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Alleviation of Drought Stress in Wheat Using Exogenous *Ulva prolifera* Extract Produced by Enzymatic Hydrolysis

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Abstract: Drought is one of the major abiotic stresses that affect plant growth and reduce agricultural productivity. Use of algal extract as a biostimulant is gaining increased attention from researchers. This study aimed to investigate the potential of *Ulva prolifera* extract (UE) as a biostimulant when enzymatically extracted under conditions of water deficit. UE treatments (0.02%, 0.06%, and 0.1%) significantly improved the shoot length, root length, and dry weight of roots after 120 h of drought stress relative to that in treatment with the negative control. An increase in catalase (CAT) and peroxidase (POD) activity was also observed that resulted in improved antioxidant capacity. Application of 0.1% UE reduced the malondialdehyde (MDA) content by 30.06% compared with that in the negative control. In addition, the soluble sugar and protein content in wheat treated with 0.1% UE was increased by 23.10% and 93.51%, respectively, resulting in adjustment of the osmotic pressure. Results suggest that UE could significantly enhance the drought tolerance of wheat. This study provides a basis for increasing the value of UE as a biostimulant.

Keywords: *Ulva prolifera* extract; enzymatic hydrolysis; antioxidant capacity; osmoprotectants; drought tolerance

1 Introduction

Wheat is the most important source of food and raw materials after rice; however, drought is the major cause of yield losses in wheat. Water deficits dramatically affect plant metabolism, resulting in altered cell membrane permeability and damaged organelles, and negatively affect normal physiological activities [1]. Therefore, it is crucial to develop techniques to increase crop yield and quality under drought conditions. Various exogenous stimulants have been studied for improving the drought resistance of plants [2,3]. At present, the utilization of plant biostimulants, compounds that have positive effects on plant growth and stress tolerance, seems to be a promising solution; among plant biostimulants, marine resources are attracting attention, particularly alga [4,5].

Extensive studies have proven that algal extract can act as a plant stimulant by virtue of its diverse biological components, including polysaccharides, phenols, mannitol, betaine, and phytohormones [6]. Sharma et al. [7] reported that *Gracilaria dura* extract increased wheat crop yield under drought conditions by 70% through increasing accumulation of abscisic acid (ABA), thereby activating ABA-response genes. Some extracts of brown alga can also enhance drought tolerance by stimulating growth, and affecting the photosynthetic performance and related gene expression [6,8]. *Ulva prolifera*, a green algae distributed globally from intertidal to upper subtidal zones, is a sustainable and important natural resource. It is largely available, with up to 500 thousand tons collected in the coastal area of Qingdao, China, in 2016 [9]. Notably, *Ulva prolifera* has unique sulfated polysaccharides consisting of xylose, galactose, arabinose, rhamnose, and glucose, with sulfate groups at the C-3 of rhamnose [10]. It has been demonstrated to effectively alleviate damage caused by NaCl stress in maize [11]. Therefore it is necessary and meaningful to explore effective ways to make full use of *Ulva prolifera*.

Due to the large quantities of interconnecting polysaccharides in algal cell walls, extraction yields of soluble bioactive substance, such as polysaccharide, soluble protein and mannitol, are limited. Conventionally, algal extract is prepared by water extraction or chemical processes. The water extraction method is usually completed by using high temperatures for a long time; it is time inefficient and results in algal extract of inferior quality [12]. Chemical processes, including alkaline and acid hydrolysis, can destroy some bioactive components, such as polyphenols [13]. In contrast, the enzyme-assisted method can achieve cell wall breakdown for a more effective release of intracellular bioactive compounds. Charoensiddhi et al. [14] showed that the extract of the brown alga, *Ecklonia radiata* produced via enzymatic hydrolysis significantly increased total phlorotannin content and antioxidant activity. This extraction process was completed under mild conditions without damage to the structure of any active compounds. Therefore, the enzyme-assisted method is a more efficient way of preparing algal extracts [15].

At present, *U. prolifera* extract has the potential to be promoted as eco-friendly biostimulants but no information is available on application of *U. prolifera* extact in plant. In this study, we evaluated the ability of enzymatically obtained *U. prolifera* extract (UE) to improve drought tolerance in wheat by measuring plant growth parameters, assessing the oxidation system, and determining the content of osmoregulatory compounds. This study might provide evidence for enhancing the application of *U. prolifera* extract in wheat.

2 Experiment

2.1 U. prolifera Extract and Chemicals

U. prolifera from the coast of Qingdao, China were used to prepare alga powder. It was firstly dissolved by hot water at 100°C for 45 min. Then it was continually degraded by enzymes from *Alteromonas* sp. A321 (provided by the Applied Microbiology Laboratory, Ocean University of China) at 37°C for 4 h (pH 7.0). The enzymatic hydrolysates of *U. prolifera* was centrifuged for 15 min to collect the supernatant and named UE. The UE composed of 4.06% (w/v) soluble solids, three different concentration of UE (0.02, 0.06, and 0.1% soluble solids) were prepared using distilled water.

