - DW_1)}{(TW_1 - DW_1)} \times 100$$

Soil water potential was measured using a WP4C Dewpoint Potentiameter (METER Group). Fresh weight of the aboveground biomass was recorded at harvest, and dry weight was measured after drying at 70 °C for three days.

Generative growth was assessed by maintaining the plants until 46 days after sowing. During this period, the time from sowing to bolting (appearance of the bolting stem) and the time to first flower opening were recorded. At 46 days after sowing, the aboveground biomass was separated into vegetative (rosette) and generative (flowering stem) components. Fresh and dry weights were determined as described for vegetative growth.

2.3. Statistical Analyses

All data were statistically analyzed using GraphPad Prism (version 10.6.1, GraphPad Software, Boston, MA, USA). For continuous data, homogeneity of variances was assessed using the Brown–Forsythe test and normality was evaluated using the Shapiro–Wilk test. If both assumptions were met, one-way ANOVA was performed with Tukey's HSD post hoc test. If the assumption of equal variances was violated, a Brown–Forsythe and Welch ANOVA was conducted followed by Dunnett's T3 multiple comparisons test. When the data did not meet the normality assumption (or both assumptions), a nonparametric Kruskal–Wallis test was applied with Dunn's multiple comparisons test. Discrete data were analyzed using the nonparametric Kruskal–Wallis test with Dunn's multiple comparisons test.

3. Results

In the present study, *Arabidopsis thaliana* was used to screen five microalgal species for biostimulant activity across the full life cycle of the plant, including germination, vegetative growth, and generative growth. In addition to evaluate the biostimulant activity under optimal growth conditions, germination assays and potting soil assays for vegetative growth were also assessed under drought stress conditions. Furthermore, different application methods and concentrations were applied.

3.1. Biostimulant Effects of Different Microalgae on A. thaliana Germination and Vegetative Growth

Germination rate, expressed as the percentage of germinated seeds at 44 h after sowing (point at which germination increased exponentially), is presented in Table 1. Although *A. thaliana* germinated rapidly, as indicated by the high germination percentage in the mock-treated control (0 g/L), no significant positive effects on germination were observed in any of the microalgal treatments. In contrast, several treatments induced significant negative effects. The highest concentration of *M. gaditana* (0.5 g/L) resulted in a significant reduction in the germination rate. A similar but non-significant trend was observed for *C. vulgaris*. In the case of *D. salina*, a significant reduction in germination by 29% was observed at 0.1 g/L compared to the mock-treated control at 44 h after sowing. At 0.5 g/L, the reduction increased to 81% relative to the control.

Table 1. Germination rates of *Arabidopsis thaliana* on solid medium supplemented with different microalgae species 44 h after sowing. Data are presented as mean ± standard deviation (n = 3). Different letters denote statistically significant differences ($p < 0.05$) among concentrations within each species according to one-way ANOVA followed by Tukey's HSD post hoc test. The mock-treated control was shared across all microalgal treatments.

Microalgal Species	0 g/L	0.01 g/L	0.1 g/L	0.5 g/L
L. platensis	88.1 ± 2.2 A	91.9 ± 3.4 A	94.1 ± 5.2 A	93.9 ± 2.1 A
C. vulgaris	88.1 ± 2.2 AB	94.3 ± 3.5 A	93.8 ± 4.0 A	80.6 ± 5.5 B
D. salina	88.1 ± 2.2 A	91.3 ± 1.3 A	59.0 ± 9.1 B	6.8 ± 6.2 C
M. gaditana	88.1 ± 2.2 A	84.9 ± 2.5 A	83.9 ± 5.1 A	55.0 ± 3.4 B
I. galbana	88.1 ± 2.2 A	92.7 ± 3.1 A	93.8 ± 4.2 A	91.4 ± 6.4 A

Five days after sowing, root and stem lengths were measured. While stem length did not exhibit any significant differences among treatments, root length was notably affected by the microalgal applications (Figures 2 and S1). Treatments with *L. platensis* and *C. vulgaris* at a concentration of 0.01 g/L resulted in significant increases in root length by 16% and 15%, respectively, compared to the mock-treated control. In contrast, the lowest concentration of the other microalgal species did not produce significant effects. A consistent trend across all species was the significant reduction in root length observed at the highest concentration (0.5 g/L), with decreases ranging from 40% to 79% relative to the control.

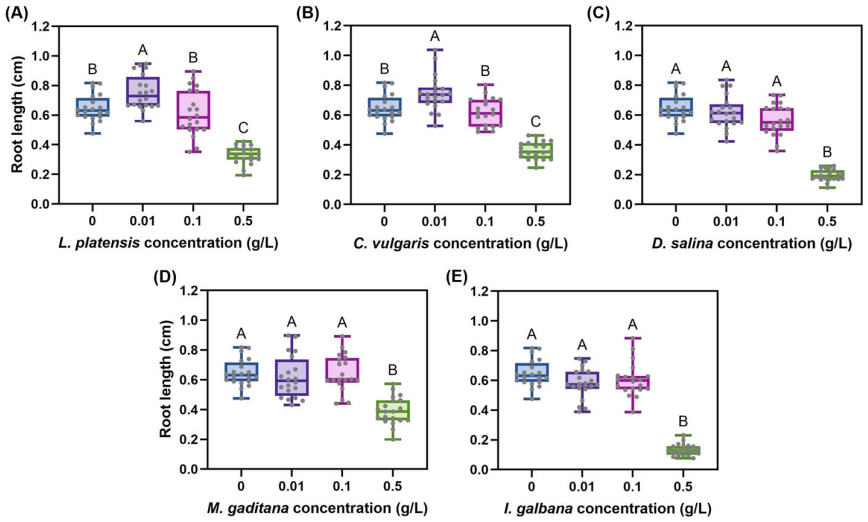

Figure 2. Root length of 5-day-old *Arabidopsis thaliana* plants grown on solid medium supplemented with different concentrations of specific microalgae species. (**A**) *Limnospira platensis*, (**B**) *Chlorella vulgaris*, (**C**) *Dunaliella salina*, (**D**) *Microchloropsis gaditana*, and (**E**) *Isochrysis galbana*. Box plots represent the median, minimum, and maximum values; gray dots represent individual data points (n = 20). Different letters indicate statistically significant differences ($p < 0.05$) according to one-way ANOVA with Tukey's HSD post hoc test (**B**), Brown–Forsythe and Welch ANOVA with Dunnett's T3 multiple comparisons test (**A**,**C**), or nonparametric Kruskal–Wallis test with Dunn's multiple comparisons test (**D**,**E**). The mock-treated control was shared across all microalgal treatments.

