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RESEARCH

Ulvan long-term biostimulant effects on *Arabidopsis thaliana* seed treatment via medium supplementation

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Abstract

This study investigates the long-term effects of ulvan, a sulfated polysaccharide extracted from the green seaweed *Ulva*, on the growth and yield of *Arabidopsis thaliana*. While previous studies have demonstrated the short-term biostimulant effects of ulvan, well characterised ulvan long-term impact on plant development and seed yield remains unclear. The effects of a well-characterized crude ulvan extract on early germination, photosynthetic efficiency, root and shoot development, and final seed yield were determined. The results indicate that ulvan application at the time of sowing significantly enhances root elongation, shoot area, and photosynthetic activity, ultimately leading to a 43% increase in seed yield, suggesting a role in modulating metabolic pathways. Proteomic analysis further reveals significant upregulation of stress-related and growth-associated proteins, suggesting ulvan role in modulating metabolic pathways. Additionally, a comparative analysis of ulvan extracted two years apart demonstrates its biochemical consistency, reinforcing its potential for agricultural applications. These findings highlight ulvan promise as a sustainable biostimulant capable of reducing dependency on synthetic fertilizers while improving crop performance. Further research is needed to explore its efficacy across different crops and environmental conditions to optimize its integration into sustainable agricultural practices.

Keywords Ulvan · *Ulva* · Biostimulant · *Arabidopsis* · Sustainable agriculture · Seed yield · Proteomics

Introduction

Global agriculture is facing unprecedented challenges. The ever-increasing global population, projected to surpass 9.7 billion by 2050, places immense pressure on existing agricultural systems to enhance food production sustainably (Giller et al. 2021; Beltran-Peña and D'Odorico 2022; White and Gleason 2022). Historically, the Green Revolution of the mid-twentieth century drastically improved crop yields through the adoption of high-yielding crop varieties, mechanization, and extensive use of synthetic fertilizers and pesticides. While these advancements successfully mitigated hunger for decades, they also introduced significant environmental issues. Soil degradation, water contamination, loss of biodiversity, and greenhouse gas emissions have become critical concerns resulting from excessive agrochemical usage. In response to these challenges, there is an urgent need for sustainable agricultural practices that balance productivity with environmental stewardship.

Plant biostimulants have emerged as promising tools in sustainable agriculture, offering a path to enhance crop productivity while minimizing ecological harm. Biostimulants are natural substances or microorganisms that, when applied to plants, stimulate natural processes to improve nutrient uptake, stress tolerance, and overall plant health (Yakhin et al. 2017). Among these, seaweed-derived extracts have garnered significant attention due to their bioactive compounds capable of promoting plant growth and resilience (Gandhi et al. 2024; Santos et al.

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2024; Weisser et al. 2024). Seaweeds, particularly green macroalgae of the genus *Ulva*, are rich in sulfated polysaccharides, with ulvan being a predominant component (Robic et al. 2009b; Kidgell et al. 2019). Ulvan complex structure, composed composed mainly by rhamnose, rhamnose-sulphate, rhamnose and uronic acids along with its sulfated groups, endows it with a range of biological activities beneficial to plant development. Developing useful application from *Ulva* biomass hold promisse to find useful solution for this common marine waste, which great green tides in many global locations (Fan et al. 2014; Zhang et al. 2019; Obolski et al. 2022).

Previous research has highlighted ulvan's potential as a plant biostimulant (Shefer et al. 2022; Osorio et al. 2024). Studies have demonstrated its ability to enhance seed germination, stimulate root and shoot development, and improve plant resistance to biotic and abiotic stresses (Paulert et al. 2021; Shefer et al. 2022). However, much of the existing literature focuses on the immediate effects of ulvan application, leaving significant knowledge gaps regarding optimal application strategies, long-term impacts on plant growth, and yield outcomes. Understanding these factors is crucial for transitioning ulvan from experimental studies to practical agricultural solutions. In addition, as ulvan is a natural material, we compared its composition from two batches of *Ulva* harvest with a two-year difference to assess consistency and variability in its bioactive properties.

The research presented in this study aims to address these knowledge gaps by investigating the effects of ulvan crude extract on *Arabidopsis thaliana*, a well-established model organism in plant biology. Specifically, this study explores how varying application timings influence early-stage germination, subsequent plant development, and final seed yield. By dissecting these parameters, we seek to optimize ulvan use as a sustainable biostimulant that can potentially reduce the reliance on synthetic agrochemicals and contribute to global food security.

Our previous work (Shefer et al. 2022) has established the chemical and biophysical profile of ulvan extracts and their immediate impact on plant growth. We demonstrated that ulvan biostimulant properties are dose-dependent and influenced by environmental factors such as light intensity. Notably, ulvan application at the time of sowing significantly enhanced root elongation and shoot area in *A. thaliana*, leading to increased seed yield at plant maturity. These findings suggest ulvan promise as a natural growth enhancer; however, they also highlight the need for a deeper understanding of its mechanisms and long-term benefits.

This study builds upon our previous research by conducting a comprehensive analysis of ulvan application strategies. We examine the optimal concentration of ulvan needed to maximize growth benefits without inducing negative effects. Additionally, we investigate the critical timing for ulvan application to determine when plants are most responsive to its active components. By exploring these factors, we aim to develop a robust framework for ulvan use in agriculture, extending its benefits beyond model plants to economically significant crops.

Successful optimization of ulvan application could lead to reduced dependency on chemical fertilizers, lower production costs for farmers, and decreased environmental impact. Furthermore, ulvan natural origin aligns with the growing demand for organic and sustainable farming practices, offering a viable solution for eco-conscious agricultural production. Ultimately, this work contributes to the broader goal of achieving sustainable intensification in agriculture—producing more food on existing farmland while preserving natural resources and reducing environmental harm also find novel solutions for marine waste biomass.

Materials and methods

Ulva spp. biomass cultivation

Ulva spp. collected from a beach in Haifa was cultivated under controlled conditions using macroalgae photobioreactors (MPBR) incorporated into a building's south wall under daylight conditions in December 2018 and April 2020. A detailed description of the cultivation system has been published previously (Chemodanov et al. 2017). The macroalgae mixture contained *U. rigida* and *U. fasciata* (Krupnik et al. 2018), sister taxa according to the results of *tufA* and ITS2 analysis (Melton and Lopez-Bautista 2021). The growth media was based on Mediterranean seawater. To the medium, ammonium nitrate (NH_4NO_3) and phosphoric acid (H_3PO_4) (Haifa Chemicals Ltd., Israel) were added to adjust total nitrogen and phosphorous contents to 6.4 and 0.97 g m^{-3}, respectively, and the pH to 8.2. The solution was agitated under CO_2 bubbling throughout the biomass cultivation period at a bubbling flow rate of 2–4 L min^{-1}. Before further use, fresh *Ulva* biomass was harvested, washed of minerals and epiphytes with deionized water, and centrifuged at 2,800 rpm to remove excess water. A single biomass batch was used to produce the ulvan to reduce material variability in the *A. thaliana* biostimulant experiments.

Ulvan extraction

A crude extraction of the ulvan from collected and washed *Ulva* spp. biomass was carried out based the protocol described by Robic et al. (2009c) with some modifications. First, 400 g of the fresh *Ulva* spp. biomass was ground with 1 L distilled water (DW) to form a wet homogeneous paste, from which surplus water was removed by squeezing it out into a filter bag. From the resultant wet material,

the pigments were removed with 2 L of ethanol at room temperature for 24 h. Following ethanol treatment, filtering, and air drying, ulvan was extracted for 2 h in an aqueous ammonium oxalate ($[NH_4]_2[C_2O_4]$, 20 mM, pH ~ 7) solution with vigorous stirring and heating (75 ± 5 °C) (Alves et al. 2013). The supernatant was collected by centrifugation, and its volume was reduced by ~ 90% on a rotary evaporator at 60 ± 5 °C/60 mmHg. To obtain a dry extract, the concentrated supernatant solution was dialyzed for 24 h against deionized water, using 3.5 kDa dialysis membranes and s subsequently lyophilized. The resulting dry ulvan crude extract was divided into 15 mL and 50 mL tubes containing 150 mg and 500 mg of the material, respectively. The tubes of ulvan powder were further sterilized with a low-intensity γ-irradiation treatment (25 kGy (Munarin et al. 2013), Soreq Nuclear Research Center, Israel) and stored at −20 °C before further use. Two extractions were performed two years apart, ulvan18 (December 2018) and ulvan20 (April 2020). The two extractions were used to compare the chemical composition of ulvan from different biomass cultivation years. Only ulvan18 was used for plant experiments.

Ash and elemental analysis

Elemental carbon, hydrogen, nitrogen, and sulfur (CHNS) analysis was performed using a Thermo Scientific CHNS Analyzer (Flash2000). A 2–3 mg sample was weighed with 8–10 mg vanadium in a tin crucible. The combustion temperature was 950 °C, and the carrier gas was helium (99.999%, flow rate 140 mL min^{-1}) with the addition of O_2 at 250 mL min^{-1} for 5 s. Cystine, BBOT ((2,5-Bis(5-tert-butyl-benzoxazol-2-yl) thiophene), sulphanilamide, and methionine were used as standards. The analysis was performed at the Technion-Israel Institute of Technology, Chemical, and Surface Analysis Laboratory.

