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The role of seaweed extract on improvement drought tolerance of wheat revealed by osmoprotectants and DNA (cpDNA) markers

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Abstract

Drought stress is one of the most severe abiotic stresses affecting adversely plant growth, crop production, and various metabolic processes. Using seaweed extract in mitigating water stress adverse effects is highly important for plant production. The present study discussed the physiological role of seaweed extract (*Sargassum denticulatum*) in improving wheat tolerance to water stress.

Water stress (40% of field capacity) caused significant decreases in wheat plant growth parameters (shoot height, fresh, and dry weights of the shoot) as well as with significant decreases in chlorophyll content and starch. Total soluble sugars, free amino acids, proline, and phenolic compounds contents increased in stressed wheat plants irrigated every three weeks compared with control plants. The foliar application of seaweed extract 2% enhanced all growth and yield parameters and more accumulation of the organic solutes in leaves of water-stressed plants. These increases correlated with significant increases in total phenolic contents as compared with control plants. The trnL intron and psbA-trnH intergenic regions of cpDNA were amplified from extracted total genomic DNA. The results indicated that the variation among psbA-trnH intergenic region was more than trnL intron region to distinct the variation of wheat treatments as responsible to water deficit.

Foliar spray of seaweeds extract was effective in improving wheat performance by enhancing compatible osmolytes, antioxidant compounds and enhancing variation among non-coding chloroplast DNA (cpDNA) regions trnL intron and psbA-tnH as a response to water deficit.

Keywords cpDNA · Drought · *Sargassum* · Seaweeds extract · Wheat

1 Introduction

One of the most important crops in the world is wheat. So, it is necessary to increase wheat production to satisfy the needs of the rapid population growth (Fred et al. 2006). Nowadays, an increase in wheat crop production occupies the main objective of agriculture policy in the Egyptian government, by improving wheat varieties and improving agricultural practices. Marine algae are one of the most important marine resources in the world and are generally used as human food, animal feed and considering raw material for several industries. More than fifteen million metric tons of seaweed products is used annually as nutrient supplements and biostimulants in agricultural and horticultural crops production (FAO 2006). It is used as biofertilizers in agriculture and horticulture (Al-Shakankery et al. 2014). These products improve seeds germination, increase plant tolerance to environmental stresses, and enhance crop yield (Kumari et al. 2011). Beneficial effects from the use of seaweed extracts as natural regulators have induced increased crop yield and plant vigor to resist adverse environmental effects (Featonby-Smith and Van Staden 1983). Drought is considered a crucial factor that affects growth and crop production in arid and semiarid regions. Some plants have a set of physiological adaptations that allow tolerating water stress conditions. From these adaptations, drought induced an accumulation of total phenolic compound, total free amino acids, and a marked increase in the proline content (Abdelgawad et al 2015). Zhang and Erivn (2008) used seaweed extract to raise plant tolerance to environmental stresses. They

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reported that the application of seaweed extract improves seedling growth of wheat plants during growth period. Abd El-Baky et al. (2014) focused on water relations, photosynthesis, and the accumulation of specific metabolites. Despite reducing photosynthetic activity, accumulation of organic acids, osmolytes, as well as changes in carbohydrate metabolism are typical physiological and biochemical responses to stress. Synthesis of osmoprotectants, osmolytes, or compatible solutes is one of the mechanisms that plants have evolved for adaptation to water deficit, which acts as osmotic balancing agents, accumulated in plant cells in response to stress (Sadak et al. 2010). Chloroplasts are one of the most important organelles in green plants, seaweed, etc., which act as environmental sensors. Chloroplasts play an important role in many plants cell functions, including photosynthesis, carbon fixation, and stress response (Zdravka et al. 2017). Furthermore (Sun and Guo 2016) mentioned that the chloroplast represents a major organelle that can be very important when studying the role of desiccation stress. Besides the very important role of the chloroplast in cellular communication, retrograde signaling plays an important role in the adaptive responses of plants to stress. Non-coding chloroplast DNA (cpDNA) regions tend to evolve more rapidly than do coding regions, by the accumulation of insertions/deletions at a rate at least equal to that for nucleotide substitutions (Wolfe et al. 1987; Zurawski and Clegg 1987; Clegg and Zurawski 1992). The trnL-F intergenic spacer and psbA-trnH intergenic regions of cpDNA are non-coding characters, and this region is more variable than the coding regions. Some studies on the non-coding regions of cpDNA showed higher variations and more often mutation than that of coding regions (Taberlet et al. 1991; Jansen et al. 2008; Skuza et al. 2020). On the other hand, the trnH-psbA intergenic spacer region is one of the most variable regions of the plastid genome in terms of having the highest percentages of variable sites (Shaw et al. 2007) and can offer high levels of species discrimination (Kress et al. 2005; Shaw et al. 2007).