Potential biostimulant activity during the vegetative growth phase of *A. thaliana* was assessed by measuring, leaf RWC, soil water potential, and aboveground fresh and dry weights. Fresh weight of plants treated with the different microalgal species, concentrations, and application methods is shown in Figure 3. Dry weight measurements yielded nearly identical trends (Figure S2). Applications of *D. salina* and *M. gaditana* did not produce significant effects on vegetative growth compared with the mock-treated control. In

contrast, *I. galbana* showed strong growth-promoting activity. Soil application at 0.1 g/L yielded the highest response, with a 59% increase in fresh weight compared to controls. All foliar concentrations significantly promoted growth, with gains of up to 46%. In contrast, irrigation treatments caused a dose-dependent reduction, with the highest concentration (0.5 g/L) decreasing fresh weight by 16%. For *C. vulgaris*, soil applications displayed the opposite trend, with fresh weight increasing progressively from 8% at 0.05 g/L to 49% at 0.5 g/L, the latter being significantly higher than the control. Irrigation treatments followed a similar pattern, although only the 0.1 g/L concentration resulted in a significant increase. *L. platensis* demonstrated broad-spectrum biostimulant activity across all application methods and concentrations. Foliar treatments produced the strongest responses, with fresh weight increases of up to 80% relative to controls. Soil and irrigation-based applications were less pronounced but still substantial, reaching maximum increases of 33% and 54%, respectively. These responses were comparatively high relative to the other microalgal species. Leaf RWC and soil water potential showed minimal variation among treatments (Figures S3 and S4).

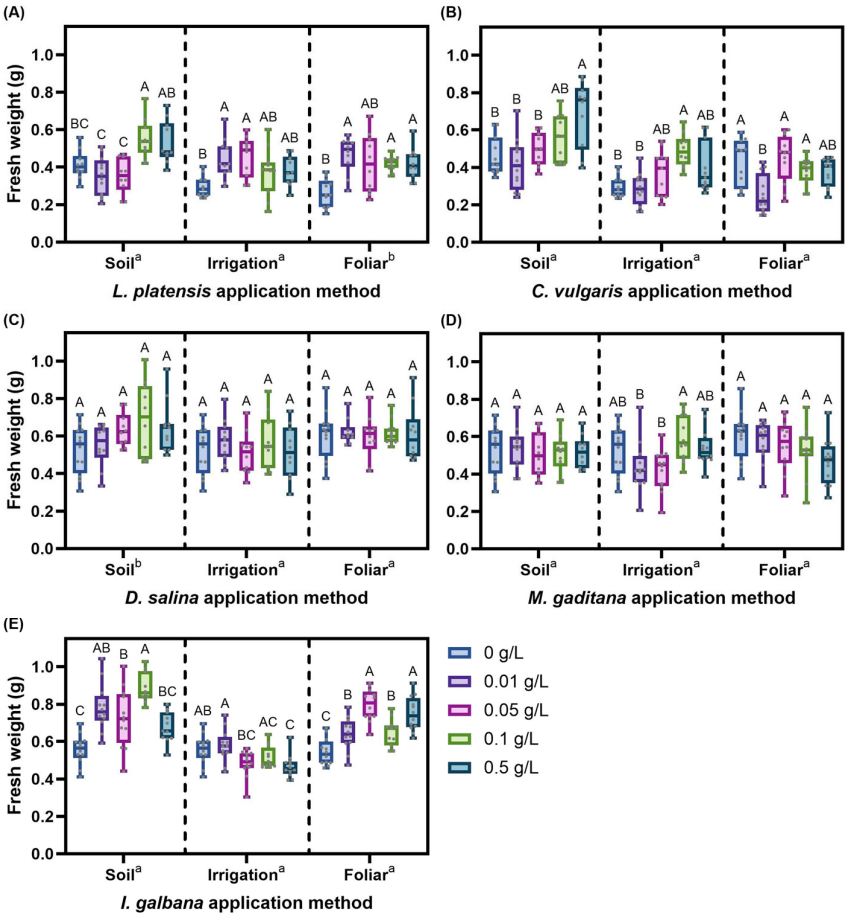

Figure 3. Vegetative growth, measured as aboveground fresh weight, of 24-day-old *Arabidopsis thaliana* plants grown in potting soil and treated with five different microalgae species. (**A**) *Limnospira platensis*, (**B**) *Chlorella vulgaris*, (**C**) *Dunaliella salina*, (**D**) *Microchloropsis gaditana*, and (**E**) *Isochrysis galbana*. Box plots represent the median, minimum, and maximum values; gray dots represent individual data points (n = 12). Different letters indicate statistically significant differences ($p < 0.05$) among concentrations within each application method according to one-way ANOVA with Tukey's HSD post hoc test (denoted as "[a]") or Brown–Forsythe and Welch ANOVA with Dunnett's T3 multiple comparisons test (denoted as "[b]"). Mock-treated controls for soil and irrigation treatments were shared but are shown twice for clarity, except for *L. platensis* and *C. vulgaris*.

3.2. Biostimulant Effects of Different Microalgae on *A. thaliana* Generative Growth

To evaluate the complete life cycle of *A. thaliana*, potting soil assays were conducted in which plants were maintained well into the generative phase, up to 46 days of age. During the transition from vegetative to generative growth, the emergence of the bolting stem and the time of first flower opening were recorded (Figure 4). Irrigation-based applications of microalgae frequently resulted in a significant delay in both bolting and flowering. Specifically, *D. salina* applied via irrigation at 0.1 g/L and 0.5 g/L, as well as *I. galbana* at nearly all tested concentrations, significantly delayed bolting and flowering by approximately two days on average. *C. vulgaris*, applied via irrigation at all concentrations except 0.5 g/L, significantly delayed bolting time; however, no corresponding significant effect on flowering time was observed. Foliar treatments did not generally influence bolting or flowering time. An exception was *I. galbana* applied as a foliar spray at 0.01 g/L, which caused a slight but significant one-day delay in both bolting and flowering time.