Polyethylene glycol-6000 (PEG-6000), trichloroacetic acid (TCA) used in the analysis were from Sigma Chemicals Co. The other reagents were of analytical grade.

2.2 Composition Analysis of the Alga Extract

The composition of UE was analyzed as follows. Total water-soluble sugar was measured by phenol sulfuric acid method [16]. Protein content was estimated according to Lowry [17]. Mannitol was extracted and analyzed based on the method developed by Cameron et al. [18]. Phenolic compounds were assayed using the Folin-Ciocalteu method [19]. Analysis for reducing sugar was performed using the 3,5-dinitrosalicyclic acid method according to Gereniu et al. [20].

2.3 Plant Material and Treatments

The present study was carried out with a drought-tolerant wheat cultivator (*Triticum aestivum* L. Jimai 22). About 300 wheat seeds were sterilized in 0.1% sodium hypochlorite solution for 20 min and triple-washed in distilled water subsequently. Then seeds were soaked in distilled water for 12 h and transferred into planting tray. They were cultivated in Hoagland solution in a growth incubator, setting as follows: a day/night cycle of 12 h/12 h at 25°C/20°C respectively, with 60% relative humidity. Seven days later, the 20% (w/v) PEG-6000 with −0.5 MPa of water potential was used in Hoagland solution to establish drought condition [21]. Wheat seedlings were divided into five groups, which were continued to be cultivated in (1) Hoagland solution (Control), (2) Hoagland solution mixed with 20% PEG-6000 (Negative control), (3) Hoagland solution mixed with 20% PEG-6000 and 0.02% UE (P + 0.02%), (4) Hoagland solution mixed with 20% PEG-6000 and 0.06% UE (P + 0.06%), (5) Hoagland solution mixed with 20% PEG-6000 and 0.1% UE (P + 0.1%), respectively.

Seedlings of each group were harvested randomly at 120 h after the PEG-6000 treatment. They were stored at −80°C for the later evaluation index.

2.4 Determination of Growth Parameter

Seedlings from each group were collected to evaluate the growth parameter. Shoot length and root length of wheat seedlings were estimated immediately, after which the roots and shoots were dried at 80°C for 24 h to determine the dry weights.

2.5 Determination of Chlorophyll

Chlorophyll (Chl) of wheat seedlings (0.5 g) was extracted with 95% ethanol. The chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl a + b) content in the seedlings were measured according to Zou et al. [22].

2.6 Antioxidant Enzyme Activity Assay

Leaves (0.5 g) from each group was homogenized using 5 ml cold sodium phosphate buffer solution (pH 7.8). The mixture was then centrifuged at 3000 g for 5 min, after which the supernatant were immediately used for detection of enzyme activity. To determine antioxidant enzyme activity, the method described by Zou et al. [22] was used. The peroxidase (POD) activity and catalase (CAT) activity were assayed at 470 nm and 240 nm, respectively.

2.7 Estimation of Lipid Peroxidation

Leaves (0.5 g) were homogenated in 10% (w/v) TCA and centrifuged subsequently. The MDA content was detected referring the method from Liu et al. [23]. Its content was calculated according to the following formula.

$$\text{MDAcontent}(\mu\text{mol/g FW}) = \frac{6.542 \times (A_{532} - A_{600}) - 0.559 \times A_{450}}{V_S \times FW} \times V_t \quad (1)$$

where V_t is the total extraction volume of leaves, V_s is the extraction volume for measurement, FW is the fresh weight of leaves for the test.

2.8 Determination of Soluble Sugar and Soluble Protein Content

The soluble sugar of 0.5 g leaves was extracted according to Zou et al. [22] and then determined using the phenol sulfuric acid method according to Dubois et al. [16]. Soluble sugar concentration was quantified by comparison against a glucose standard curve. The content was calculated as mg/g FW.

Soluble protein of 0.5 g leaves was extracted by the method of He et al. [24] and measured using coomassie blue at 595 nm based on Bradford [25] successively. Soluble protein concentration was quantified using bovine serum albumin as the standard. The content was expressed as mg/g FW.

2.9 Statistical Analysis

The data of differences among treatments were obtained by the determination of nine seedlings. Statistical procedures were conducted by one-way analysis of variance followed by Tukey's multiple comparison test, $P < 0.05$ was considered to be statistical significant. Each value is the mean ± SE.

3 Results and Discussion

3.1 Plant Growth Parameter

Under prolonged exposure to drought, water balance may destabilize, thereby disrupting normal cellular activities and suppressing plant growth [26]. Our results show that shoot length, root length, and dry weight of the negative control were significantly lower than those of the control ($P < 0.05$; Fig. 1). However, application of UE improved these three parameters. The chemical constituents of UE are presented in Tab. 1. Relative to that in the negative control, the shoot length in UE treatments (0.02%, 0.06%, and 0.1%) was increased by 9.91%, 4.95%, and 9.90%, respectively. Notably, the root length in response to 0.02% UE treatment was higher than that of both the negative control and control groups. Moreover, the 0.02% and 0.06% UE treatments significantly increased the dry weight of shoots and roots compared with the negative control, while no difference was observed compared to the control (Fig. 1).