The hypothesis of this study is that *Sargassum denticulatum* extract may promote plant growth under drought stress and may affect chloroplast genome.

Therefore, the current investigation first intended to study the possible effects of local seaweed extracts with low doses on wheat growth under water stress. The second aim is to study seaweed's efficiency of chloroplast response to a severe and prolonged water deficit.

2 Materials and methods

This work was carried out through two experiments; the first experiment was a preliminary (laboratory) experiment that aimed to study the effect of different concentrations of *Sargassum denticulatum* extractions on the growth of wheat plants seedling and to choose the best concentration of seaweeds for the treatment of plants in the second experiment. The second was a greenhouse experiment to study the differential responses of wheat plants, Giza 168 cultivar (*Triticum aestivum* L.) for water stress and foliar treatment with seaweed extract (*Sargassum denticulatum*). The work was conducted in Faculty of Women for Arts, Science and Education, Botany Department Ain shams University, Cairo, Egypt, during two successive winter seasons 2017/2018 and 2018/2019. Wheat seeds, Giza 168 cultivar were obtained from the Agricultural Research Centre, Ministry of Agriculture, and Land Reclamation, Egypt. The marine seaweeds *Sargassum denticulatum* were collected from Ras Sedr (Lat. 29° 37′ N; Long 32° 41′ E).

2.1 Preparation of seaweed algal extracts – Samples of seaweeds were air-dried (26–30 °C) with indirect light for 10 days followed by oven-drying for 48 h at 60 °C. Dried seaweeds were crushed and powdered with a mixer grinder, and then, the powdered seaweed samples were stored in the airtight container for the future use. To 500 g of powdered seaweeds, 5000 ml of sterile distilled water was added, and contents were stood at room temperature for 60 min {ratio 1:10 (w/v)}. Then the extracts were filtered through a filter paper and stored at 4 °C for further experimental studies. The filtered was used as 10% (w/v) aqueous seaweed extract. Three different concentrations, i.e., 0% control, 1%, 2%, and 3% for *S. denticulatum,* was prepared using distilled water (Anisimov et al. 2013).

2.2 Algal analysis – Pigments (chlorophyll a, chlorophyll b, and carotenoids) were estimated by spectrophotometer according to Timothy et al. (2013). The elements, calcium, potassium, magnesium, sodium, manganese, copper, zinc, iron, phosphate, and nitrogen, were estimated by the Inductively Coupled Plasma-Emission Spectrometry (ICP-ES) with Ultra Sonic Nebulizer (USN) (Eaton and Franson 2005). Amino acids were detected according to George (2019). Total protein content was determined as Bradford (1976). Total carbohydrates content was estimated by phenol–sulfuric acid method (Dubois et al. 1956), and total soluble lipid content was determined by a method described by Van Handel (1985). Phenols contents were determined according to Singleton et al. (1999). Growth hormones, abscisic acid, cytokinin, gibberellic acid, salicylic acid, and brassinosteroids, were detected according to Arteca et al. (1980).

2.3 Preliminary experiment – A homogenous lot of seeds of the wheat plants were selected for uniformity of size, shape, and viability. Before germinating, the seeds were surface sterilized by soaking for 3 min in 2.5% sodium hypochlorite solution and washed several times with distilled water. The sterilized seeds were presoaked in distilled water (control) and different concentrations of algal extract (1, 2, and 3% for *S. denticulatum*) for 12 h.

Subsequently, the seeds were allowed to drain for one hour. The seeds were transferred to sterile Petri dishes containing two sheets of Whitman number 1 filter paper moistened with water. Each Petri dish contained 20 seeds, and each treatment was replicated 3 times. The seeds were allowed to germinate at 25 °C in the darkness. The germination percentage was recorded after 7 days. At the end of the experimental period (14 days), seedlings, shoot, and root length as well as fresh and dry matter were recorded.