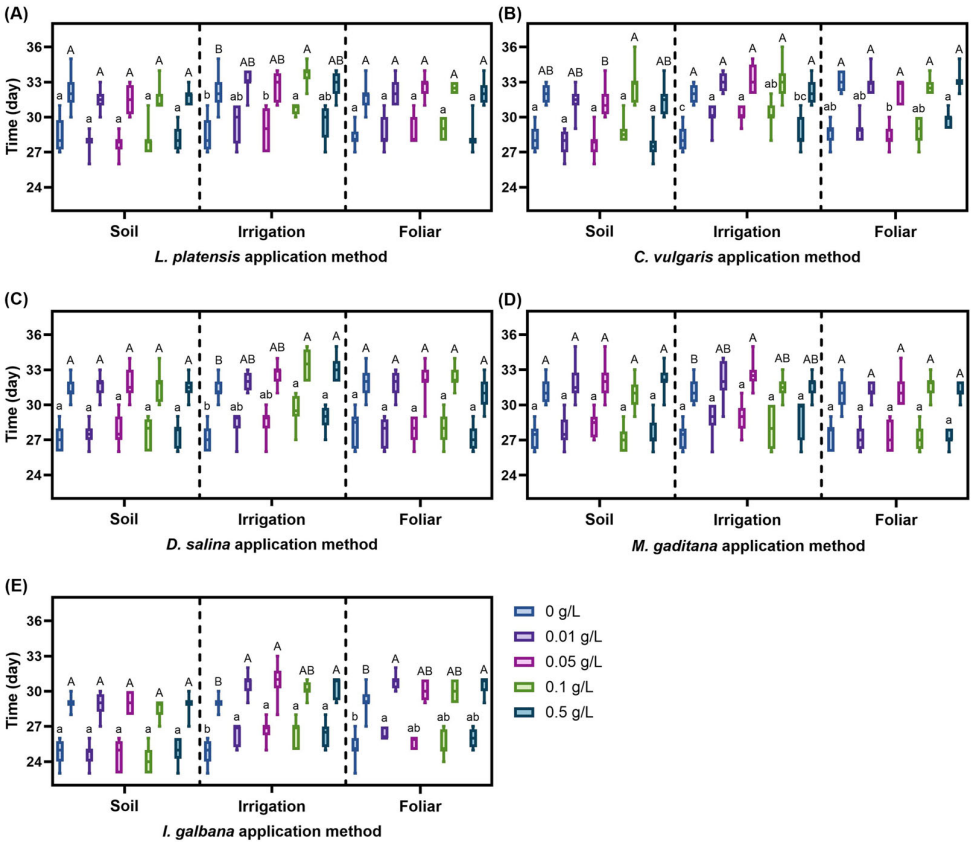

Figure 4. Time until bolting and flowering of *Arabidopsis thaliana* plants grown in potting soil and treated with five different microalgae species. (**A**) *Limnospira platensis*, (**B**) *Chlorella vulgaris*, (**C**) *Dunaliella salina*, (**D**) *Microchloropsis gaditana*, and (**E**) *Isochrysis galbana*. Time until bolting (**left**) and time until flowering (**right**) are presented side-by-side. Box plots represent the median, minimum, and maximum values (n = 12). Different lowercase letters indicate significant differences among concentrations of time until bolting within each application method, while uppercase letters denote significant differences among concentrations of time until bolting within each application method ($p < 0.05$) according to Kruskal–Wallis test with Dunn's multiple comparisons test. Mock-treated controls for soil and irrigation treatments were shared but are shown twice for clarity.

Aboveground fresh weight of 46-day-old *A. thaliana* plants, separated into vegetative (rosette) and generative (flowering stem) components, is shown in Figure 5. A recurring trend was observed whereby vegetative and generative biomass tended to change in parallel, with increases or decreases occurring simultaneously. Soil application of *D. salina*

significantly reduced growth at all tested concentrations, with rosette fresh weight decreasing by ~25% and flowering stem fresh weight by ~23% relative to the mock-treated control. Similar negative effects were observed for *M. gaditana*, both as a soil amendment at all concentrations and as a foliar treatment at 0.01 and 0.1 g/L. In contrast, irrigation-based treatments of *I. galbana* (all concentrations) significantly increased vegetative fresh weight, while increases in flowering stem biomass were significant at concentrations between 0.1 g/L and 0.5 g/L for *I. galbana*. Interestingly, while vegetative biomass remained largely unaffected for *C. vulgaris* and *L. platensis*, flowering stem biomass was significantly reduced by up to 37% compared to mock-treated plants, across all application methods except foliar *L. platensis*.

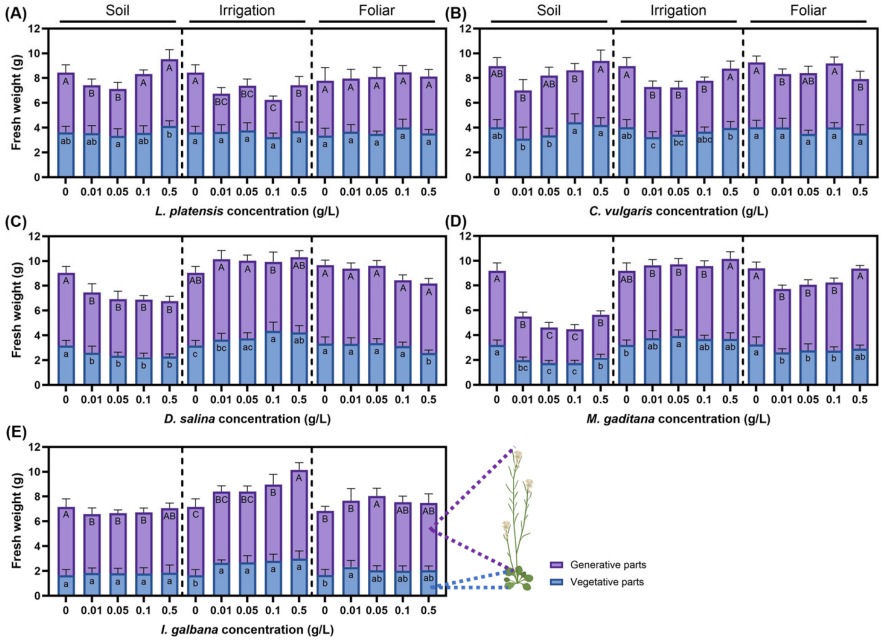

Figure 5. Generative (purple) and vegetative (blue) growth, measured as fresh weight, of 46-day-old *Arabidopsis thaliana* plants grown in potting soil and treated with five different microalgae species. (**A**) *Limnospira platensis*, (**B**) *Chlorella vulgaris*, (**C**) *Dunaliella salina*, (**D**) *Microchloropsis gaditana*, and (**E**) *Isochrysis galbana*. Bars represent means ± SD (n = 12). Different lowercase letters indicate significant differences among concentrations of vegetative parts within each application method, while uppercase letters denote significant differences among concentrations of generative parts within each application method ($p < 0.05$) according to one-way ANOVA with Tukey's HSD post hoc test. Mock-treated controls for soil and irrigation treatments were shared but are shown twice for clarity.