Elemental analysis for minerals was performed using microwave-assisted acid digestion followed by inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis. An aliquot of each sample (~ 150 mg) was digested with HNO_3 and H_2O_2 in an Ethos Easy laboratory microwave oven (Milestone, Italy). The samples were dissolved completely. The element concentration was analyzed on HR Dual-View ICP-OES PQ9000 (Analytik, Germany). Measurements were calibrated with ICP standards (Merck, Germany). Element concentrations that exceeded the linear dynamic range were diluted and reanalyzed. Dilutions were prepared using calibrated pipettes. The calibration verification standard was continuously measured to check instrument stability.

For ash fraction determination, each aliquot was digested in a glass crucible at 550 °C for 5 h until a constant weight was obtained. The ash weight was determined in a dry state at 105 °C. Three replicates of each aliquot were analyzed. Analyses for minerals and ash were performed in the Interdepartmental Equipment Unit of Hebrew University, Rehovot.

Fourier-transform infrared (ATR-FTIR) spectroscopy

Attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy (Tensor 27, Bruker, USA) equipped with a standard attenuated total reflection attachment (ATR, Pike Technologies, USA) was used to analyze the solid freeze-dried ulvan extract samples (Osorio et al. 2024). The FT-IR spectra of the extracts within the spectral range 4000–400 cm^{-1} (4 cm^{-1} resolution) was measued using a single reflectance horizontal ATR cell (Golden Gate with a diamond crystal) equipped with a system to compress the dried homogenized dry ulvan samples onto the crystal surface. Each sample was analyzed three times. The spectra were compared to those in the literature performed on similar *Ulva* seaweeds, focusing on the major peaks of the spectrum and analyzed functional groups accordingly.

Monosaccharide composition determination

The monosaccharides glucose, rhamnose, xylose, and uronic acid in the hydrolysates were quantified using acid hydrolysis and high-pressure ion chromatography (HPIC), following previously reported protocols (Robin et al. 2017; Shefer et al. 2017). Briefly, Dionex ICS-5000 (Thermo Fischer Scientific), Carbopac MA1 (Thermo Fischer Scientific,) and its corresponding guard column were used for separation. An electrochemical detector with AgCl as a reference electrode was used for detection. A trinary solvent system was used for elution (Table S1). The column temperature was maintained at 30 °C and a flow rate of 0.25 mL min^{-1}. Calibration curves were constructed for rhamnose, glucose, xylose, and uronic acid (0–100 µg mL^{-1}), and sample measurements were compared to standard (Sigma-Aldrich, Israel) concentrations. Each sample was analyzed three times.

Arabidopsis thaliana plant material and growth conditions

All the *A. thaliana* lines used in this work were of Col-0 (wild type Columbia-0, CS70000 ABRC) variety. The seeds were sterilized for 2 h in chlorine gas, generated by mixing 3 mL of 32% HCl with 50 mL of 11% NaClO. After sterilization, the seeds were held in the laminar hood for 10–15 min before sowing. 8–10 seeds were sowed onto 12 × 12 cm square plates containing half-strength Murashige and Skoog (MS) medium (0.43% MS salt [Sigma Aldrich], 0.05% MES [Sigma Aldrich], 0.8% of plant agar [Merck] and 1% sucrose [Bio-Lab, Israel]). The pH of the medium was adjusted to 5.7 using 1 M KOH before autoclaving. Each plate contained 35 mL of media, 30 mL of the agar + MS,

and 5 mL of ulvan at the tested concentration. The control plate contained 35 mL of agar-MS.

After sowing the seeds, the plates were sealed with Micropore paper tape (3 M, USA) to prevent water loss. They were placed in a dark refrigerator (4 °C) for 2 to 3 days (stratification) to improve and synchronize the germination. After stratification, we transferred the plates to the growth chamber, maintaining constant environmental conditions: 16 h of light (in the control, approx. 150 µmol photons m^{-2} s^{-1}) and 8 h of darkness, 60% relative humidity, and temperature of 22 °C for three weeks.

The entire process, including seed sterilization, preparation of ulvan solution with the agar-MS medium, and establishing the plates, was performed in a sterile environment to prevent the growth of microbial or fungal contaminants. A new set of seeds from the same batch for each treatment was used.

Preparation of an agar-MS medium supplemented with ulvan crude extract

A 1% ulvan stock solution was prepared by adding 15 mL of autoclaved double distilled water (DDW) to a 15-mL tube containing 150 mg sterilized dried ulvan; the solution was thoroughly mixed using a vortex. This stock solution was used to prepare the different ulvan concentrations, mixing the solution with agar-MS at the required ratios.

Examine the timing effect of ulvan supplementation on seed germination

After determined the range of bioactive ulvan concentration, in the previous paper, 0.2 mg mL^{-1} was identified as the optimal ulvan concentration for stimulating *A. thaliana* germination, we tested the timing of adding ulvan biostimulant during the seedling growth process. For this purpose, 0.2 mg mL^{-1} ulvan was added at two consecutive time points. First, the same method as in previous experiments was used, adding the ulvan solution to the agar-MS medium when sowing the seeds at time 0, thereby testing the effect of ulvan on seed imbibition. Second, addition the ulvan to the medium a week after sowing the seeds, thereby testing the effect of ulvan on leaf development stage.

The same batch of ulvan (ulvan18) and the same batch of WT *Arabidopsis* Col-0 seeds in all experiments.

Table 1 outlines the experimental design used to evaluate the effect of ulvan supplementation on different stages of *A. thaliana* germination. Six treatment groups were established based on the timing and presence of ulvan (0.2 mg mL^{-1}) in the growth medium. Group 1 (S.C) served as the untreated control, while Group 2 (S.U) received ulvan at sowing. Groups 3 to 6 assessed the impact of transferring seedlings to new media one week post-sowing. Group 3 (Sp.C) involved seedlings not previously treated, transferred to control medium. Group 4 (Sp.U) comprised untreated seedlings transferred to ulvan-supplemented medium. Group 5 (Sp.U.C) included seedlings initially treated with ulvan but transferred to unsupplemented medium, while Group 6 (Sp.U.U) was treated with ulvan both at sowing and post-transfer. This design allowed for the disentanglement of initial and sustained ulvan effects and the isolation of medium transfer as a confounding factor.

The rationale for the group divisions was to neutralize the effect of the transition to the new agar MS medium after one week, allowing us to focus on the ulvan effect. The transfer made it necessary to create media with new ulvan for groups 4 and 6. The environmental conditions of light, temperature, and humidity were kept constant for all treatments. All plates in each experiment were sown and opened for scanning at the same time after

Table 1 Experimental design for evaluating ulvan supplementation timing on *Arabidopsis thaliana* germination and development. S.C – seeds only (control), S.U – seeds exposed to ulvan at time 0, Sp.C – seedlings control—not exposed to ulvan, Sp.U – seedlings from seeds not exposed to ulvan transferred to agar with ulvan, Sp.U.C – seedlings from seeds exposed to ulvan transferred to agar without ulvan, Sp.U.U – seedlings from seeds exposed to ulvan transferred to agar with ulvan. "+" means ulvan was added at that stage. "−" means no ulvan was added at that stage

Group number	Group Name	Time 0	After one week	
		Ulvan18 (0.2 mg mL^{-1})	Transfer to new agar-MS	Transfer to new agar – MS + ulvan18 (0.2 mg mL^{-1})
1	S.C	-	-	-
2	S.U	+	-	-
3	Sp.C	-	+	-
4	Sp.U	-	+	+
5	Sp.U.C	+	+	-
6	Sp.U.U	+	+	+

approximately 3 weeks. They were immediately measured for photosynthetic parameters and the leaf area scanning at the phenomics unit, followed by scanning for root length analysis. Next, they were thoroughly dried at 65 °C for 3 days and weighed.

Effect of ulvan on A. thaliana photosynthetic parameters

The photosynthetic parameters using the Plant ScreenTM Phenotyping System (Photon Systems Instruments)—Chlorophyll Fluorescence Imaging Unit were examined in the TAU Phenomics center.The fluorescence imaging station uses an enhancement of the FluorCam FC-800MF Pulse Amplitude Modulated (PAM) camera system (Photon Systems Instruments). The unit pauses for 15 min while all trays are in complete darkness and then examines the plates individually for the FluorCam to monitor fluorescence kinetics and the RGB camera for plant morphometric analysis. In this work, we analyzed the following photosynthetic parameters: $F_v/F_{m(QY\ max)}$, F_v/F_m_Lss, NPQ_Lss, and Rfd_Lss and one morphological characteristic of shoot area. $F_v/F_{m(QYmax),}$ in which $F_v = (F_{max}\text{-}F_0)$, is an indicator of the maximum quantum yield of PSII photochemistry. In healthy *A. thaliana* plants, this parameter should be ~ 0.83, while lower values can indicate stress (Maxwell and Johnson 2000; Bresson et al. 2015). F_v/F_m_Lss, in which $F_v = (F_m$_Lss-$F_0)$, denotes the PSII quantum yield of the light-adapted sample at steady-state and is expected to be lower than F_m (QY_{max})(Murchie and Lawson 2013).

NPQ_Lss is the non-photochemical quenching (NPQ; at steady-state) of chlorophyll fluorescence, a process that counteracts the build-up of excess energy in PSII by dissipating the excitation energy into heat (Ware et al. 2015). Rfd_Lss is an indicator of the potential photosynthetic activity of leaves, correlated with the net photosynthetic CO_2 assimilation (Lichtenthaler and Miehé 1997). These parameters were chosen because they indicate the seedlings photosynthetic condition and enable comparison between the treated and untreated seeds (Lichtenthaler et al. 2005, Brestic and Zivcak 2013; Ware et al. 2015; Singh et al. 2017).