2.4 Greenhouse experiment – Wheat seeds that were previously sterilized were grown in pots (diameter 35 cm and depth 40 cm) containing 7 kg soil. Characteristics of the soil were as follows: texture, sandy loam; pH, 7.7; ECe, 0.23 dS m-1, and organic matter, 0.41%. Before planting calcium superphosphate (15.5% P_2O_5) and potassium sulfate (48% K_2O) at the rate of 3.0 and 1.50 g/pot were added, respectively. The fertilization with ammonium sulfate (20.5% N) at the rate of 6.86 g/pot was added in two equal doses, and the first one was added after 2 weeks from sowing and the second 2 weeks later. Ten seeds per pot and five replicates were used for each treatment. Five plants after seedling were let to grow in each pot. Preliminary experiments showed that a 2% concentration of seaweed extract (*S. denticulatum*) exhibited the best performance for wheat plants seedling. Therefore, it was chosen to treat plants in the pot experiment. Treatment sets were as follows:

2.5 First set: control and irrigation intervals – Irrigation every week, soil moisture content depleted from 100 to 70% of field capacity (FC). Irrigation every two weeks, soil moisture content depleted from 100 to 55% of FC. Irrigation every three weeks, soil moisture content depleted from 100 to 40% of FC (soil FC was determined on dry weight basis of irrigated pots after keeping saturated soil for 24 h under free drainage).

2.6 Second set: seaweeds treatments – The second set was subjected to the same water regime as in the first set, and after two weeks from sowing, wheat seedlings were sprayed with seaweed extract 2% (*S. denticulatum*) and then sprayed again after 45 days from sowing. The first group was sprayed with distilled water only at the same times as the second group was sprayed with seaweed extract.

At 70 days after sowing, samples from the two sets were taken to study growth parameters in terms of morphological parameters (plant height, fresh, and dry weight), photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) of leaves, carbohydrate (total soluble sugars and starch), and some osmoprotectants as proline, total free amino acid, and phenolic compounds contents.

2.7 Yield determination – At maturity, plant samples were collected randomly to record yield and its components such as plant height, spike length, number of spikelet's/spikes, the weight of grain/plant, and 1000 seeds weight.

2.8 Phytochemical analysis – Photosynthetic pigments content, total chlorophyll a and b, and carotenoid contents in fresh leaves were determined using the method of Lichtenthaler and Buschmann (2001). Total soluble sugars (TSS) were extracted and determined by Yemm and Willis (1956). Starch was measured as reducing sugars by Nelson's method (Nelson 1944) and expressed in maltose equivalents. Proline content was assayed according to the method described by Bates et al. (1973). Free amino acid content (FAA) was extracted according to the method described by Vartanain et al. (1992) and determined by spectrocolorimeter according to Yemm et al. (1955). Phenolic content was assayed by Diaz and Martin (1972).

2.9 DNA extraction and amplification – Total genomic DNA was extracted from fresh leaves using the DNeasy plant mini kit (Qiagen). The universal primer pairs for trnL-F (C + D Primers) and trnH-psbA regions were amplified as recommended by Taberlet et al. (1991) and Hamilton (1999), (Table 1 and Fig. 3). PCRs were carried out in 25 µl reaction volumes and included 2 µl of genomic DNA, 3 µl of a 10 × reaction buffer, 3 µl of 2 mM MgCl2, 3 µl of 1 mM dNTP solution, 3 pmol of each primer, and 0.25 U RedTaqTM polymerase (Sigma-Aldrich, St. Louis, USA). The final volume was adjusted with deionized distilled water. The cycling parameters included an initial denaturation step at 96 °C for 5 min followed by 30 cycles of 96 °C for 30 s, 50 °C for 30 s, and 72 °C for 40 s. A final extension step at 72 °C for 5 min completed the reactions. Negative controls were kept for all the primers which included all the components of the reaction mixture except template DNA, and it was replaced with nuclease-free water. Amplified products were observed on 1.5% agarose gel (1XTBE) with ethidium bromide staining.

2.10 Statistical analysis – The data were statistically analyzed using a one-way analysis of variance (ANOVA 1) as described by Snedecor and Cockran (1969). The means were compared by LSD using SPSS (version 16).

Table 1 Sequences of the chloroplast trnL (UAA) gene with primer combinations of (C + D) and trnH-psbA primer

Primer	Sequence 5'-3'
C	CGAAATCGGTAGACGCTACG
D	GGGGATAGAGGGACTTGAAC
trnH-psbA F	GTTATGCATGAACGTAATGCTC
trnH-psbA R	CGCGCATGGTGGATTCACAATCC

3 Results

3.1 Algal analysis – The photosynthetic pigments like chlorophyll 'a,' chlorophyll 'b,' and carotenoids content were estimated in *S. denticulatum* presented in Table 2.