In addition to fresh weight, dry weight was also determined (Figure S5). For *L. platensis*, *C. vulgaris*, and *D. salina*, the dry weight results closely mirrored fresh weight observations. However, notable differences were observed for *M. gaditana* and *I. galbana*. Foliar-applied *M. gaditana* displayed a dose-dependent increase, with flowering stem dry weight rising significantly by 11% at 0.5 g/L, an effect not detected in fresh weight measurements. In contrast, while fresh weight indicated significant increases in generative biomass for irrigation-based and foliar applications of *I. galbana*, these effects were not significant in dry weight.

3.3. Biostimulant Effects of Different Microalgae on Drought Stressed A. thaliana Germination and Vegetative Growth

In addition to optimal growth conditions, drought conditions were included to assess the biostimulant activity for the five microalgal species. In the germination assay, drought stress was simulated by supplementing the medium with 5% PEG6000. Although

this treatment significantly reduced root length, reflecting the high sensitivity of young *A. thaliana* roots to decreased external water potential, the germination rate was not affected (Table 2). Imbibition of *A. thaliana* seeds remains efficient at PEG6000 concentrations up to 10% [46,47]. Germination dynamics under drought stress mirrored those observed under optimal conditions, with the highest concentration of microalgal addition (0.5 g/L) consistently resulting in a significant reduction in germination rate.

Table 2. Germination rates of *Arabidopsis thaliana* on solid medium supplemented with different microalgae species and 5% PEG6000 to induce drought stress, measured 44 h after sowing. Data are presented as mean ± standard deviation (n = 3). Different letters denote statistically significant differences ($p < 0.05$) among concentrations within each species according to one-way ANOVA followed by Tukey's HSD post hoc test. Both the UC (unstressed control) and the mock-treated control (stressed) were shared across all microalgal treatments.

Microalgal Species	UC	0 g/L	0.01 g/L	0.1 g/L	0.5 g/L
L. platensis	89.8 ± 5.1 A	92.7 ± 3.1 A	87.8 ± 6.1 A	90.4 ± 7.6 A	67.8 ± 19.9 A
C. vulgaris	89.8 ± 5.1 A	92.7 ± 3.1 A	89.5 ± 5.5 A	84.4 ± 7.9 A	74.1 ± 19.1 A
D. salina	89.8 ± 5.1 A	92.7 ± 3.1 A	99.3 ± 1.2 A	87.1 ± 11.6 A	6.7 ± 5.5 B
M. gaditana	89.8 ± 5.1 AB	92.7 ± 3.1 A	96.5 ± 2.9 A	98.8 ± 1.1 A	75.9 ± 9.6 B
I. galbana	89.8 ± 5.1 A	92.7 ± 3.1 A	85.4 ± 11.6 A	78.4 ± 15.7 A	40.6 ± 14.6 B

Root length, however, revealed promising drought stress mitigation effects (Figure 6). At 0.01 g/L, *I. galbana* and *M. gaditana* significantly enhanced root elongation. *I. galbana* increased root length by 17% compared to mock controls, though not enough to fully counteract drought effects. Stronger responses were observed for *M. gaditana*, which increased root length by 37%, restoring root length to levels comparable with unstressed controls. For *M. gaditana*, the positive effect persisted across a broad concentration range, with root elongation compared to the mock-treated control remaining significant at 0.1 g/L (33%). Consistent with observations under optimal and salt stress conditions, the highest concentration (0.5 g/L) often significantly reduced root elongation. Stem (hypocotyl) length was unaffected by drought stress and showed no response to microalgal treatments (Figure S6).

Beyond the germination assays, the potential beneficial effects of microalgae during the vegetative phase were evaluated in potting soil assays under drought conditions. Aboveground fresh weight measurements are shown in Figure 7, with dry weight following similar trends (Figure S7). Application of *M. gaditana* to the rhizosphere (via soil amendment or irrigation) significantly increased the aboveground fresh weight of *A. thaliana* by 54% to 92% compared to the mock-treated control. At most concentrations, fresh and dry weights were statistically comparable to those of unstressed plants, indicating substantial mitigation of drought effects. Foliar treatment with *M. gaditana* at 0.5 g/L also improved growth, though less strongly, with a 34% increase in fresh weight compared to controls. Among the application methods tested, irrigation-based delivery of microalgal suspensions proved to be the most effective under drought stress. In addition to *M. gaditana* irrigation-based application, significant increases in vegetative growth were also observed for *L. platensis*, *D. salina*, and *I. galbana.* In particular, *I. galbana* applied through irrigation increased fresh weight with ~26% compared to the mock-treated plants, though significant improvements were only detected at 0.01 and 0.1 g/L. In contrast, foliar application of *I. galbana* significantly reduced biomass, with fresh weight decreases of 19–36% across all concentrations compared to the mock-treated controls. Similarly to *I. galbana*, irrigation-based application of *L. platensis* produced a plateau effect, with significant fresh weight increases ranging from 28% to 50% at concentrations between 0.05 and 0.5 g/L. Finally, *D. salina* significantly promoted growth when applied to the rhizosphere, with soil applica-

tion at 0.05–0.1 g/L and irrigation at 0.5 g/L increasing fresh weight by 66–77% compared to mock-treated controls.