Effects of ulvans on seed yield in A. thaliana plants

Next it was determined whether the ulvan biostimulant had any significant effect on the final yield of seeds in the mature *A. thaliana* plant. The objective was to assess if any significant development effects identified on the seedling stage and the seedlings affected the mature plant and its seed yield.

The three-week-old seedlings were transferred to pots with planting mixture and Osmocote (Smart-Release Plant nutrition). The plant was allowed to reach full growth and inflorescence and the siliques to turn completely brown before harvesting the seeds (approximately three months). The seeds were collected from each plant separately and kept them in 2.5-mL Eppendorf tubes.

Two groups of 16 three-week-old seedlings were tested. The first group was supplemented with ulvan18 upon seed sowing in the agar-MS medium (0.2 mg mL^{-1} media), while the second group was a control. After three weeks, both groups were moved from the medium to pots containing a plant growth medium**.** Osmocote, a fertilizer that slowly releases nutrients over time to deliver an ongoing supply of nutrition to the plants was added. The pots were maintained in a growth chamber under optimal growth conditions (16 h of light and 8 h of darkness, 60% relative humidity, a temperature of 22 °C, and light intensity of ~ 150 µmol photons m^{-2} s^{-1}). The pots were irrigated when the medium started to dry (approximately once a week). Each plant was covered with a transparent plastic bag (commercial flower sleeves) as an enclosure to separate each plant from the others and to prevent the loss of pods and seeds**.** After the plants reached maturity and the first pods began turning brown, the watering was stopped, allowing the plants to dry out completely. After the siliques had completely browned, the seeds were harvested.

To harvest the seeds: the plant was carefully cut from its base under the plastic cover, placed on parchment paper on the table, and the outside of the plastic bag was shaken to separate the seeds from the dry vegetative material. The harvested seeds were sieved from the excess dry matter and chaff in multiple steps using strainers with different mesh sizes. Finally, the harvested seeds of each plant were transferred to 2.5-mL Eppendorf tubes and stored at 4 °C until use.

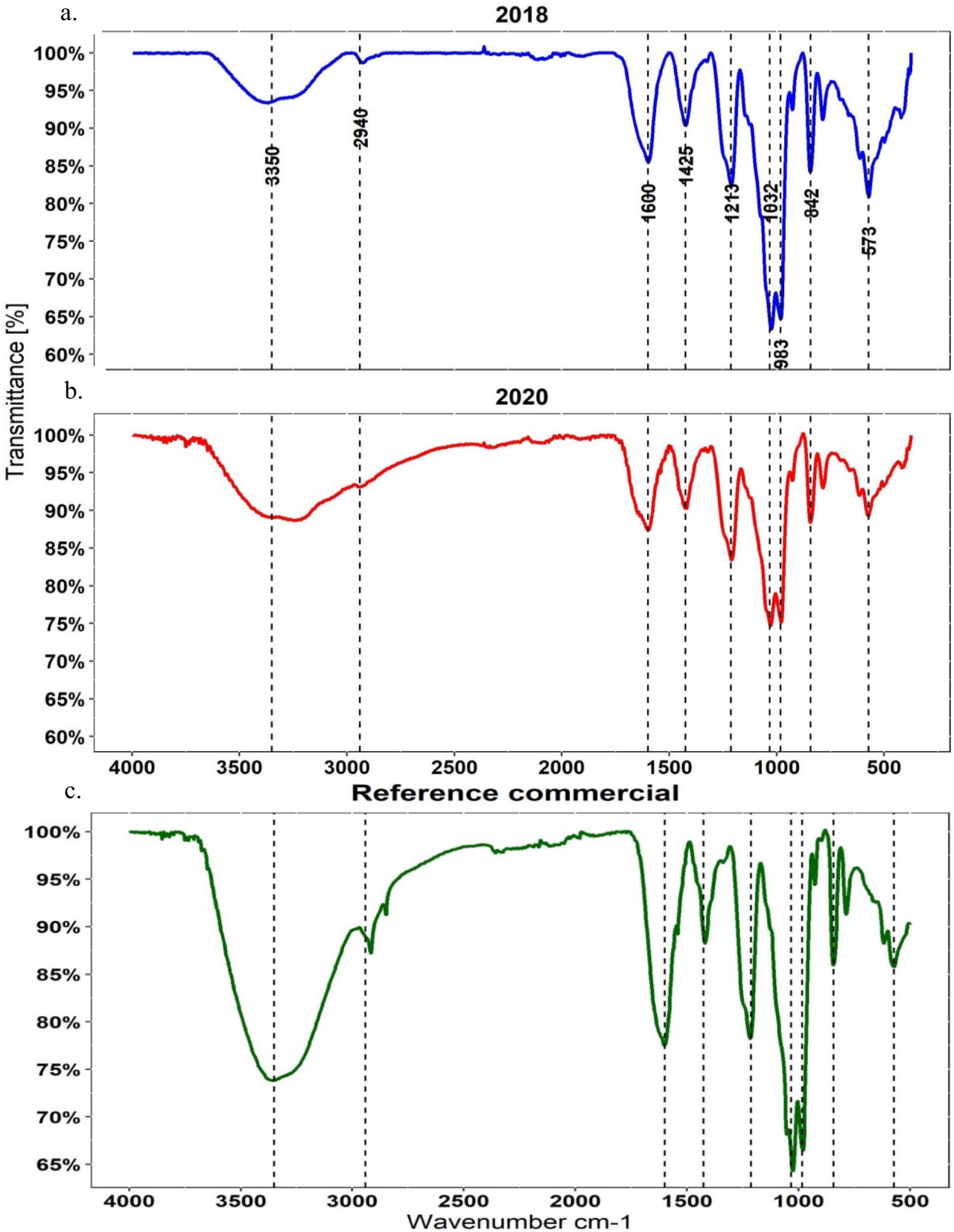
To measure the areas of the seeds, ImageJ SW was used. Thirty seeds from each treatment were scanned using the scanner (WorkForce GT-1500 scanner, EPSON) at 300 dpi. The ImageJ SW converted the image file to an 8-bit binary file**.** Next, the "Analyze Particles" method was used to outline the seeds to their edge, numbering each seed and measuring each area in μm^2**.**

To measure the impact of ulvan on the yield, six portion were picked with sizes of seeds: 100, 200, 300, 400, 500, and 1000 seeds from each group of plants. Each portion was weighed individually. This process allowed building a curve that correlated the number of seeds with weight using the linear equation:

$$N = m \cdot W - b \quad (1)$$

where N = number of seeds, W = total weight of seeds, m = slope, b = constant (y-intercept).

Using the equation obtained, the seeds from each plant were weighted to obtain the number of seeds.


a.
2018
100%
95%
90%
85%
80%
75%
70%
65%
60%
3350
2940
1600
1425
1213
1032
983
842
573
Transmittance [%]
b.
2020
4000
3500
3000
2500
2000
1500
1000
500
Reference commercial
c.
Wavenumber cm-1

◂**Fig. 1** FTIR spectra of ulvan extracted from *Ulva* spp. (**a**) Material obtained in the ulvan18 extraction. (**b**) Material obtained in the ulvan20 extraction. (**c**) Reference commercial "winter-heavy" ulvan (CarboSynth, UK), extracted from *Ulva armoricana* from Bretagne, France. Peak wavenumbers are shown in (a)

Ulvans effects on roots and shoots as measured with untargeted mass spectrometry

For the detection of ulvan effects on roots and shoots, 6 *A. thaliana* plants were used from which sampled roots and shoots, which were frozen and ground. The samples were brought to 8 M urea, 400 mM ammonium bicarbonate, 10 mM DTT, vortexed, sonicated for 5 min at 90% with 10–10 cycles, and centrifuged. The protein amount was estimated using Bradford readings. 20 µg protein from each sample was reduced to 60 ºC for 30 min, modified with 37.5 mM iodoacetamide in 400 mM ammonium bicarbonate (in the dark, at room temperature, for 30 min), and digested in 2M urea, 100 mM ammonium bicarbonate with modified trypsin (Promega) at a 1:50 enzyme:substrate ratio, overnight at 37 °C. Additional second digestion with trypsin was done for 4 h at 37 °C. The tryptic peptides were desalted using C18 tips (Harvard Apparatus, USA), dried, and re-suspended in 0.1% formic acid. The peptides were resolved by reverse-phase chromatography on 0.075 X 180-mm fused silica capillaries (J&W Pharmlab, USA) packed with Reprosil reversed-phase compound (Dr. Maisch GmbH, Germany). The peptides were eluted with a linear 180-min gradient of 5 to 28%, a 15-min gradient of 28 to 95%, and a 25-min gradient at 95% acetonitrile with 0.1% formic acid in water at flow rates of 0.15 µL min^{-1}. Mass spectrometry was performed using a Q-Exactive Plus mass spectrometer (Thermo Fischer Scientific) in positive mode using a repetitively full MS scan, followed by collision-induced dissociation (HCD) of the 10 most dominant ions selected from the first MS scan. The mass spectrometry data from all of the biological repeats were analyzed using MaxQuant software 1.5.2.8 versus the *A. thaliana* proteome from the UniProt database with 1% false discovery rate (FDR). The data were quantified by label-free analysis using the same software, based on extracted ion currents (XICs) of peptides, enabling quantitation from each LC/MS/MS run for each peptide identified in any of the experiments. The identified proteins appear in https://github.com/GolbergLab/Ulvan_Arabidopsis and Data are available via ProteomeXchange with identifier PXD061726.

Differential expression analysis of proteins as quantified with mass spectrometry

Differential expression per protein was calculated with Wilcoxon rank-sum test for independent samples (*scipy.stats.ranksums*). False Discovery Rate (FDR) was calculated as ratio of expected and observed values for a given p-value. Ulvan-Control ratio for each protein was estimated as ratio of its intensity means in ulvan and control groups of samples.