The macro- and micronutrients as mg/g of the brown marine alga *S. denticulatum* revealed the presence of nitrogen (168.3 mg/g), phosphorus (26.12 mg/g), potassium (73.54 mg/g), magnesium (60.67 mg/g), calcium (78.93 mg/g), copper (0.012 mg/g), manganese (0.441 mg/g), zinc (0.737 mg/g), and iron (6.91 mg/g). Among the elements estimated, nitrogen (168.3 mg/g) as macronutrients and iron (6.91 mg/g) as micronutrients were found to be abundant in the alga. Similarly, in the case of phytohormones analysis (µg/g), cytokinin (84.51) was found to be more when compared to abscisic acid (57.93), gibberellic acid (38.25), salicylic acid (6.35), and brassinosteroids (2.19). The percentage of phenolic compound recorded in *S. denticulatum* was higher (15.2) than protein, carbohydrate, and lipid (11.87, 7.65, and 3.19), respectively. The chemical properties of the extract for seaweed *S. denticulatum* have been analyzed, and their values are given in Table 3.

Amino acids analysis of *S. denticulatum* (Table 4) led to the detection of 17 amino acids with reasonably resolved separations. The seaweeds contained all the essential amino acids at different proportions. The total amino acid content was (3.76 µg/g) and the highest essential amino acid was glutamic (0.56 µg/g), and the most limiting amino acid was histidine followed by cysteine.

3.2 Preliminary experiment – The effect of different concentrations of *S. denticulatum* extractions on germination percentage and growth parameter of wheat seedling is shown in Table 5. The highest rate of germination (97.2%) was found on application of the *S. denticulatum* liquid fertilizer at 2% concentration, followed by 93.3 and 92.5%, the result obtained on using 1 and 3%, respectively, as compared with the control seeds (92.3%). The highest shoot length (21 cm) and root length (19.7 cm) were observed at 2% concentration of alga. The maximum total fresh weight (0.40 g/plant) and total dry weight (0.022 g/plant) were recorded on seeds treated with 2% concentration of alga liquid fertilizer as compared to the control values (0.32 g/plant) for total fresh weight and total dry weight (0.012 g/plant). In our experiments, the use of 2% seaweed liquid extracts of *S. denticulatum* significantly promoted the rate of growth on seedling of wheat. Concentrations of (1 and 3%) were found to show lower effect on all the parameters studied.

4 Pot experiment

4.1 Changes in yield parameters – Data illustrated in Table 6 show that plant length, spike length, spike weight, number of spikelets/spike number of seeds/spike, seeds yield/plant (cm), weight of seeds/spike (g), and weight of 1000 seeds (g) of plant attained the highest values for control plants and were declined progressively by increasing water intervals. A significant increase was detected in all plants treated with 2% *S. denticulatum* extractions compared with control. Seaweeds sprayed plants gave significantly higher yield and yield components than non-sprayed plants.

5 Phytochemical analysis

5.1 Changes in pigments, TSS, and starch contents – Changes in total soluble sugars and starch contents of wheat leaves treated with seaweeds and grown under normal and water deficit are presented in Table 7. The data show a decrease in pigments content (Chl. a, Chl. B, and carotenoids) in wheat leaves under water stress. However, 2% of *S. denticulatum* extract increased the pigment content when compared to control plants under the same conditions. Data clearly show that wheat plant sprayed with 2% *S. denticulatum* extractions significantly increased the starch contents of leaves as compared to plants under the same water regime and without spraying with the extract.

5.2 Changes in proline, FAA, and phenolic contents – Data presented in Figs. 1 and 2 showed the effect of foliar

Table 2 Photosynthetic pigments of *S. denticulatum* (µg /L)

Chlorophyll (a)	7.2
Chlorophyll (b)	Nil
Carotenoids	3.58

Table 3 Chemical composition of *S. denticulatum* extracts

Macronutrients (mg/g dry wt.)		Micronutrients (mg/g dry wt.)		Plant growth hormones (µg/g dry wt.)		Biochemical composition (%)	
Nitrogen	168.30	Copper	0.012	Abscisic acid	57.93	Protein	11.87
Phosphorus	26.12	Manganese	0.441	Cytokinins	84.51	Carbohydrate	7.65
Potassium	73.54	Zinc	0.737	Gibberellic acid	38.25	Lipid	3.19
Magnesium	60.67	Iron	6.91	Salicylic acid	6.35	Phenolic compound	15.20
Calcium	78.93			Brassinosteroids	2.19		