Other studies have identified similar results. The application of *Ascophyllum Nodosum* extract significantly improved the growth parameters of sweet orange trees, and extracts of *Sargassum latifolium* and *Ulva lactuca* increased root depth, shoot height and leaf area in wheat under drought stress [27,28]. Furthermore, there is evidence that some components in algal extract, including polysaccharides, auxins, and cytokinins, could affect cellular metabolism, and thus enhance growth and crop yield under abiotic stress [22,29,30]. In general, we found that UE application stimulated shoot and root growth to varying degrees during drought conditions.

3.2 Chlorophyll Content

The availability of water and CO_2 is the main prerequisite for photosynthesis, a process directly linked to the growth and survival of plants. Drought stress causes stomatal closure and results in inadequate water supply to plants and inhibition of photosynthesis. Chlorophyll content is widely used as an index of tolerance against abiotic stress, and tends to be significantly decreased in plants exposed to drought conditions [26]. The effects of UE on chlorophyll content in wheat are illustrated in Fig. 2; there were no significant differences among the five treatments.

In some studies that evaluated the effect of algal extract on plant growth under different condition, the chlorophyll content of plant sometimes would be effected and further affect photosynthesis. But in our study, the extract showed no significant effect on chlorophyll content of wheat. It might be explained that difference in algal species and processing techniques, which would produce difference in chemical compositions of algal extract, caused an impact on its function. The concentration of algal extract, the time of treatment, and method of application to plant may be related to this observation. Moreover, the effects of different extracts on plant may be different in photosynthesis, C metabolism, nitrogen metabolism, enzyme activity, etc. [28,31,32].

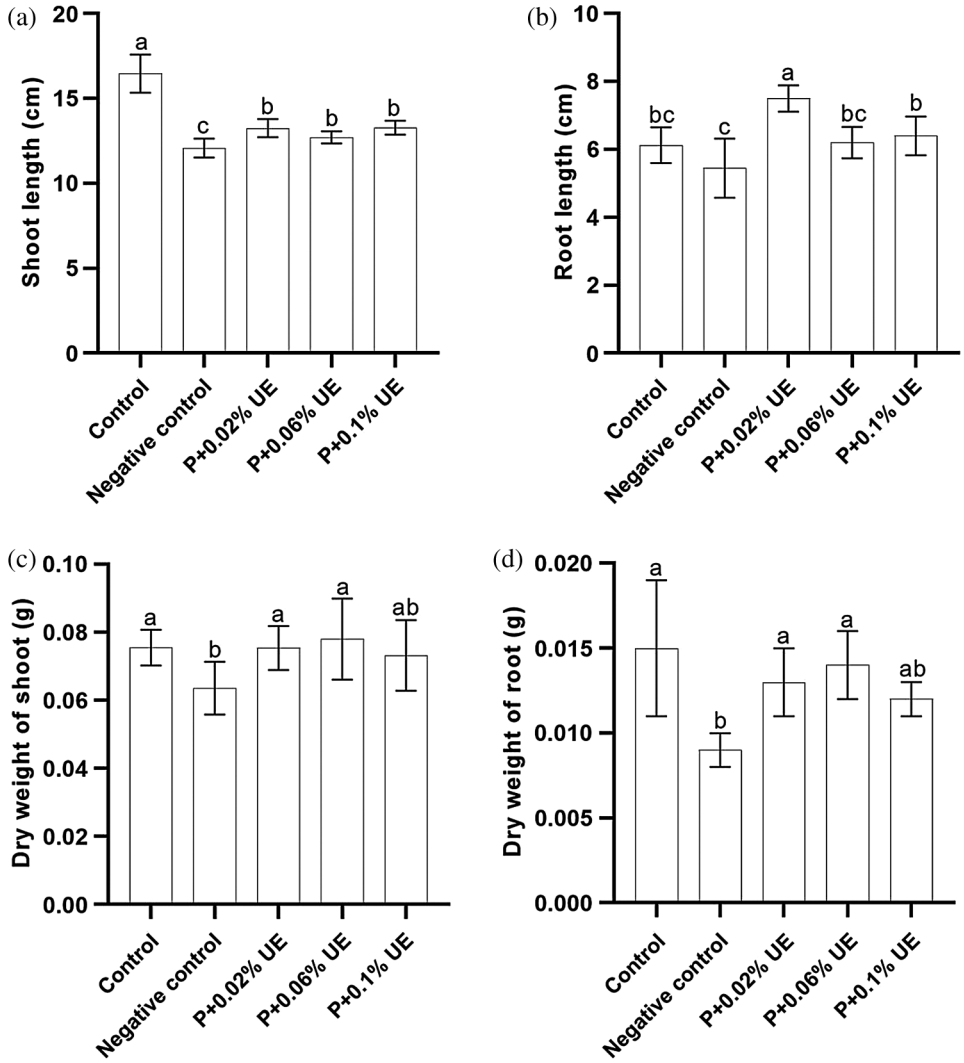

Figure 1: Effect of UE on shoot length (a), root length (b), dry weight of shoot (c) and dry weight of root (d) in wheat seedlings. Different letters indicate significant differences at $P < 0.05$