Statistical analysis

The results were analyzed using either two-way ANOVA, in which there were more than two parameters to compare, or a paired Student's t-test, with two parameters. The ANOVA test was completed using RStudio (RStudio: Integrated Development for R. RStudio, PBC, USA). The equal variance assumption of homogeny (by running residuals vs. fitted and scale-location plots) was made and using the normal QQ-plots to verify normal distribution before conducting ANOVA.

Results

Ulvan extraction

To verify ulvan purity and the consistency of the extraction method, two ulvan extractions (Ulvan18 and Ulvan20). The yield of the extracted ulvan was ~ 14.5% w/w and ~ 8.7% w/w from dry material in the first and second extraction steps.

Chemical and biophysical profile of the ulvan extract

Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) was performed to verify the consistency between the two extractions and compare them to previously published ulvan extraction results (Fig. 1). The FTIR spectra of the ulvan18 and ulvan20 extractions revealed a high degree of similarity (peaks are listed in Table 2).

The prominent wavelength peaks identified through ATR-FTIR spectroscopy for ulvan extracts obtained in 2018 and 2020 are shown in Table 2. The table aligns these peaks with corresponding functional group assignments, highlighting chemical consistency across batches. Peaks at 573 and 1032 cm^{-1} are attributed to symmetric stretching of C–O–C bonds in carboxylic groups, while the 842 cm^{-1} peak corresponds to C-O-S bonds, characteristic of sulfate presence in ulvan. Additional peaks at 1213 and 1600 cm^{-1} indicate the presence of sulfate (S = O) and uronic acid-derived carboxylic groups, respectively. Peaks at 2940 and 3350 cm^{-1} are consistent with C-H and O–H bond stretching, typical of sugar and hydroxyl-rich polysaccharides.

Table 2 The prominent FTIR wavelength peaks obtained from both ulvan extractions (ulvan18 and ulvan20)

Peak wavenumber (cm^{-1})	Functional group assignment
573, 1032	symmetric stretching of C–O–C bonds of carboxylic groups
842	stretching of C-O-S bonds, usually found in ulvan due to the presence of the sulfate groups
983	stretching of C-O bonds in sugars
1213	stretching of the S = O bond of the sulfate groups
1425	asymmetric stretching of O-C-O bonds of carboxylic groups
1600	carboxylic groups of the uronic acid moieties
2940	stretching of C-H bond
3350	stretching of O–H bonds of the hydroxyl groups

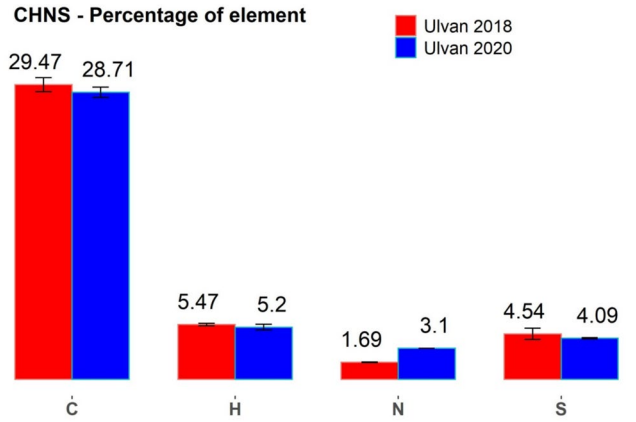


Fig. 2 CHNS elemental analysis of the two ulvan extracts obtained in 2018 (red) and 2020 (blue). n = 3. Standard deviation error bars are shown

Elemental analysis (CHNS)

The chemical composition of the ulvan18 and ulvan20 extracts was compared by elemental combustion analysis of carbon, hydrogen, nitrogen, and sulfur (Fig. 2). The results of the CHNS analysis of the materials obtained by both extractions showed high content similarity for carbon (~ 29% C), hydrogen (~ 5.25% H), and sulfur (~ 4.3% S). The main observed in this study difference between the two extracts was the nitrogen content, which was 83% higher in the ulvan20 extraction.

Elemental analysis for minerals and ash content

The elemental analysis results for minerals performed on both ulvan extracts (18 and 20) are presented as mg kg^{-1} for each element per weight of ulvan. Although there are some substantial elemental content differences between the two extractions (Table 3), it is essential to emphasize that the overall elemental concentrations are minimal. The ulvan18 Al, Fe, K, and Na contents were higher (300%, 155%, 183%, and 335%, respectively), and the Ca, Cd, and P were lower (62%, 9%, and 44%, respectively) than in ulvan20. The Mg content was the highest of all minerals measured and was similar in both extracts. The Ca, Na, K, and P contents were less than Mg but greater than the remaining mineral elements. The results of ash analysis showed a low level of ash in both extracts, 9.74% ± 0.08 of dry ulvan18 and 12.14% ± 0.34 of dry ulvan20, respectively.

HPIC analysis of monosaccharides and uronic acid

Four repeats (n = 4) of HPIC analysis were used to determine the monosaccharide fraction in the ulvan18

Table 3 Elemental analysis of mineral and ash content of ulvan extracts

Element	Ulvan18			Ulvan20			Method LOQ (mg kg^{-1})
	Concentration (mg kg^{-1} avg (n = 3))	std	CV%	Concentration (mg kg^{-1} avg (n = 3))	std	CV%	
Al	21.20	0.85	3.99	7.05	0.45	6.41	0.62
Ca	1447.76	3.22	0.22	2311.20	31.49	1.36	0.096
Cd	0.68	0.01	1.31	7.25	0.03	0.41	0.013
Fe	24.39	0.16	0.66	15.68	0.50	3.18	0.071
K	2061.60	21.69	1.05	1124.15	19.91	1.77	0.149
Mg	24,342.08	257.58	1.06	24,379.17	292.79	1.20	0.519
Na	1077.30	8.74	0.81	321.09	4.63	1.44	0.087
P	806.77	18.72	2.32	1818.44	39.25	2.16	1.716
Ash, % of dry	9.74	0.08	0.85	12.14	0.39	3.25	

extract. The most abundant monosaccharides in the powder were rhamnose (111.1 ± 8.9 mg g^{-1}), followed by glucose (54.6 ± 2.2 mg g^{-1}) and xylose (23.6 ± 7.9 mg g^{-1}), and uronic acid (glucuronic acid and iduronic acid) (194.4 ± 23.4 mg g^{-1}) (Fig S1).

The effect of ulvan treatment on phenotypic characteristics of A. thaliana.

The effect of ulvan treatment on phenotypic characteristics is shown in Fig. 3: root length (Fig. 3a), shoot area (Fig. 3b), and total seedlings weight (Fig. 3c) after three weeks for all six treatment groups, as outlined in Table 1.

Results shown in Fig. 3, suggest that the ulvan positively affected the germination of *A. thaliana* seeds. The roots of the treated seedlings (Fig. 3a) were significantly longer (+13.6%, mean 5.55 cm) than the control (mean ~ 4.88 cm). The treated seedlings (Fig. 3b) also had a higher shoot area (+45.2%, 58.06 mm^2) than the control (33.98 mm^2). No significant difference in the overall weight of any of the treated and untreated seedlings was observed, possibly resulting from the young age of the seedling (three weeks): the total weight is not high enough to exhibit significant differences. Although in the 4 treatments with the new agar-MS medium (groups 3–6), there were no significant differences for either characteristic, root length and shoot area, as expected, were