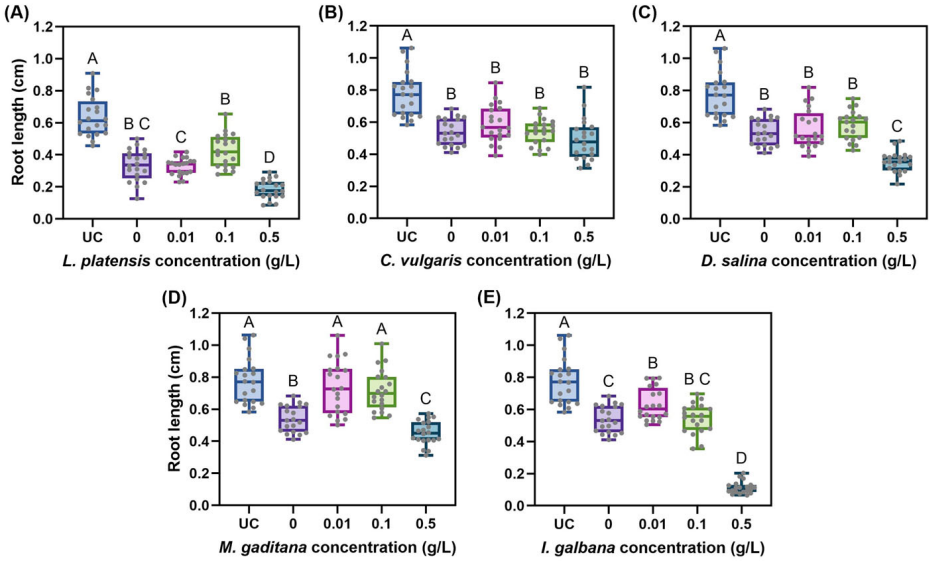

Figure 6. Root length of 5-day-old *Arabidopsis thaliana* plants grown on solid medium supplemented with different concentrations of specific microalgae species and 5% PEG6000 to induce drought stress. (**A**) *Limnospira platensis*, (**B**) *Chlorella vulgaris*, (**C**) *Dunaliella salina*, (**D**) *Microchloropsis gaditana*, and (**E**) *Isochrysis galbana*. Box plots represent the median, minimum, and maximum values; gray dots represent individual data points (n = 20). Different letters indicate statistically significant differences ($p < 0.05$) according to one-way ANOVA with Tukey's HSD post hoc test (**B**), or Brown–Forsythe and Welch ANOVA with Dunnett's T3 multiple comparisons test (**A**,**C**–**E**). Both the UC (unstressed control) and the mock-treated control (stressed) were shared across all microalgal treatments, except for *L. platensis*.

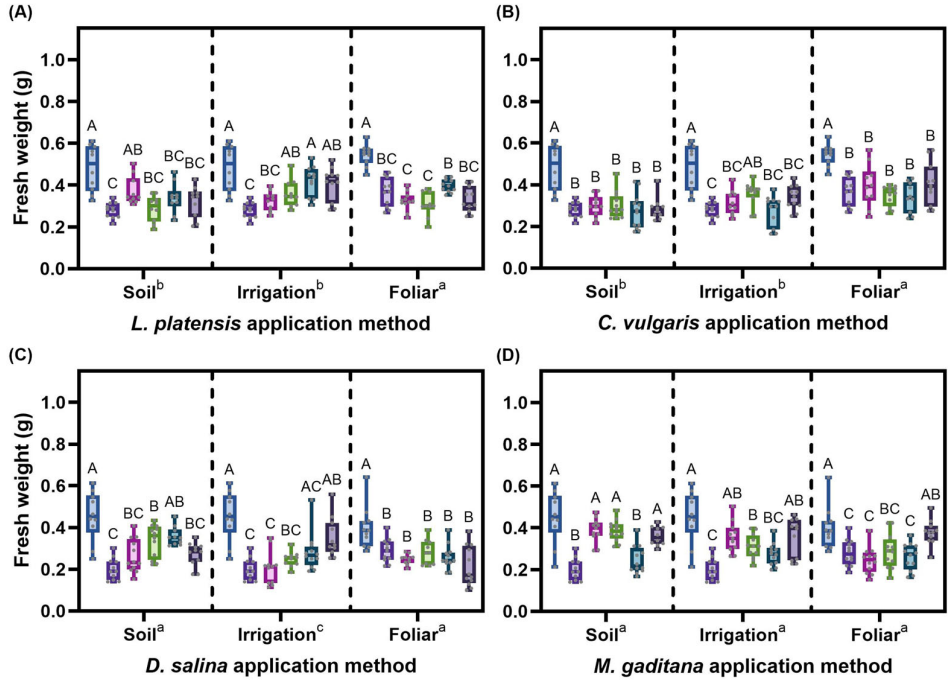

Figure 7. *Cont.*

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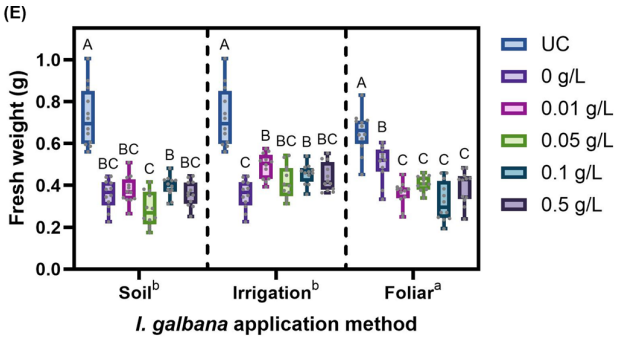

Figure 7. Vegetative growth, measured as aboveground fresh weight, of 24-day-old *Arabidopsis thaliana* plants grown in potting soil and treated with five different microalgae species. (**A**) *Limnospira platensis*, (**B**) *Chlorella vulgaris*, (**C**) *Dunaliella salina*, (**D**) *Microchloropsis gaditana*, and (**E**) *Isochrysis galbana*. Drought stress was applied by reducing irrigation amount from 17 mL to 5 mL. Box plots represent the median, minimum, and maximum values; gray dots represent individual data points (n = 12). Different letters indicate statistically significant differences ($p < 0.05$) among concentrations within each application method according to one-way ANOVA with Tukey's HSD post hoc test (denoted as "[a]"), Brown–Forsythe and Welch ANOVA with Dunnett's T3 multiple comparisons test (denoted as "[b]"), or Kruskal–Wallis test with Dunn's multiple comparisons test (denoted as "[c]"). Both the UC (unstressed control) and the mock-treated controls (stressed) for soil and irrigation treatments were shared but are shown twice for clarity.

Leaf RWC for the different treatments is presented in Figure 8. Drought stress induced a significant reduction in RWC, decreasing from an average of 87% in unstressed plants to 77% in mock-treated plants. Although *M. gaditana* soil and irrigation-based applications significantly increased vegetative growth, a significant reduction in RWC was observed for these treatments. Specifically, RWC declined from 79% in mock-treated plants to around 70% in all irrigation treatments and at 0.01 g/L and 0.5 g/L soil applications. A similar pattern was observed for irrigation with *D. salina*: while only the 0.5 g/L treatment significantly increased biomass, all concentrations reduced RWC by ~8% compared to the control, with the reduction significant at 0.05 g/L. In contrast, irrigation with *I. galbana* and *L. platensis* enhanced biomass without significantly altering leaf RWC.