Table 1: Constituents of *U. prolifera* extract

Constituent	Content (mg/ml)
Total sugar	13.49 ± 1.30
Soluble protein	3.42 ± 0.14
Mannitol	0.26 ± 0.01
Polyphenol	0.33 ± 0.01
Reducing sugar	5.74 ± 0.02

3.3 Antioxidant Enzyme Activity

The overproduction of reactive oxygen species (ROS) is one of the earliest biochemical responses of eukaryotic cells to drought stress, which causes serious damage to cellular structures and deregulates

metabolic processes [33]. An enzyme defense system is one of the effective strategies used to minimize the effects of oxidative stress. Peroxidase (POD) and catalase (CAT) are important enzymes that are involved in the elimination of H_2O_2 [34]. Wheat seedlings of the negative control group showed higher CAT and POD activity in response to a water deficit than those in the control group (Fig. 3). Notably, CAT activity in 0.06% UE-treated wheat further rose by 42.35% compared with that in the negative control. Treatment of seedlings with 0.1% UE resulted in effective functioning of POD, and its activity was increased by 33.07%.

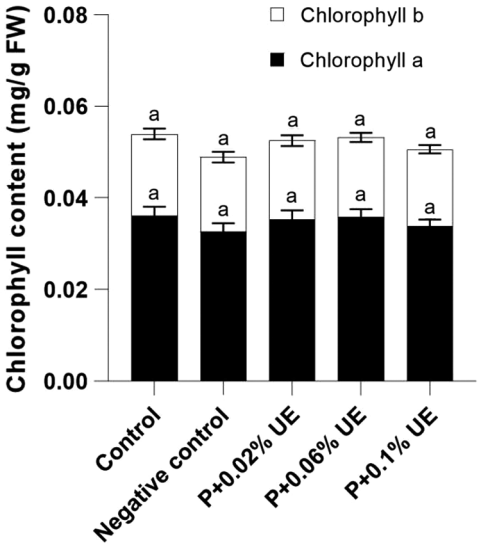

Figure 2: Effect of UE on chlorophyll content in wheat seedlings. Different letters indicate significant differences at $P < 0.05$

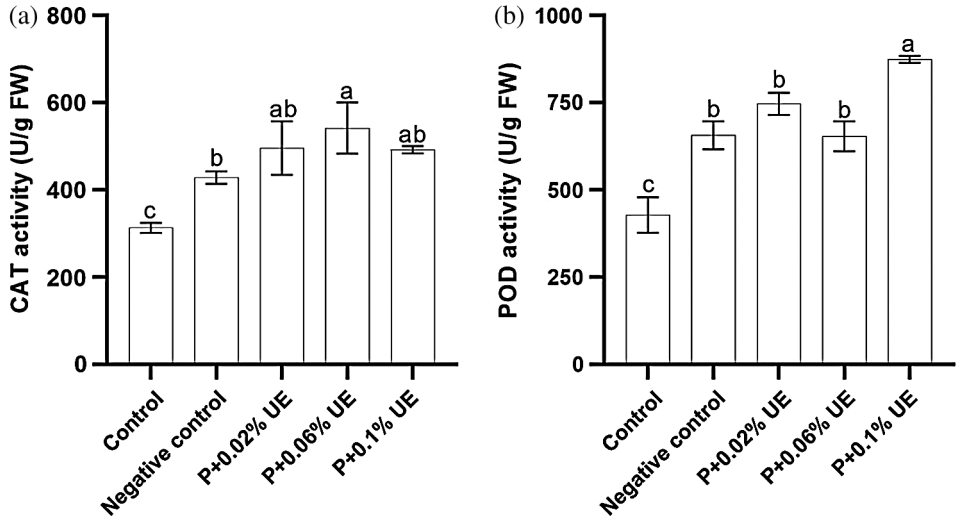

Figure 3: Effect of UE on CAT (a) and POD (b) activities in wheat seedlings. Different letters indicate significant differences at $P < 0.05$

Kappaphycus alvarezii extract decreases oxidative damage in maize under conditions of drought stress by enhancing the activity of antioxidant enzymes [31], while *U. lactuca* is reported to increase CAT activity

in wheat under conditions of salinity stress [35]. Reports have revealed that the polysaccharides in algal extract, such as κ-carrageenan, can regulate antioxidant enzyme activity, to improve abiotic stress tolerance in plants [36]. Additionally, *Pyropia yezoensis* polysaccharides with lower molecular weight are more effective at inducing superoxide dismutase, CAT, and POD activity [21]. *Alteromonas* sp. A321 enzymes show highly efficient and specific *U. prolifera* polysaccharide degradation [37], and liquid fermentation by *Alteromonas* sp. A321 is known to result in production of oligosaccharides from *U. prolifera* tissues [38]. Our results indicate that the oligosaccharides in UE might improve the antioxidant capacity of wheat by increasing CAT and POD activity under conditions of drought stress.