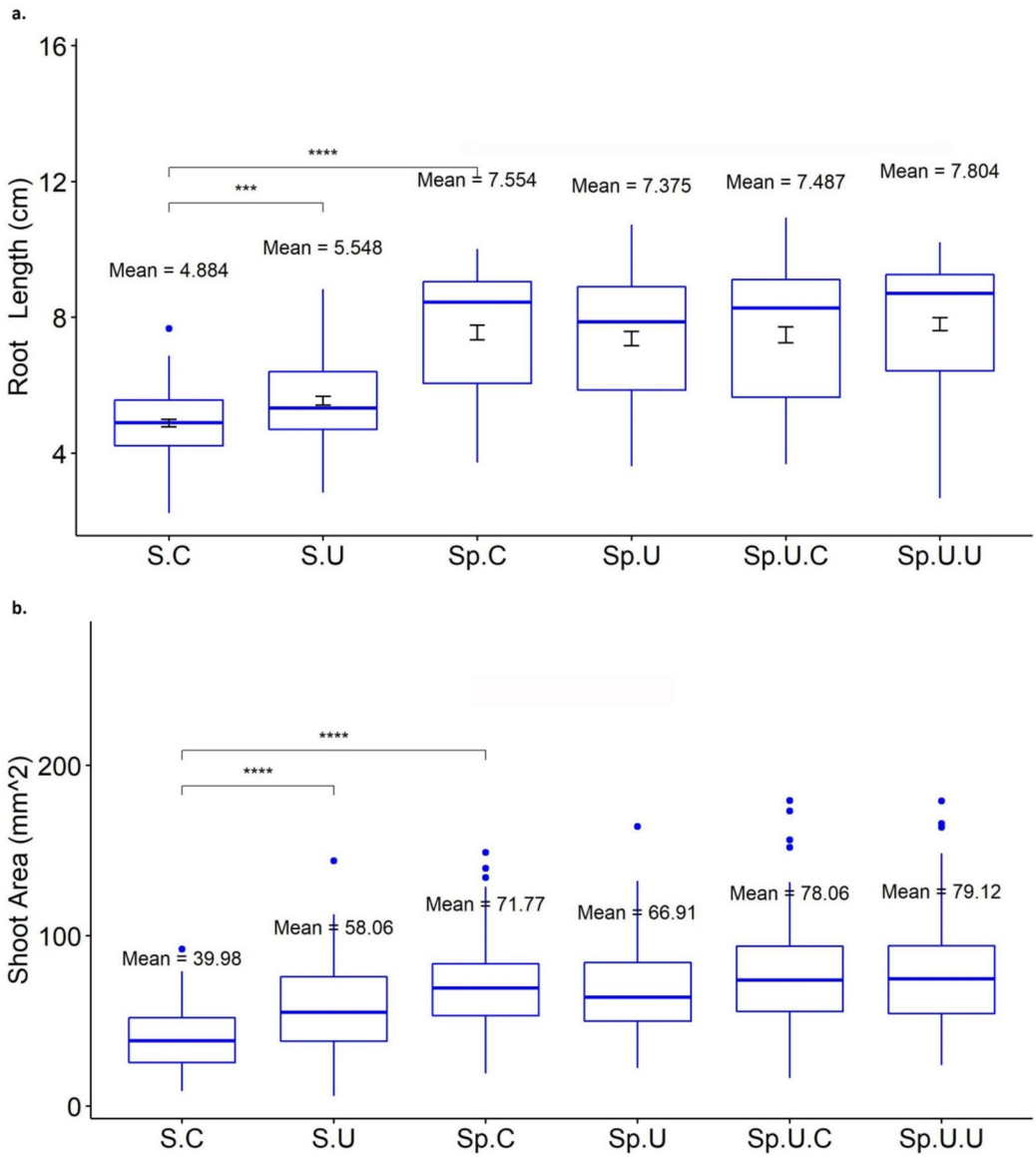


Fig. 3 Effect of ulvan treatment on phenotypic characteristics of *A. thaliana*. (**a**) Root length and (**b**) shoot area of the seedlings after three weeks for all six treatment groups (outlined in Table 1)

significantly greater in this group of four treatments than groups 1 and 2 that were not transferred to a new medium. This difference may be due to the addition of nutrients in the fresh medium.

The effect of ulvan treatment on photosynthetic characteristics of A. thaliana.

Four photosynthetic parameters were analyzed using phenomics:

$F_v/F_{m(QY\ max)}$, an indicator of maximum quantum yield of PSII photochemistry. In healthy *Arabidopsis* plants, this parameter should be ~ 0.83, while lower values can indicate stress (Maxwell and Johnson 2000; Bresson et al. 2015).

F_v/F_m_Lss, denoting the PSII quantum yield of the light-adapted sample at steady-state, expected to be lower than F_m (QY_{max}).

NPQ_Lss, non-photochemical quenching (at steady-state) of chlorophyll fluorescence (NPQ), a process that counteracts the build-up of excess energy in PSII by dissipating the excitation energy into heat (Ware et al. 2015).

Rfd_Lss, an indicator of the potential photosynthetic activity of leaves, correlated with the net photosynthetic CO_2 assimilation(Lichtenthaler and Miehé 1997).

Photosynthetic parameters following ulvan treatment (Fig. 4) were also analysed. $F_v/F_{m(QY\_Max)}$ is shown in Fig. 4a, F_vF_m_Lss is shown in Fig. 4b, NPQ_Lss is shown in Fig. 4c, and Rfd_Lss is shown in Fig. 4d. Treatment groups 1–6 are defined in Table 1. The statistical analysis showed some minor significant differences. The seeds kept in the same medium (groups 1 and 2), and the seeds that underwent medium replacement after one week (groups 3 and 4), were healthy regardless of the treatment, as indicated by their photosynthetic parameters.

Effect of early-stage ulvan treatment on seed yield in mature Arabidopsis thaliana plants.

To assess whether the ulvan-induced stimulation observed after three weeks exerted prolonged effects on seed yield in mature *A. thaliana* plants, two groups (one ulvan-treated 0.2 mg mL^{-1} during the seed sowing stage and one control), each comprising 20 three-week-old seedlings, were evaluated. All plants were allowed to complete their development, dry fully, and were subsequently harvested for seed collection. Seed size and weight variation between the two treatments was initially examined. Samples of thirty seeds per treatment group were collected, and the area of each seed was measured. No significant difference in seed area was found between the groups ($p = 0.85$) (Fig S2). The mean seed area of the control group was 0.1057 mm^2 (SEM = 0.0036), while the ulvan-treated group had a mean seed area of 0.1048 mm^2 (SEM = 0.0034).

Subsequently, a calibration curve was constructed, and the total seed yield from all plants in each group was weighed (Fig S3). A significantly higher seed yield was recorded in the ulvan-treated group compared to the control group. Specifically, the ulvan-treated plants exhibited approximately 43% greater seed yield than the control plants (Fig. 5).

Based on these results, its was concluded that the addition of ulvan in the early stages of *A. thaliana* seed germination enhances its growth parameters resulting in higher seed yields.

Proteins differential expression

Proteomics profiles of roots and shoots were measured in 3 control and in 3 ulvan samples during two years. Overall 5814 proteins were detected in the roots and 5482 proteins detected in the shoots in ulvan treated plants and controls.

In roots, on year 2020 up-regulation in 315 proteins (FDR = 0.48) and down-regulation in 355 proteins in ulvan treated plants (FDR = 0.43) was observed. On year 2021 up-regulation in 259 proteins (FDR = 0.58) and down-regulation in 175 proteins in ulvan treated plants (FDR = 0.86) was observed. Overall, 21 proteins (FDR = 0.36) were up-regulated and 6 proteins (FDR = 1.26) were down-regulated in ulvan treated samples in both years. Table 4 presents 5 top up- and down-regulated proteins in ulvan treated plants in roots.

In shoots, on year 2020 up-regulation in 380 proteins (FDR = 0.40) and down-regulation in 310 proteins in ulvan-treated samples (FDR = 0.49) were observed. On year 2021 up-regulation in 306 proteins (FDR = 0.49) and down-regulation in 160 proteins in ulvan-treated samples (FDR = 0.94) were observed. Overall, 24 proteins (FDR = 0.31) were up-regulated and 12 proteins (FDR = 0.63) were down-regulated in ulvan-treated samples in both years. Table 5 presents 5 top up- and down-regulated proteins in ulvan-treated samples in shoots.

These findings suggest that ulvan enhances photosynthetic efficiency, oxidative stress tolerance, and osmotic balance, while potentially redistributing energy resources within the plant.

Discussion

Using a biostimulant from a natural source to enhance vegetation and fruit yield is essential environmentally and economically. These biostimulant activities have been extensively studied in recent years. Seaweed extracts, in combination with other substances, are known to accelerate plant growth. However, the mechanisms of these effects are still unclear, especially when considering a specific combination of multiple factors, each of which may have a different effect (Rehim et al. 2021).