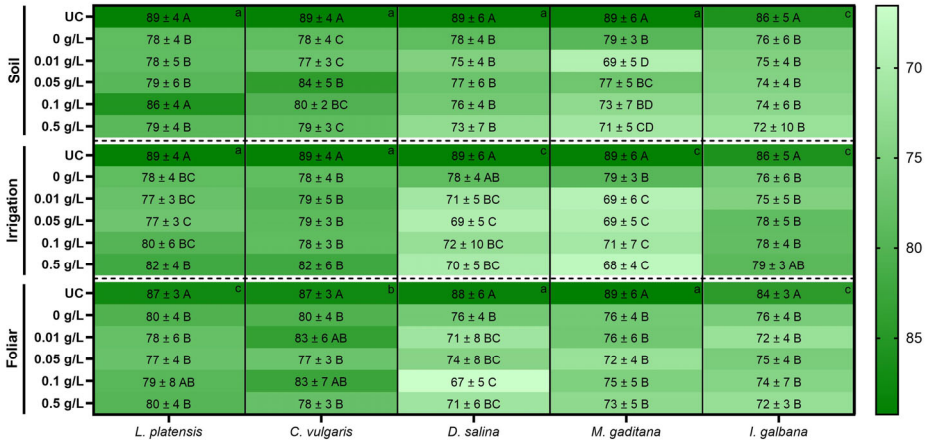

Figure 8. Leaf relative water content (%) of 24-day-old *Arabidopsis thaliana* plants grown in potting soil and treated with five different microalgae species. *Limnospira platensis*, *Chlorella vulgaris*, *Dunaliella salina*, *Microchloropsis gaditana*, and *Isochrysis galbana*. Drought stress was applied by reducing irrigation amount from 17 mL to 5 mL. Heatmap colors represent the mean values, with numerical labels indicating means ± SD (n = 12). Different letters indicate statistically significant differences ($p < 0.05$) among concentrations within each species and application method according to one-way ANOVA with Tukey's HSD post hoc test (denoted as "[a]") or Brown–Forsythe and Welch ANOVA with

Dunnett's T3 multiple comparisons test (denoted as "[b]"), or nonparametric Kruskal–Wallis test with Dunn's multiple comparisons test (denoted as "[c]"). Both the UC (unstressed control) and the mock-treated controls (stressed) for soil and irrigation treatments were shared but are shown twice for clarity.

Drought conditions significantly reduced soil water potential from an average of −0.09 MPa in unstressed controls to −0.39 MPa in mock-treated drought-stressed soils (Figure 9). Several microalgal treatments further influenced soil water potential, particularly when applied via soil or foliar methods. Soil applications of *L. platensis* (0.01–0.1 g/L) significantly reduced soil water potential, with the lowest value recorded at −0.53 MPa at 0.01 g/L. Similarly, foliar applications of *D. salina* (0.1–0.5 g/L) and *I. galbana* (0.01–0.5 g/L) significantly decreased soil water potential to average values of −0.50 MPa and −0.73 MPa, respectively. In contrast, foliar application of *L. platensis* significantly increased soil water availability, with improvements of up to 51% compared to the mock-treated control.

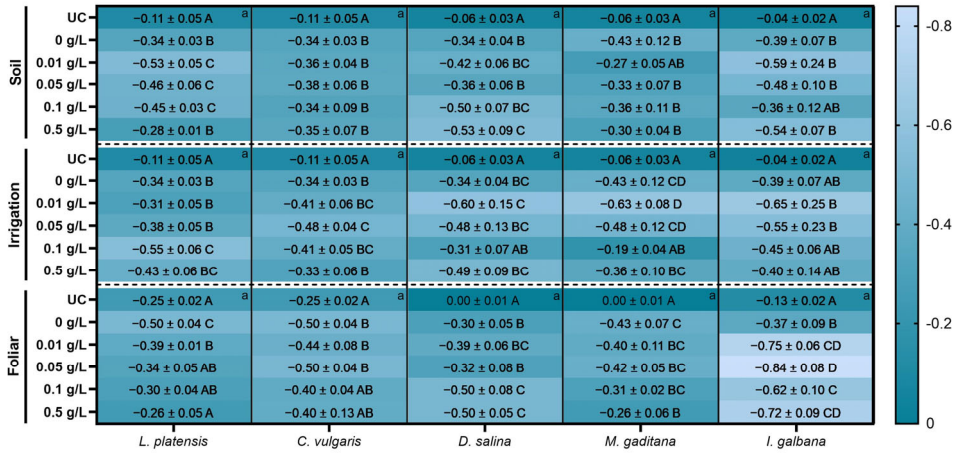

Figure 9. Soil water potential (MPa) of 24-day-old *Arabidopsis thaliana* plants grown in potting soil and treated with five different microalgae species. *Limnospira platensis*, *Chlorella vulgaris*, *Dunaliella salina*, *Microchloropsis gaditana*, and *Isochrysis galbana*. Drought stress was applied by reducing irrigation amount from 17 mL to 5 mL. Heatmap colors represent the mean values, with numerical labels indicating means ± SD (n = 5). Different letters indicate statistically significant differences ($p < 0.05$) among concentrations within each species and application method according to one-way ANOVA with Tukey's HSD post hoc test (denoted as "[a]"). Both the UC (unstressed control) and the mock-treated controls (stressed) for soil and irrigation treatments were shared but are shown twice for clarity.

4. Discussion

4.1. Application Method and Concentration Shape Microalgal Biostimulant Efficacy in A. thaliana

The concentration of microalgae proved to be a critical factor in determining their biostimulant potential, with certain concentrations even exerting detrimental effects. A consistent observation across all germination assays was the inhibitory effect of the highest microalgal concentration (0.5 g/L) on both germination rate and root length. This significant reduction was evident for nearly all tested species and aligns with previous findings. For instance, a recent study reported that *C. vulgaris* significantly increased the mean germination rate of wheat by 17% and root length by 62%. However, these effects were reversed at the highest concentration (0.5 g/L), resulting in significant reductions compared to the control [48]. Similar patterns have been reported for *Scenedesmus* sp. on watercress (*Lepidium sativum*) [49], *Planktochlorella nurekis* on various crops [50], and *L. platensis* on milkweed (*Calotropis procera*) [51]. Given the origin of the selected microalgae in the current study, four marine and two freshwater—brackish species, it could be hypothesized that the inhibitory effects at higher concentrations are linked to elevated media EC. However, the