3.4 Lipid Peroxidation Degree

Malondialdehyde (MDA), a product of ROS-induced lipid peroxidation, is an indicator of oxidative damage to the cell membrane. Drought stress causes an increase in MDA content due to excess ROS [26]. In this study, MDA content of wheat in the negative control group under drought conditions was increased by 10.71% compared with that in the control (Fig. 4). The application of 0.02% and 0.1% UE significantly decreased MDA content relative to that in the negative control. In particular, wheat treated with 0.1% UE had the lowest MDA content, with a reduction of 30.06% in comparison to that in the control.

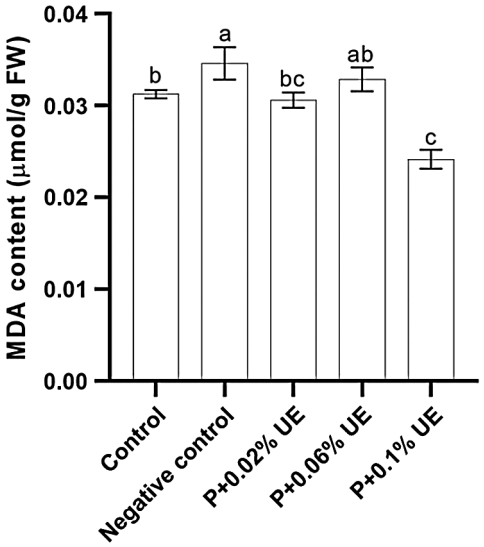

Figure 4: Effect of UE on MDA content in wheat seedlings. Different letters indicate significant differences at $P < 0.05$

UE treatments appear to mitigate lipid peroxidation under conditions of drought stress (Fig. 4). Mansori et al. [39] also observed a similar positive effect when *U. rigida* extract was applied to *Salvia officinalis* L. to alleviate oxidative damage. Antioxidant enzymes reduce free radical content, thereby reducing lipid oxidation levels. The POD activity of wheat was significantly improved and the lowest MDA content was observed with application of 0.1% UE. The increased POD activity in UE-treated wheat (Fig. 3) may primarily enhance ROS elimination, thereby lowering the accumulation of MDA. This result is similar to that of Yang et al. who found that wheat treated with exogenous abscisic acid also had lower MDA content with significantly improved POD activity [40]. Therefore, it can be assumed that appropriate UE treatments could decrease lipid peroxidation through the improvement of the enzyme defense system.

3.5 Estimation of Osmoprotectant Content

Drought stress can induce osmotic stress, thereby leading to turgor loss and poor water absorption. The accumulation of compatible solutes is considered another important mechanism for increasing drought tolerance in plants by reducing water potential [41,42]. In this process, osmoprotectants such as total soluble protein and sugar play an important role in protecting normal metabolic processes [43]. In our study, drought stress led to a higher total soluble protein and sugar content than that in the control (Fig. 5). We observed a protein content of 3.59 mg/g FW in the negative control, while the protein content in UE-treated (0.02%, 0.06%, and 0.1%) wheat was increased to 6.71 mg/g FW, 7.65 mg/g FW, and 6.95 mg/g FW, respectively. In response to 0.1% UE treatment, the soluble sugar content in wheat showed a 23.10% increase relative to that of the negative control under drought conditions.

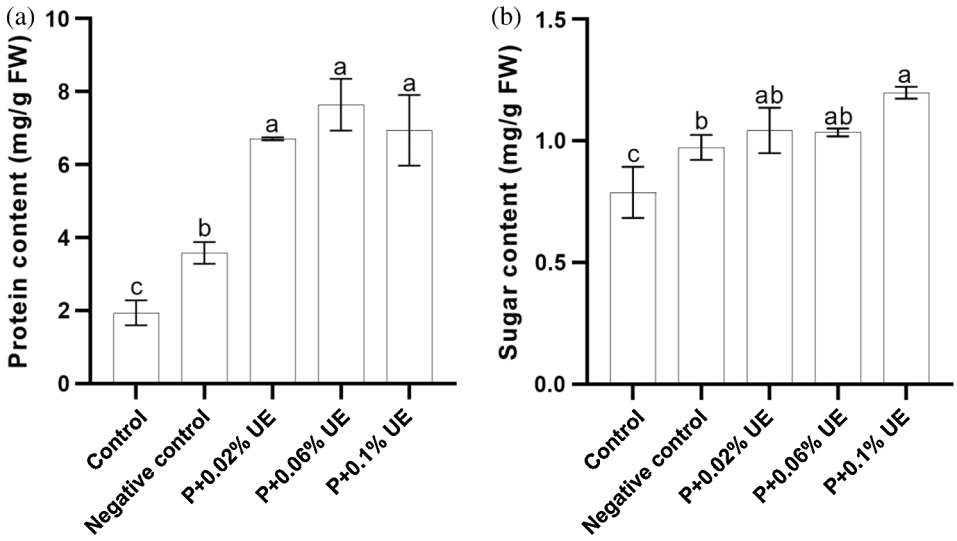

Figure 5: Effect of UE on soluble protein (a) and sugar (b) content in wheat seedlings. Different letters indicate significant differences at $P < 0.05$