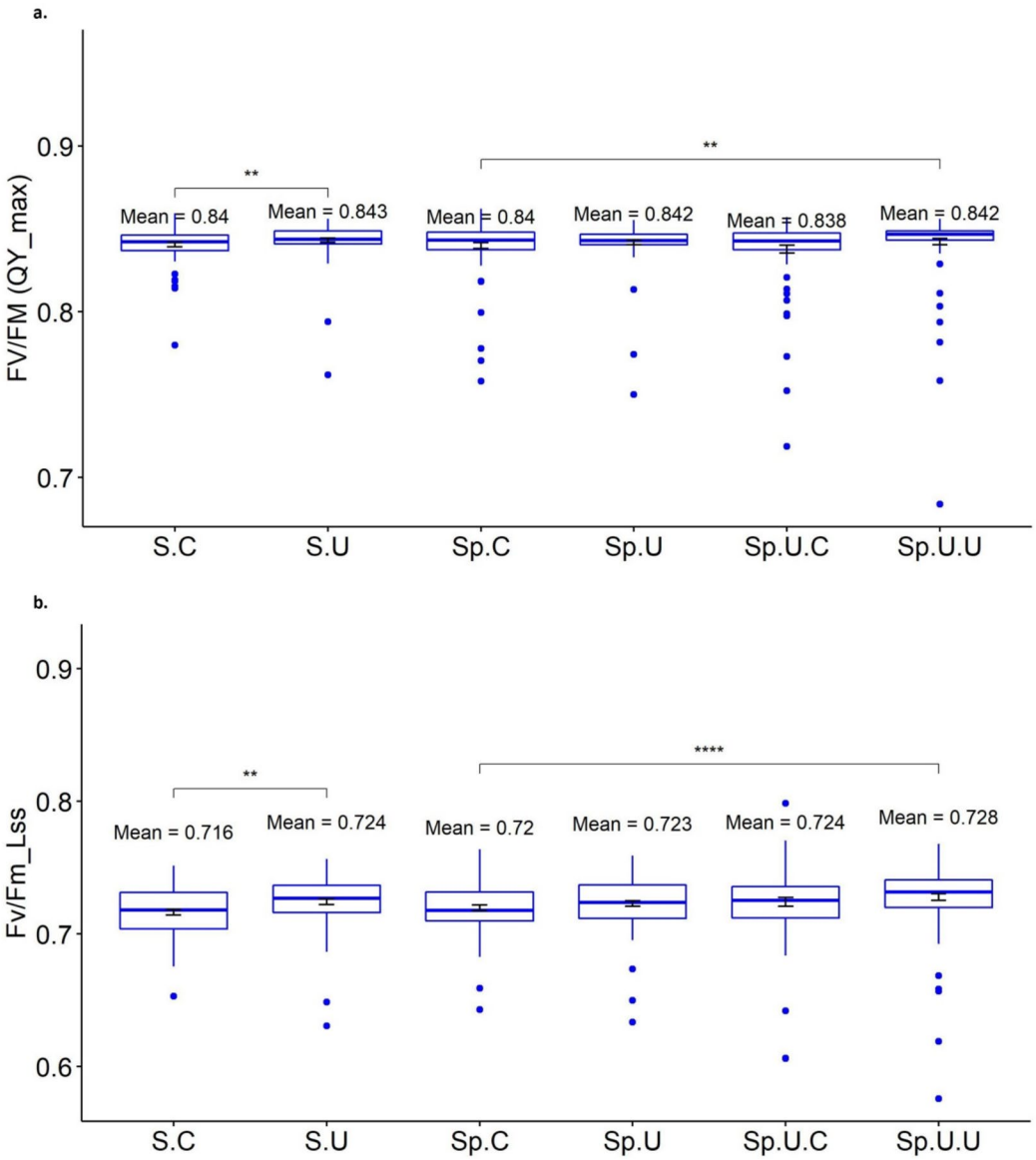


Fig. 4 Effect of ulvan treatment on four main photosynthetic parameters of *A. thaliana*: (**a**) $F_v/F_{m(QYmax),}$ (**b**) F_v/F_{m-}Lss, (**c**) NPQ_Lss, and (**d**) Rfd_Lss. Treatment groups 1–6 are described in Table 1

This study examined the efficacy of a biostimulant derived from *Ulva* spp. This genus are widely distributed along the world's coastlines. This study focused on extracting a specific type of molecule, the sulfated polysaccharide ulvan, unique to the cell wall of *Ulva*. The sulfated polysaccharide molecules are known to have bioactive potential (Cluzet et al. 2004; Hernández-Herrera et al. 2014; Mzibra et al. 2018; Borba et al. 2019; Benítez García et al. 2020). Most of the polysaharides studied have originated on products from red and brown seaweeds, and studies of the green seaweed polysaccharides have mainly been focused on the potential to reduce abiotic and biotic stresses of plants and less on accelerating their germination stages.

Previous ulvan analyses have shown a wide variation in ulvan yield and physicochemical properties, both affected by growth conditions and extraction protocols (Abdel-Fattah and Edrees 1973; Hernández-Garibay et al. 2011; Yaich et al. 2017; Tabarsa et al. 2018; Zhong et al. 2020). It was suggested that these variations in the ulvan physicochemical properties lead to variations in its biological activities (Kidgell et al. 2019) and the mechanisms by which it affects various biological systems (Lahaye and Robic 2007). Therefore, for comparative studies, there is a need for a well-characterized ulvan extraction methodology (Kidgell et al. 2019). Herein, this challenge was addressed by using *Ulva* spp. biomass cultivated in a closed system (Chemodanov

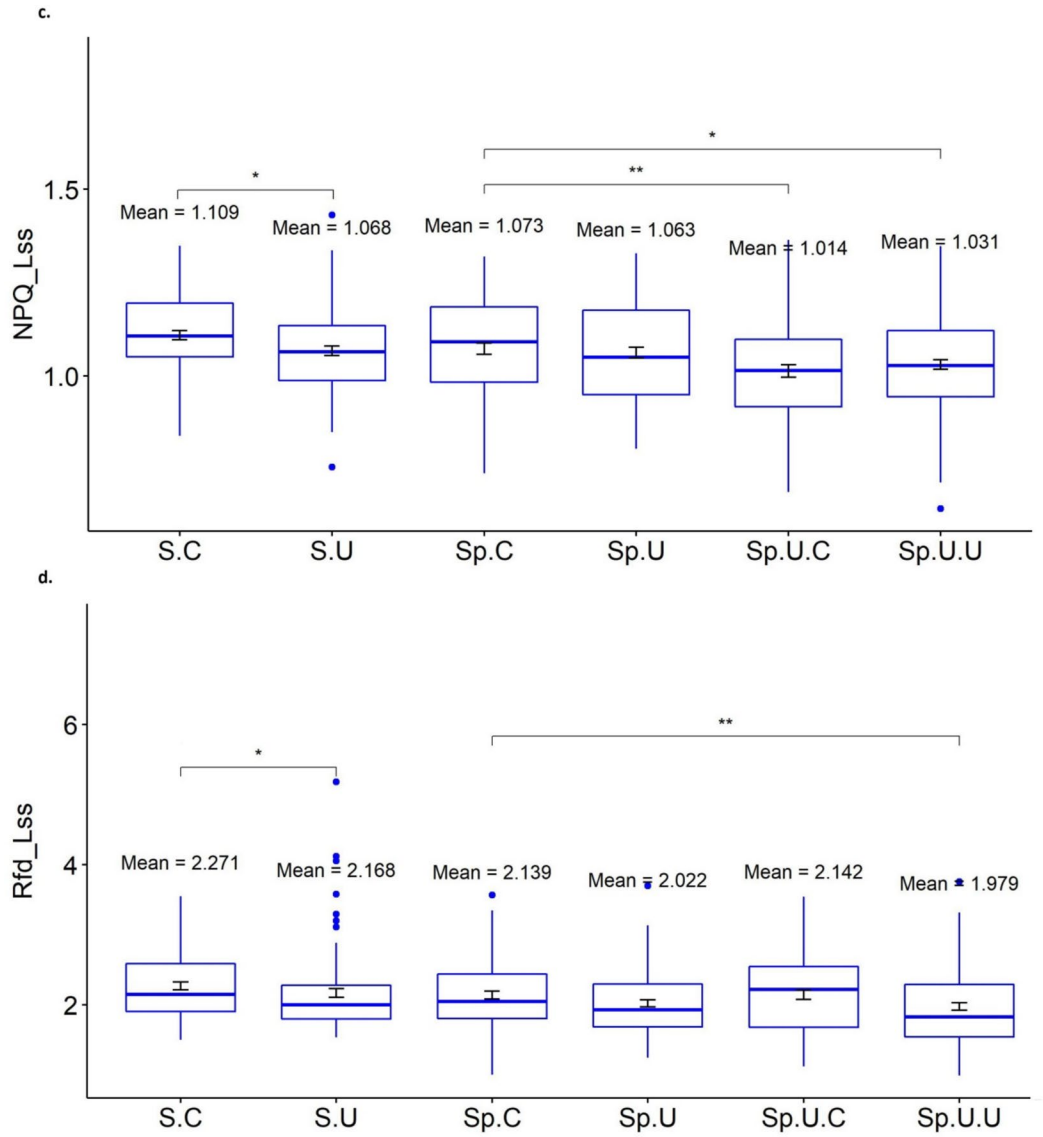


Fig. 4 (continued)

et al. 2017) and providing a thorough chemical profile of the extract. Two ulvan extractions were performed (2018 and 2020) using *Ulva* spp. from the same cultivation system. The extraction procedure for both biomasses was similar. The properties of the final extracts were compared to verify that the extracted material and its biophysical and biochemical properties were similar. This extraction was used to compare both ulvan extracts and determine reproducibility for the following studies.

The first aim of this work was to develop an extraction method to obtain high-quality, crude ulvan extract from local *Ulva* spp. and characterize its composition. To achieve a simplified, non-destructive extraction method, the following parameters (based on a modified protocol from Robic et al. 2009c) were applied: (i) the temperature under 80 °C during the entire extraction process, (ii) avoiding using acidic extraction steps, and (iii) shortening the extraction time (up to two hours when using a high-temperature process). These three parameters have been previously determined to affect the quality and yield of the extracted ulvan (Kidgell et al. 2019).

Based on published ulvan characteristics, the quality of ulvan extracts was tested on two separate occasions (autumn of 2018 and spring of 2020), comparing yield, FTIR characterization, the nutrient and mineral content, monosaccharide content, and molecular weight.

Regarding yield, the reported extraction method resulted in ~ 9–14% w/w, within the reported range of other ulvan extraction methods (Alves et al. 2010; Thanh et al. 2016; Wahlström et al. 2020; Kidgell et al. 2021). Moreover, the

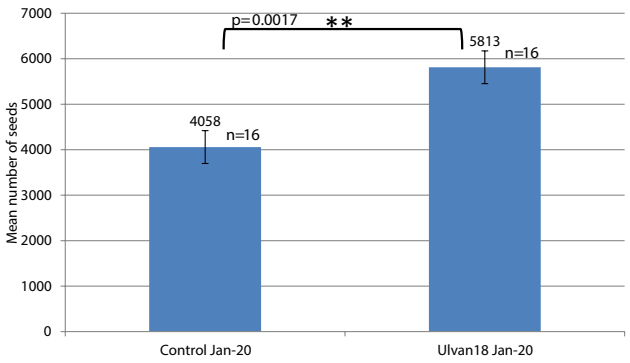


Fig. 5 *Arabidopsis thaliana* plant yield for seeds sown in January 2020. Sixteen plants were treated with ulvan18 at 0.2 mg mL^{-1} during the seed sowing stage, in the plant medium and 16 were untreated and used as controls. (The calibration curve is shown in Fig S3). Unpaired t-test results of the number of seeds in the ulvan-treated groups vs. the control show a significant difference between the plant groups (n = 16, p = 0.0017)

FTIR spectra of extracted in this study ulvans were similar to those of a commercial (CarboSynth, UK) "winter-heavy" ulvan, extracted from *Ulva armoricana* collected in Bretagne, France (Wahlström et al. 2020), and to other reported ulvan extracts (Robic et al. 2009a; Sharma et al. 2012; Van Oosten et al. 2017; Wahlström et al. 2020).