EC of the germination media (Table S2) increased by only approximately 0.1–0.2 mS/cm across the different microalgal treatments. These increases are minor compared with the baseline EC under optimal conditions (0.962 mS/cm) and remain far below the salt stress thresholds reported for *A. thaliana*, where germination is inhibited at ≥75 mM NaCl (~7.5 mS/cm) and root elongation, which is more sensitive, is affected from ≥50 mM NaCl (~5.0 mS/cm) [52,53]. Moreover, the freshwater microalga *C. vulgaris*, which did not alter medium EC, also showed strong inhibitory effects at 0.5 g/L. Alternatively, pH may have contributed, as it was strongly affected by several microalgae. In particular, *L. platensis* increased the pH by approximately 1.5 units, which could have altered nutrient availability [54]. These pH shifts likely stem from residual cultivation medium, with *L. platensis* traditionally grown at pH 9.0–10.5 [55,56]. This underlines the complexity of microalgal biomass, which contains intracellular components with biostimulant activity but also residues from growth media. It also highlights the importance of monitoring pH when applying microalgal biostimulants in water-based systems. Still, pH alterations alone cannot fully explain the consistent reduction in germination and root length at 0.5 g/L across species, since similar inhibitory effects were observed with algae such as *D. salina*, which did not alter the pH. Taken together, these findings, supported by literature, suggest that the observed growth inhibition is not primarily attributable to increased salinity or pH, but rather to specific bioactive compounds that, at high concentrations, exert phytotoxic effects [49]. The aqueous environment of the germination assay likely amplifies this effect by enhancing compound bioavailability and increasing direct root exposure [57].

In addition to concentration, biostimulant efficacy was found to be strongly influenced by the application method. This was evident both within microalgal species, across environmental conditions, and at different plant developmental stages. For instance, *I. galbana* significantly increased the fresh weight of *A. thaliana* under optimal conditions by an average of 34% when applied via soil or foliar treatments, whereas no such effect was observed with irrigation-based application. Likewise, application method determined biostimulant performance under environmental and plant-developmental contexts. Irrigation-based applications predominantly increased drought tolerance and influenced bolting and flowering time. The importance of application method for microalgal efficacy has also been highlighted in previous studies. For example, selenium-enriched *C. vulgaris* improved growth and seed quality more effectively when applied as a foliar spray rather than via soil in common bean [58]. Comparable findings have been reported for foliar application of *C. vulgaris* in lettuce [59] and tomato [60,61]. The distinction between foliar and root-based applications (soil and irrigation-based) is relatively straightforward: foliar sprays deliver bioactive compounds directly to the phyllosphere, whereas root-based applications interact with the rhizosphere. However, differences between soil and irrigation treatments are less obvious. Soil applications involve a single, relatively high dose of microalgal biomass incorporated directly into the substrate (e.g., ~100 mg per pot at the highest concentration), enabling slow degradation and sustained compound release. In contrast, irrigation-based treatments provide smaller, repeated doses (e.g., ~8.5 mg per pot per irrigation event at the highest concentration), ensuring a continuous but lower-level supply of fresh bioactive molecules [10,36]. These mechanistic differences between application methods likely contribute to the variation in observed biostimulant effects, even when using the same microalgal species. They may reflect differences in compound bioavailability, persistence, and mode of action [62,63]. Consequently, application strategy is a critical parameter that must be considered when evaluating or optimizing the performance of microalgal biostimulants.

4.2. Microalgal Species-Specific Biostimulant Activity in A. thaliana: Identification in Traditional and Novel Species

The screening of the five microalgal species revealed that some exhibit broad biostimulant activity across multiple developmental stages, whereas others act as more narrowly defined specialists. *L. platensis*, together with *C. vulgaris*, represents the most well-documented microalga among those tested. Although not consistently yielding the strongest effects, *L. platensis* demonstrated positive vegetative responses under both optimal and drought conditions. This is consistent with previous findings where *L. platensis* mitigated drought and other abiotic stresses in crops such as mandarin (*Citrus reticulata*) [64], grapevine [24], broad bean [40], *Petunia x* hybrida via foliar application [65], and wheat through soil application [66]. While most studies did not investigate mechanisms of action in detail, some reported increased antioxidant enzyme activities, proline accumulation, and reduced membrane damage [64,66]. Such effects could explain why leaf relative water content and soil water potential remained unchanged, while biomass under drought stress increased, suggesting improved water-use efficiency [67]. This mechanism has also been observed in mandarin treated with foliar applications of living *L. platensis* [64]. Biochemical analyses were not performed in the present study; hence, these hypotheses remain speculative. In the present study, *L. platensis* was also able to enhance root and vegetative growth under optimal conditions, a phenomenon previously documented in tomato [63], lettuce [68], milkweed [69], and pea (*Pisum sativum*) [70]. However, no enhancement of generative growth was observed in *A. thaliana*, despite several reports describing such effects in other species [24,63,71]. This discrepancy may be attributed to the varying extraction and application methods reported in the literature. For instance, one study reported improved tomato fruit lycopene content using *L. platensis* hydrolysates delivered via chitosan nanoparticles, whereas another found increased bean yield with foliar applications of air-dried and ground *L. platensis* powder [63,71]. These examples highlight how plant species, extraction procedures, and application strategies collectively influence biostimulant efficacy. *C. vulgaris*, by contrast, showed a more specialized biostimulant profile, with beneficial effects restricted to optimal conditions. It significantly improved root length and vegetative fresh weight via soil and irrigation-based treatments. Similar effects have been reported for *C. vulgaris* applications in lettuce [59,72], kale (*Brassica napus*) [73], and wheat [48]. Although *C. vulgaris* has been shown elsewhere to mitigate abiotic stress, those studies often employed living cells, which may explain the absence of stress-mitigating effects in the current assays [74–76].