Our results indicate that UE promotes protein and sugar synthesis to regulate osmotic pressure. Such a marked increase in total soluble sugar and protein content has also been observed during the improvement of drought resistance of maize seedlings by using exogenous urea [44]. Soluble sugars may also function as typical osmoprotectants, i.e., they are involved in stabilizing cell membranes and maintaining turgor. It is known that stoma closure due to drought stress decreases the internal CO_2 concentration in leaves, thereby affecting sugar synthesis [45]. However, Zou et al. found that chitooligosaccharide application increases stomatal conductance and soluble sugar content of wheat under conditions of salinity stress [46]. Additionally, it has been demonstrated via microarray analysis that *Ascophyllum nodosum* extract enhances the expression of carbon fixation-related genes under water deficit conditions [32]. Thus, it can be assumed that exogenous UE is beneficial for the accumulation of soluble sugars and proteins, which decrease the cell osmotic potential in response to osmotic stress.

4 Conclusions

In conclusion, the application of UE can effectively enhance the tolerance of wheat to drought stress. UE treatments stimulate the growth of seedlings, particularly the root, but cause no significant change in chlorophyll content under water deficit conditions. Appropriate UE treatments may improve plant defense systems against oxidative stress by enhancing the activity of POD and CAT, thereby reducing lipid peroxidation. The levels of soluble sugars and especially proteins that function as osmoprotectants in

wheat were also improved in response to UE treatments. Overall, this study shows the prospects of UE as an environmentally friendly biostimulant. Further work about the effect of this algal extract on different growth periods of wheat and a number of trials under field conditions would benefit.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Cruz de Carvalho, M. H. (2008). Drought stress and reactive oxygen species: production, scavenging and signaling. *Plant Signaling & Behavior, 3(3),* 156–165. DOI 10.4161/psb.3.3.5536.
2. Wang, Y., Ma, F., Li, M., Liang, D., Zou, J. (2011). Physiological responses of kiwifruit plants to exogenous ABA under drought conditions. *Plant Growth Regulation, 64(1),* 63–74. DOI 10.1007/s10725-010-9537-y.
3. Zhang, X., Li, K., Liu, S., Xing, R., Yu, H. et al. (2016). Size effects of chitooligomers on the growth and photosynthetic characteristics of wheat seedlings. *Carbohydrate Polymers, 138,* 27–33. DOI 10.1016/j.carbpol.2015.11.050.
4. Fu, X., Zhu, L., Li, L., Zhang, T., Li, M. et al. (2019). Eco-friendly preparation of chitooligosaccharides with different degrees of deacetylation from shrimp shell waste and their effects on the germination of wheat seeds. *Marine Life Science & Technology, 1(1),* 95–103. DOI 10.1007/s42995-019-00012-3.
5. Sharma, H. S. S., Fleming, C., Selby, C., Rao, J. R., Martin, T. (2014). Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. *Journal of Applied Phycology, 26(1),* 465–490. DOI 10.1007/s10811-013-0101-9.
6. Battacharyya, D., Babgohari, M. Z., Rathor, P., Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae, 196,* 39–48. DOI 10.1016/j.scienta.2015.09.012.
7. Sharma, S., Chen, C., Khatri, K., Rathore, M. S., Pandey, S. P. (2019). *Gracilaria dura* extract confers drought tolerance in wheat by modulating abscisic acid homeostasis. *Plant Physiology and Biochemistry, 136,* 143–154. DOI 10.1016/j.plaphy.2019.01.015.
8. Santaniello, A., Scartazza, A., Gresta, F., Loreti, E., Biasone, A. et al. (2017). *Ascophyllum nodosum* seaweed extract alleviates drought stress in *Arabidopsis* by affecting photosynthetic performance and related gene expression. *Frontiers in Plant Science, 8,* 1362. DOI 10.3389/fpls.2017.01362.
9. Chi, Y., Li, Y., Zhang, G., Gao, Y., Ye, H. et al. (2018a). Effect of extraction techniques on properties of polysaccharides from *Enteromorpha prolifera* and their applicability in iron chelation. *Carbohydrate Polymers, 181,* 616–623. DOI 10.1016/j.carbpol.2017.11.104.
10. Yu, Y., Li, Y., Du, C., Mou, H., Wang, P. (2017). Compositional and structural characteristics of sulfated polysaccharide from *Enteromorpha prolifera*. *Carbohydrate Polymers, 165,* 221–228. DOI 10.1016/j.carbpol.2017.02.011.
11. Liu, S., Li, B., Chen, X., Qin, Y., Li, P. (2019). Effect of polysaccharide from *Enteromorpha prolifera* on maize seedlings under NaCl stress. *Journal of Oceanology and Limnology, 37(4),* 1372–1381. DOI 10.1007/s00343-019-8150-9.
12. Hernández-Herrera, R. M., Santacruz-Ruvalcaba, F., Ruiz-López, M. A., Norrie, J., Hernández-Carmona, G. (2014). Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). *Journal of Applied Phycology, 26(1),* 619–218. DOI 10.1007/s10811-013-0078-4.
13. Friedman, M., Jürgens, H. S. (2000). Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry, 48(6),* 2101–2110. DOI 10.1021/jf990489j.
14. Charoensiddhi, S., Franco, C., Su, P., Zhang, W. (2015). Improved antioxidant activities of brown seaweed *Ecklonia radiata* extracts prepared by microwave-assisted enzymatic extraction. *Journal of Applied Phycology, 27(5),* 2049–2058. DOI 10.1007/s10811-014-0476-2.