The elemental (CHNS) analysis results of ulvan reported in this study were similar to published CHNS values (Robic et al. 2009d; Glasson et al. 2017). However, notably, the nitrogen (N) content differed between the two ulvan batches (higher N content in the 2020 extract than the 2018 extract). There is a high correlation between the nitrogen content and the protein content in seaweeds (Angell et al. 2016). Thus, the N values may reflect variability in the protein levels in our ulvan preparations. This explanation is most likely since proteins co-extract with sulfated polysaccharides (Glasson et al. 2017; Kidgell et al. 2019; Wahlström et al. 2020). Since the two studied ulvan extracts were extracted from *Ulva*

Table 4 5 most up-regulated and 5 most down-regulated proteins in roots in ulvan-treated *Arabidopsis thaliana* plants relative to control samples

Protein ID	Protein names	Gene names	Ulvan-Control ratio	
P42804	Glutamyl-tRNA reductase 1, chloroplastic	HEMA1	3.11	UP
P24704	Superoxide dismutase [Cu–Zn] 1	CSD1	2.24	UP
Q8LG89	Basic blue protein	ARPN	2.04	UP
O78310	Superoxide dismutase [Cu–Zn] 2, chloroplastic	CSD2	1.72	UP
Q9XEA1	Protein OSCA1 OS = Arabidopsis thaliana OX = 3702 GN	OSCA1	1.56	UP
Q9FZD1	Pentatricopeptide repeat-containing protein At1g26460, mitochondrial	At1g26460	0.91	DOWN
O49629	Probable plastid-lipid-associated protein 2, chloroplastic	PAP2	0.89	DOWN
Q8GWW8	Chaperone protein dnaJ GFA2, mitochondrial	GFA2	0.68	DOWN
Q9ZU66	Putative spliceosome-associated protein	emb2444	0.68	DOWN
Q9ZUN8	Putative WD-40 repeat protein	heat	0.63	DOWN

Table is sorted according to ulvan-to-control ratio, calculated as ratio between average protein intensities

Table 5 5 most up-regulated and 5 most down-regulated proteins in shoots in ulvan-treated *Arabidopsis thaliana* plants relative to control samples

Protein ID	Protein names	Gene names	Ulvan-Control ratio	
Q93VM8	Copper transporter 5	COPT5	48.43	UP
Q94AG6	S-adenosylmethionine carrier 1, chloroplastic/mitochondrial	SAMC1	19.46	UP
Q9XIE2	ABC transporter G family member 36	ABCG36	16.31	UP
Q42338	AT3G48140 protein	T24C20_20	5.45	UP
A0A2P2CLF0 P93285	Cytochrome c oxidase subunit 2	COX2	5.41	UP
Q42418	Profilin-2	PRF2	0.95	DOWN
Q9C9U9	Pathogenesis-related thaumatin superfamily protein	At1g73620	0.87	DOWN
Q9SVU5	GDSL esterase/lipase At4g28780	At4g28780	0.62	DOWN
F4HQZ1	Regulator of chromosome condensation (RCC1) family protein	At1g19880	0.61	DOWN
Q42589	Non-specific lipid-transfer protein 1	LTP1	0.51	DOWN

Table is sorted according to ulvan-to-control ratio, calculated as ratio between average protein intensities

grown in two different seasons (autumn and spring), thrse results are consistent with Robic et al. (2009c). The authors identified seasonal variability in ulvan physicochemical and rheological properties. Furthermore, they showed a significant difference in nitrogen and protein content for ulvans extracted from the same *Ulva* spp., in different seasons.

The mineral element analyses revealed low mineral and ash content in our ulvan extractions, although the exact levels differed between the two preparations. These differences likely occurred due to changes in seawater composition (in the different years and seasons during which the seaweeds were collected). Notwithstanding, it was assumed that these minute levels would not affect the bioactivity of the ulvan in our extractions. In addition, the low ash content provides further support to the notion that the extraction method results in high-quality ulvan, with low levels of impurities (Wahlström et al. 2020).

It has been reported that ulvans extracted from blade *Ulva* species, such as *U. rigida*, have high concentrations of uronic acids (Kidgell et al. 2021) and rhamnose (reported to promote cell proliferation and collagen biosynthesis) (Lakshmi et al. 2020). Using HPIC, quantities of monosaccharides (rhamnose, glucose, and xylose) and uronic acids (glucuronic acid and iduronic acid) were detected. Of note, this quantification may underestimate the actual concentration of these molecules in our extractions since the acidic hydrolysis performed in these HPIC analyses might not break all glycosidic bonds between neutral and uronic acid residues (De Ruiter et al. 1992).

The analytical results demonstrate that simplified, modified extraction method reported in this study yields crude ulvan that matches the parameters of ulvan extracted by other methods, as described in Kidgell et al. (2021).

Having defined an extraction protocol that results in high-quality, crude ulvan, the bioactivity of the ulvan and identified a concentration appropriate for effective plant growth stimulation was tested. Previous reports demonstrated that low concentrations of *Ulva* spp. or extracted ulvan enhanced plant germination. Specifically, in low concentrations of 0.2 and 0.4 mg mL^{-1}, *U. lactuca* extracts promoted the germination and growth of tomato and mung bean (Hernández-Herrera et al. 2016). In the latter case, enhanced rooting was also noted. The positive effects of low concentrations (0.2%) of *U. lactuca* extracts on seed imbibition, germination, and seedling growth of mung bean plants were also reported by (Castellanos-Barriga et al. 2017). Furthermore, polysaccharide-enriched extracts of six seaweed species (including two green seaweeds, *U. rigida* and *Codium decorticatum*) showed beneficial effects on tomato plant germination, biomass, and chlorophyll content (Mzibra et al. 2018). Accordingly, previously it was tested if low concentrations of ulvan extracted by our method could enhance plant germination. Plants of the model species *A. thaliana* and focused on its early (three weeks) germination stages at two different light intensities were used. The most significant positive effect of the ulvan extract at a lower concentration (0.14 mg mL^{-1}) was the elongation of the roots (between 120 to 215% greater than controls) in the tested light intensities. An increase in total plant weight at the high light intensity was also observed. These results show that our crude extraction method yielded bioactive ulvan. Higher ulvan concentrations (greater than 0.71 mg mL^{-1}) did not positively affect the above parameters. These results are consistent with published results that showed either no effect or even inhibitory effects of high ulvan concentrations on plant growth (Hernandez-Herrera et al. 2014; Castellanos-Barriga et al. 2017; Mzibra et al. 2018).

Having determined the range of bioactive ulvan concentration in the previous work, in this paper ulvan supplementation timing on *A. thaliana* germination was studied. The early germination stage of *A. thaliana* (first three weeks), defined by the BBCH scale (Lorenz et al. 1994), encompasses seed imbibition and, one week later, the start of leaf development (Boyes et al. 2001). The positive effects of our ulvan extract on *A. thaliana* germination (enhanced root length and shoot area, recorded at the end of the three-week germination period) occurred if we added the ulvan at the beginning of this first week (ulvan present at sowing). Adding ulvan at the beginning of the second week (after sowing) had no significant effect on these parameters. Furthermore, only minor differences in the photosynthetic parameters of the above two groups were identified, suggesting that the positive effect induced by ulvan on root length and shoot area was not the result of an ulvan-mediated effect on photosynthesis.

Treatment with ulvan led to significant changes in protein expression in *A. thaliana*, indicating its role in modulating plant stress responses and metabolic pathways. Notably, glutamyl-tRNA reductase 1, a key enzyme in chlorophyll biosynthesis, was upregulated, suggesting enhanced photosynthetic capacity (Zeng et al. 2020). Similarly, superoxide dismutase [Cu–Zn] 1 and 2 showed increased expression, indicating a potential role in reactive oxygen species (ROS) detoxification (Li et al. 2017). Protein OSCA1, an osmosensor, was also upregulated, supporting its involvement in osmotic stress regulation and water homeostasis (Yuan et al. 2014). In contrast, pentatricopeptide repeat-containing protein At1g26460 and chaperone protein dnaJ GFA2, both mitochondrial proteins, were downregulated in roots, suggesting a possible shift in energy metabolism from roots to shoots (Schmitz-Linneweber and Small 2008; Kampinga and Craig 2010). Additionally, upregulation of spliceosome-associated and WD-40 repeat proteins points to broader transcriptional and post-transcriptional regulatory effects (Stirnimann et al. 2010).

Ulvan treatment significantly altered protein expression in *A. thaliana* shoots, suggesting enhanced metabolic activity, transport efficiency, and growth regulation. Upregulation of copper transporter 5 (COPT5) and cytochrome c oxidase subunit 2 indicates improved copper homeostasis and mitochondrial energy production, supporting shoot metabolism and redox balance (Klaumann et al. 2011; Kühn et al. 2015). Increased expression of S-adenosylmethionine carrier 1 (SAMC1) and ABC Transporter G Family Member 36 suggests active participation in phytohormone signaling and metabolite transport, potentially facilitating growth regulation (Geisler et al. 2017; Ma et al. 2017).

In contrast, downregulation of pathogenesis-related thaumatin superfamily protein, and non-specific lipid-transfer protein 1 points to a shift from biotic stress defense toward a growth-prioritizing metabolic state (Liu et al. 2015; Oladzad et al. 2023). Additionally, the observed reduction in RCC1 family protein suggests potential chromatin remodeling and epigenetic adjustments in response to ulvan (Liu et al. 2019).