D. salina and *M. gaditana* demonstrated specialized biostimulant potential, particularly under drought conditions. While biostimulant activity of *M. gaditana* has received limited attention, positive effects on salt stress tolerance by *D. salina* (particularly its exopolysaccharides) have been reported in tomato, wheat, and bell pepper [29–31,77]. The improvements in drought tolerance observed in *A. thaliana*, both during germination and vegetative growth, have not yet been documented for either species. Interestingly, irrigation-based applications of *M. gaditana*, and to a lesser extent *D. salina*, significantly increased aboveground fresh weight under drought conditions, yet these effects coincided with reductions in leaf RWC. Likewise, *M. gaditana* foliar treatments did not enhance fresh weight of generative tissues but did significantly increase dry weight, suggesting reduced water content relative to controls. Together, these findings imply that biomass accumulation was promoted at the expense of water status, potentially increasing susceptibility under prolonged or more severe drought [78,79]. One possible explanation is that enhanced photosynthesis and growth were driven by prolonged stomatal opening. Such alterations in stomatal regulation following microalgal treatments have been previously reported [75]. By contrast, irrigation with *I. galbana* increased fresh weight of generative tissues without

altering dry weight. This suggests improved water status relative to controls, potentially reflecting enhanced stomatal regulation, the opposite mechanism proposed for *M. gaditana*. Moreover, *I. galbana* irrigation also improved vegetative growth under optimal conditions, providing additional evidence of its potential as a biostimulant. Given the limited research on this species, these results highlight its promise for further exploration.

4.3. A High-Throughput Platform for Standardized Comparison of Microalgal Biostimulant Activities in A. thaliana Across the Plant Life Cycle

Rapid screening of microalgae for biostimulant activity is essential to efficiently identify promising species and optimize critical parameters influencing their effectiveness, including cultivation strategies, extraction techniques, application methods, and appropriate concentrations [11]. However, previous screening approaches have often been limited in throughput due to long cultivation periods and a focus on specific developmental stages [13,14]. Using *A. thaliana* as a model organism offers several advantages for high-throughput screening. Its short life cycle enables the evaluation of generative growth within 46 days, and its small size allows high-density cultivation and replication [15]. Although *A. thaliana* has already been employed in biostimulant research, its potential for full life-cycle screening remained largely underexploited, as most studies focused on early developmental stages or specific stress responses. For example, an in vitro rooting assay similar to the germination assay used in our study evaluated microalgal biostimulants from a Nordic collection but was restricted to root development between 8 and 12 days and assessed later stages using a different plant species [16]. Similarly, in vivo cultivation of *A. thaliana* has been used to investigate drought tolerance conferred by *C. vulgaris* [75] and commercial biostimulants [17]. These studies incorporated physiological and molecular analyses but were only limited to vegetative growth. A notable gap still exists in using *A. thaliana* to assess generative responses to biostimulants, particularly those derived from microalgae [80]. Most studies targeting generative growth only focus on economically important crops such as strawberry (*Fragaria ananassa*) [81], tomato [82], and soybean (*Glycine max*) [83], which have longer cultivation cycles and require more space and resources. The *Arabidopsis*-based platform employed in our study facilitates the comparison of different biostimulant activities in one plant species (standardization) across the full plant life cycle while remaining flexible and adaptable to diverse environmental conditions and additional phenotypic or physiological measurements.

It is important to notice that the transferability of findings to economically important species can vary widely. For example, while *C. vulgaris* has been shown to enhance drought tolerance in *A. thaliana* [75], and similar effects were observed in broccoli (*Brassica oleracea*) [84] and guar (*Cyamopsis tetragonoloba*) [74], such consistency is not universal. Several studies have already reported differences in biostimulant responses between *A. thaliana* and crop species, as well as among cultivars of the same species [85,86].

Nevertheless, we propose that the *Arabidopsis*-based screening platform offers a valuable, standardized system for comparing the biostimulant activity of different microalgal species. Using *A. thaliana* for high-throughput screening enables direct comparison across multiple microalgal species, diverse concentrations, and treatments, supporting informed decisions about which candidates should be advanced to more dedicated time- and resource-intensive, follow-up studies in economically relevant crops.

5. Conclusions

A high-throughput screening method was employed to evaluate the biostimulant activity of five microalgal species in *A. thaliana* across different environmental conditions, application methods, and concentrations. The method enabled standardized assessment

over the full life cycle of *A. thaliana* and proved flexible and easily adaptable to various experimental parameters. The results underscored the critical importance of application strategy and dosage. The highest concentration (0.5 g/L) consistently inhibited germination and root elongation, while irrigation-based applications were generally the most effective for enhancing drought tolerance and exerted the strongest influence on bolting and flowering time. These findings emphasize the need to optimize application rates to maximize efficacy while avoiding phytotoxicity. The screening outcomes revealed two general patterns: some microalgae exhibited broad-spectrum, generalist biostimulant activity, while others acted as specialists, effective under specific conditions or developmental stages. *L. platensis* emerged as a generalist, enhancing vegetative growth under optimal and drought conditions. Similarly, *I. galbana* showed general biostimulant activity, improving both vegetative (optimal and drought conditions) and generative growth. In contrast, *D. salina* and *M. gaditana* promoted germination, early developmental and vegetative growth of plants under drought stress, indicating a more specialized biostimulant activity. *C. vulgaris* also demonstrated a specialist profile, with activity largely limited to root and vegetative growth under optimal conditions. Several of the observed biostimulant effects have not yet been reported in the literature, underscoring the added value of a high-throughput screening approach for expanding knowledge of biostimulant properties across a wider portion of the vast diversity of microalgal species. This work and the accompanying screening method enable standardized and rapid comparison of microalgal biostimulant activity in *A. thaliana*, supporting informed decisions for further, targeted validation in economically important crops.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/phycology6010001/s1. Table S1: Chemical composition of the 'Arabidopsis standard' nutrient solution used in the germination assays; Table S2: Electrical conductivity (EC), pH, and water potential (Ψ) of a tested subset of germination media without agar for each microalgal species and environmental condition; Figure S1: Stem length of 5-day-old *Arabidopsis thaliana* grown on microalgae-supplemented solid medium; Figure S2: Aboveground dry weight of 24-day-old *Arabidopsis thaliana* treated with five microalgal species; Figure S3: Leaf relative water content of 24-day-old *Arabidopsis thaliana* treated with five microalgal species; Figure S4: Soil water potential of 24-day-old *Arabidopsis thaliana* treated with five microalgal species; Figure S5: Vegetative and generative dry weight of 46-day-old *Arabidopsis thaliana* treated with five microalgal species; Figure S6: Stem length of 5-day-old *Arabidopsis thaliana* under PEG-induced drought stress and microalgal treatment; Figure S7: Aboveground dry weight of 24-day-old drought-stressed *Arabidopsis thaliana* treated with five microalgal species.

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Abbreviations

The following abbreviations are used in this manuscript:

MS	Murashige and Skoog
PEG6000	Polyethylene glycol 6000
RWC	Relative water content
EC	Electrical conductivity

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