15. Wijesinghe, W. A. J. P., Jeon, Y. (2012). Enzyme-assistant extraction (EAE) of bioactive components: a useful approach for recovery of industrially important metabolites from seaweeds: a review. *Fitoterapia, 83(1),* 6–12. DOI 10.1016/j.fitote.2011.10.016.
16. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry, 28(3),* 350–356. DOI 10.1021/ac60111a017.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193,* 265–275.
18. Cameron, M. C., Ross, A. G., Percival, E. G. V. (1948). Methods for the routine estimation of mannitol, alginic acid, and combined fucose in seaweeds. *Journal of Chemical Technology & Biotechnology, 67(4),* 161–164. DOI 10.1002/jctb.5000670410.
19. Deng, J., Xu, Z., Xiang, C., Liu, J., Zhou, L. et al. (2017). Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives. *Ultrasonics Sonochemistry, 37,* 328–334. DOI 10.1016/j.ultsonch.2017.01.023.
20. Gereniu, C. R., Saravana, P., Getachew, A., Chun, B. (2017). Characteristics of functional materials recovered from Solomon Islands red seaweed (Kappaphycus alvarezii) using pressurized hot water extraction. *Journal of Applied Phycology, 29(3),* 1609–1621. DOI 10.1007/s10811-017-1052-3.
21. Rong, W., Qi, L., Wang, A., Ye, X., Du, L. et al. (2014). The ERF transcription factor TaERF 3 promotes tolerance to salt and drought stresses in wheat. *Plant Biotechnology, 12(4),* 468–479. DOI 10.1111/pbi.12153.
22. Zou, P., Lu, X., Jing, C., Yuan, Y., Lu, Y. et al. (2018). Low-molecular-weightt polysaccharides from *Pyropia yezoensis* enhance tolerance of wheat seedlings (*Triticum aestivum* L.) to salt stress. *Frontiers in Plant Science, 9,* 427. DOI 10.3389/fpls.2018.00427.
23. Liu, H., Zhang, Y., Yin, H., Wang, W., Zhao, X. et al. (2013). Alginate oligosaccharides enhanced *Triticum aestivum* L. tolerance to drought stress. *Plant Physiology and Biochemistry, 62,* 33–40. DOI 10.1016/j.plaphy.2012.10.012.
24. He, J., Li, R., Sun, X., Wang, W., Hu, J. et al. (2018). Effects of calcium alginate submicroparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *Polymers, 10(10),* 1154. DOI 10.3390/polym10101154.
25. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72(1–2),* 248–254. DOI 10.1016/0003-2697(76)90527-3.
26. Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development, 29(1),* 185–212. DOI 10.1051/agro:2008021.
27. Castellanos-Barriga, L. G., Santacruz-Ruvalcaba, F., Hernández-Carmona, G., Ramírez-Briones, E., Hernández-Herrera, R. M. (2017). Effect of seaweed liquid extracts from *Ulva lactuca* on seedling growth of mung bean (*Vigna radiata*). *Journal of Applied Phycology, 29(5),* 2479–2488. DOI 10.1007/s10811-017-1082-x.
28. Hernández-Herrera, R. M., Santacruz-Ruvalcaba, F., Zañudo-Hernández, J., Hernández-Carmona, G. (2016). Activity of seaweed extracts and polysaccharide-enriched extracts from *Ulva lactuca* and *Padina gymnospora* as growth promoters of tomato and mung bean plants. *Journal of Applied Phycology, 28(4),* 2549–2560. DOI 10.1007/s10811-015-0781-4.
29. Sergiev, I., Todorova, D., Shopova, E., Jankauskienė, J., Jankovska-Bortkevič, E. et al. (2019). Exogenous auxin type compounds amend PEG-induced physiological responses of pea plants. *Scientia Horticulturae, 248,* 200–205. DOI 10.1016/j.scienta.2019.01.015.
30. Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P. et al. (2009). Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulation, 28(4),* 386–399. DOI 10.1007/s00344-009-9103-x.
31. Ibrahim, W. M., Ali, R. M., Hemida, K. A., Sayed, M. A. (2014). Role of *Ulva lactuca* extract in alleviation of salinity stress on wheat seedlings. *Scientific World Journal, 2014(8),* 1–11. DOI 10.1155/2014/847290.