It was shown that ulvan exerts biostimulant activity similar to other reported materials added to cucumber, tomato, lettuce, red clover (*Trifolium pratense*), or perennial ryegrass (*Lolium perenne*) seeds before sowing (Vendruscolo et al. 2016; Qiu et al. 2020). Nevertheless, which specific growth mechanisms are affected by ulvan addition at the first week of germination us unknown. Since this week is characterized by the initiation of seed-specific germination metabolism that prepares the seed for radicle protrusion (Silva et al. 2017), it can be speculated that ulvan affects the metabolic processes of germination. It can be infered at least two explanations for such an effect. The first involves ulvan-mediated induction of a gene expression signature similar to that of methyl jasmonate (MeJA) treatment as ulvan activates the jasmonic acid (JA) hormonal pathway of *Arabidopsis*. It might be that *Ulva*-derived hormones are present in the crude ulvan preparations. Indeed, it was suggested that environmental fluctuations contribute to the synthesis of hormones, endogenous cytokinins, auxins, and abscisic acid (ABA) in *U. fasciata* (Stirk et al. 2009). In this respect, such hormones affect several seed development processes, including pattern formation, weakening of the embryo surrounding tissues, cell division, and expansion (Locascio et al. 2014; Carrera-Castaño et al. 2020; Xu et al. 2020). It was also demonstrated that cytokinin and auxin, present in a commercial biostimulant used to treat sweet corn seeds, contributed to vegetative development. The second explanation is that sugars, like hormones, are known to improve plant growth since mung beans showed a significant increase in dry matter yield and root numbers upon treatment with polysaccharides (Hernández-Herrera et al. 2016). Since ulvan is a sulfated polysaccharide, it may induce the same effect through a similar mechanism.

Additional support for the biostimulant activity of our ulvan extracts was supplied by a preliminary experiment that assessed the seed yield of mature plants. It was found that ulvan addition, restricted to sowing time and the first week, had a profound positive effect on the seed yield, which we quantified approximately three months after the initial ulvan treatment. The seed yield was ~ 43% higher for the ulvan-treated group than the control, untreated group. This result is consistent with a previous biostimulant study with *Arabidopsis*, using an extract from the brown seaweed *Ascophyllum nodosum*, which showed root length increase up to 55% and 34% on days five and seven post-treatment, respectively (Rayorath et al. 2008). The same study also reported an increase (up to 30%) in the average fresh weight of the plant (at seven days post-treatment). The authors provided evidence that *A. nodosum* extracts modulate the concentration and localization of auxins, which could account, at least in part, for the enhanced plant growth (Rayorath et al. 2008). This explanation may also be relevant to our results. However, it should bed should emphasized that the ulvan biostimulant activity in our experiment was quantified after a more extended period (3 months versus 1 week). Moreover, to th best of our knowledge, this is the first to report a stimulatory effect on *A. thaliana* seed yield as a result of early ulvan treatment.

Future experiments should define the exact processes affected by ulvan addition during sowing and the following week. This goal can be achieved by mapping shorter ulvan incubation times needed to observe its biostimulant activity and monitoring the seed's metabolic changes using different techniques (e.g., proteomics of the treated seeds). A complementary approach would be to analyze our ulvan preparations' fractions to investigate the different components contributing to the biostimulant activity in the first germination phase. In this respect, the SEC-MALS and AEX techniques applied to fractionate and analyze the ulvan should assist such investigations. Future studies should also test combinations of ulvan extracts with other features, such as environmental factors, site conditions, crop species, light, and temperature, to systematically assess synergistic positive effects on crop production.

The economic promise of ulvan-based biostimulants derives from multiple factors: higher yield, improved seed germination rates, more effective disease control. Building on these agronomic outcomes, the next crucial step is to assess the economic implications of adopting algae-based biostimulants, particularly in fruit and vegetable sectors valued globally at approximately US$ 234 billion in 2024. Below, we frame the discussion in terms of economic viability, social welfare considerations, and strategic prioritization for adoption.

The diffusion of new agricultural technologies typically follows a gradual process, with early adopters being those

farmers who anticipate the greatest benefits, measured by increased profits adjusted for risk (Zilberman et al. 2012). Detecting the producers by profitability considerations adjusted for risk helps identify where to introduce ulvan first. Over time, as production costs decline and knowledge about the technology spreads—partly through feedback from these early adopters—a broader range of farmers across various regions begin to adopt the innovation. While yield increases typically benefit farmers, standard economic theory warns that a sizable jump in total market output can drive down prices. In highly competitive commodity markets with elastic demand, a significant supply expansion may reduce the per-unit price enough that farmers' total revenue, and thus profit, could decline. However, from a societal perspective, expanded supply typically raises consumer surplus because lower prices benefit end-users. Therefore, even if individual farm profitability becomes ambiguous (or slightly reduced) when supply expands significantly, social welfare—the total gain to society, encompassing both consumer surplus and producer surplus—often increases.

Not all crops exhibit the same market conditions. High-value horticultural crops such as almonds, grapes, specialty vegetables, and off-season fruits typically have more inelastic demand, higher profit margins, and greater potential for product differentiation. Producers of these crops could adopt ulvan-based biostimulants to attain yield gains or shortened cultivation cycles, capturing sizable economic rents because market prices tend not to plummet with moderate output increases. In contrast, low-margin field crops—wheat, corn, soybean—often exhibit significant price sensitivity to supply expansions. If a biostimulant meaningfully boosts yields in a very large acreage commodity like corn, the market price could fall enough to offset individual farmers' extra gains in output. As a result, farmers growing these commodity crops may prove less motivated to adopt new inputs unless they also confer clear cost savings or quality differentials that justify a premium price.

Beyond yield gains, the results of this study reveal that ulvan biostimulants shorten the growing cycle by increasing the growth rate. This shorter cycle allows: Multiple cultivation runs in the same season, enhancing annual returns, and suitability for shorter optimal climate windows, enabling crop production in regions previously considered marginal (particularly relevant under climate change). These advantages can be especially pivotal for high-value fruits such as mangoes or premium vegetables with tight market windows (e.g., off-season or holiday demand) where timing influences market prices. By accelerating production and potentially expanding suitable growing regions, farmers can capture more market opportunities and address the increase in supply during periods of high demand and high WTP.

Beyond yield, the ability of ulvan to enhance disease resistance (Paulert et al. 2021) creates further savings on pesticides and reduces crop losses—additional profit drivers. Moreover, these qualities reinforce sustainability credentials and open doors to branding produce as eco-friendly or organically grown, which can command price premiums.

One of the challenges of biostimulants is their reliability and stability. The impact might change with temperature, season, etc. The main breakthrough of the current research is that ulvan effects remain stable for extended periods and is not affected by seasons. A consistent mean yield improvement (e.g., from 5% average effect in older biostimulants to around 7% with ulvan) coupled with a lower standard deviation (e.g., from 1% down to 0.5%) reduces risk for the farmer. In practical terms, less yield fluctuation supports contractual guarantees from input suppliers or insurance mechanisms and increases the farmer's willingness to pay and adopt algae-based biostimulant. Stable performance is, therefore, integral to adoption: when farmers can count on reliable returns, they are more inclined to incorporate new inputs into their management practices.

Current research often defaults to testing innovations on major commodities (e.g., corn), yet the low margin environment disincentivizes adoption of premium inputs. Future experiments should thus shift toward vegetables, fruits with seasonal price premiums, or niche crops where ulvan stable, guaranteed improvements in yield and quality can yield a higher rate of return. For instance, trials on premium specialty vegetables, orchard produce (almonds, mangoes), or even emerging high-value segments (e.g., cannabis) could better demonstrate the profitability of biostimulants.

To summarise, ulvan-based biostimulants from green macroalgae present a compelling option for boosting crop performance and, under many market conditions, producer profitability. Although robust yield increases can sometimes lower market prices—especially in elastic demand markets—the overall social welfare often rises, benefiting consumers through lower prices and farmers through at least partial gains in surplus. Furthermore, stable efficacy across storage times de-risks adoption, making ulvan-based biostimulants highly attractive. Over time, the cost of the production will decline and farmers will gain profitability utilizing it, which will benefit a range of farmers and regions where it is grown. Furthermore, feedback from farmers on the performance of the ulvan and the way to use it with different crops- will enhance the likelihood of adoption and the willingness to pay for the additive.

Conclusions

This study presents a modified and simplified ulvan extraction method from local to Israel *Ulva* spp. Furthermore, our investigation revealed that enrichment of plant nutrition with low doses of this extract resulted in biostimulation of a plant model (*A. thaliana*) by affecting its early germination stage. This activity on seed germination has pronounced effects on later stages of plant development, evident by the increase in seedling productivity (significant enhancement of seedling root length and shoot area) and seed yield of the mature plants. This study contributes to the environmental trend of applying natural fertilizers to reduce the use of synthetic materials. Our results can inform seed enhancement technologies, such as seed priming and seed coating, developed by the agricultural seed industry to accelerate and synchronize germination and improve seed vigor, seedling emergence, and establishment. Prioritizing high-value horticultural crops for further experimentation will likely clarify the best-fit markets where ulvan multiple benefits—yield, quality, shortened growth cycles, disease control—translate into the largest economic gains. In so doing, it becomes more feasible to design effective deployment strategies, contract structures, and farmer outreach programs that advance both farm-level profitability and broader societal benefits through more abundant, sustainable agricultural production.

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Data availability All proteomics data are available at https://www.github.com/GolbergLab/Ulvan/_Arabidopsis and raw data are available via ProteomeXchange with identifier PXD061726.

Declarations

Competing interests The authors declare no competing interests.

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