32. Jannin, L., Arkoun, M., Etienne, P., Laîné, P., Goux, D. et al. (2013). *Brassica napus* growth is promoted by *Ascophyllum nodosum* (L.) Le Jol. seaweed extract: microarray analysis and physiological characterization of N, C, and S metabolisms. *Journal of Plant Growth Regulation, 32(1),* 31–52. DOI 10.1007/s00344-012-9273-9.
33. Ajithkumar, I. P., Panneerselvam, R. (2014). ROS scavenging system, osmotic maintenance, pigment and growth status of *Panicum sumatrense* Roth. under drought stress. *Cell Biochemistry and Biophysics, 68(3),* 587–595. DOI 10.1007/s12013-013-9746-x.
34. Hasanuzzaman, M., Mahmud, J. A., Anee, T. I., Nahar, K., Islam, M. T. (2018). *Drought stress tolerance in wheat: omics approaches in understanding and enhancing antioxidant defense. abiotic stress-mediated sensing and signaling in plants: an omics perspective*. Singapore: Springer.
35. Trivedi, K., Vijay Anand, K. G., Kubavat, D., Patidar, R., Ghosh, A. (2018). Drought alleviatory potential of *Kappaphycus* seaweed extract and the role of the quaternary ammonium compounds as its constituents towards imparting drought tolerance in *Zea mays* L. *Journal of Applied Phycology, 30(3),* 2001–2015. DOI 10.1007/s10811-017-1375-0.
36. Zheng, F., Chen, L., Zhang, P., Zhou, J., Lu, X. et al. (2020). Carbohydrate polymers exhibit great potential as effective elicitors in organic agriculture: a review. *Carbohydrate Polymers, 230,* 115637. DOI 10.1016/j.carbpol.2019.115637.
37. Li, Y., Wang, J., Yu, Y., Lia, X., Jiang, X. et al. (2013). Production of enzymes by *Alteromonas sp*. A321 to degrade polysaccharides from *Enteromorpha prolifera*. *Carbohydrate Polymers, 98(1),* 988–994. DOI 10.1016/j.carbpol.2013.07.016.
38. Chi, Y., Qiao, L., Zuo, S., Yan, H., Du, C. et al. (2018b). Submerged liquid fermentation of *Ulva prolifera* Müller tissue and applications for improving growth performance in juvenile abalone (*Haliotis discus hannai Ino*). *Journal of Applied Phycology, 30(4),* 2639–2647. DOI 10.1007/s10811-018-1413-6.
39. Mansori, M., Chernane, H., Latique, S., Benaliat, A., Hsissou, D. et al. (2016). Effect of seaweed extract (*Ulva rigida*) on the water deficit tolerance of *Salvia officinalis* L. *Journal of Applied Phycology, 28(2),* 1363–1370. DOI 10.1007/s10811-015-0671-9.
40. Yang, D., Peng, D., Yang, W., Yin, Y., Li, Y. et al. (2016). Application of abscisic acid regulates antioxidant enzymes activities and modulates endosperm cell division in winter wheat. *Canadian Journal of Plant Science, 96(2),* 283–295. DOI 10.1139/cjps-2015-0368.
41. Krasensky, J., Jonak, C. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany, 63(4),* 1593–1608. DOI 10.1093/jxb/err460.
42. Hasanuzzaman, M., Banerjee, A., Bhuyan, M., Roychoudhury, A., Mahmud, J. et al. (2019). Targeting glycinebetaine for abiotic stress tolerance in crop plants: physiological mechanism, molecular interaction and signaling. *Phyton-International Journal of Experimental Botany, 88(3),* 185–221. DOI 10.32604/phyton.2019.07559.
43. Blum, A. (2016). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant, Cell & Environment, 40(1),* 4–10. DOI 10.1111/pce.12800.
44. Gou, W., Zheng, P., Tian, L., Gao, L., Zhang, L. et al. (2017). Exogenous application of urea and a urease inhibitor improves drought stress tolerance in maize (*Zea mays* L.). *Journal of Plant Research, 130(3),* 599–609. DOI 10.1007/s10265-017-0933-5.
45. Kulkarni, M., Soolanayakanahally, R., Ogawa, S., Uga, Y., Selvaraj, M. G. et al. (2017). Drought response in wheat: key genes and regulatory mechanisms controlling root system architecture and transpiration efficiency. *Frontiers in Chemistry, 5,* 106. DOI 10.3389/fchem.2017.00106.
46. Zou, P., Li, K., Liu, S., Xing, R., Qin, Y. et al. (2015). Effect of chitooligosaccharides with different degrees of acetylation on wheat seedlings under salt stress. *Carbohydrate Polymers, 126,* 62–69. DOI 10.1016/j.carbpol.2015.03.028.