Table 4 Amino acids content of the *S. denticulatum* (µg/g)

Total amino acids	Alanine	Arginine	Aspartic	Cysteine	Glutamic	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tyrosine	Valine
3.76	0.27	0.2	0.4	0.11	0.56	0.26	0.05	0.15	0.27	0.21	0.12	0.21	0.12	0.16	0.18	0.17	0.26

application by 2% of *S. denticulatum* extracts on proline, free amino acids, and phenolic contents in wheat plants. The exposure of wheat plants to water deficit led to significant increases in proline, free amino acids, and phenolic content of wheat leaves. In the meantime, foliar with 2% *S. denticulatum* extraction led to marked decreased in FAA and proline content compared to plants grown under the same conditions. Seaweeds foliar treatment caused significant increases in phenolic content as compared with untreated plants.

5.3 Amplification of cpDNA coding for trnL intron and psbA-trnH intergenic region – The PCR amplification of the trnL intron was successfully obtained for all foliar treatments with seaweeds extract with or without water deficit and control plants irrigated every 3 weeks. Meanwhile, amplification was not successful for the control plants irrigated every 1 and 2 weeks. The length of the amplified trnL gene was 530 bp (Fig. 3).

PCR amplification of psbA-trnH intergenic region was successfully obtained for all treatments of wheat plants except for control plants treated with a long-term dehydration period of three weeks. The samples had a PCR product of about 321 bp. Thus, it can be assumed that the chloroplast psbA-trnH intergenic region gene missing from the chloroplast genome may be involved with water-deficit responses, necessitating further investigation.

6 Discussion

The goal of this study was to see how effective *Sargassum denticulatum* is at increasing wheat drought tolerance. According to Zodape (2001) and Tarakhovskaya et al. (2007) seaweed products contain growth regulators (auxins, cytokinin, and gibberellins), amino acids, and mineral elements that positively promote plant development and division. In response to the treatment with varied concentrations of the seaweed extracts under research, statistically significant changes in shoot length, root length, and seedling, fresh and dry weights were detected. Seed soaking in 2% *Sargassum denticulatum* showed a favorable response in growth characteristics in this regard. The inclusion of several growth-promoting chemicals could explain the higher seedling growth. Seedling growth was higher with *Sargassum denticulatum* liquid fertilizer. The existence of nutritional contents in seaweeds may also contribute to their ability to promote growth (Kalaivanan and Venkatesalu 2012). Pre-soaking wheat seeds in various doses of algal extract improved all seedling growth criteria. The use of 2% alga resulted in the greatest increases in all growth criteria. Drought, the most critical environmental stress, stifles plant growth and development, reduces plant yield, and impairs agricultural plant performance more than any other factor. Due to the fall in turgor pressure, growth is one of the most drought-sensitive

Table 5 Changes in growth parameters of wheat seedling treated with different concentrations of seaweeds (*S. denticulatum*)

Treatment		After 7 days	After 14 days			
		Germination rate (%)	Shoot length (cm)	Root length (cm)	Total fresh weight (g)/plant	Total dry weight (g)/plant
Control		92.3 ± 4.56b	19.3 ± 1.24b	16.8 ± 0.98b	0.32 ± 0.012a	0.012 ± 0.001a
Concentrations of *S. denticulatum*	1	93.3 ± 4.62b	19.8 ± 1.36b	18.7 ± 1.24a	0.36 ± 0.030a	0.015 ± 0.002a
	2	97.2 ± 6.22a	21.0 ± 1.75a	19.7 ± 1.62a	0.40 ± 0.028a	0.022 ± 0.002a
	3	92.5 ± 5.44b	20.4 ± 1.65ab	18.7 ± 0.84a	0.33 ± 0.024a	0.013 ± 0.001a

Means with the same letter(s) in the same column are not significantly different at $p < 0.05$

Table 6 Changes in yield parameters of wheat plants treated with seaweed (*S. denticulatum*) and grown under normal and water deficit

Treatments		Plant length (cm)	Spike length (cm)	Spike wt. (g)	No. of spikelets/spike	No. of seeds/spike	Seeds yield/plant (g)	Wt. of seeds/spike (g)	Wt. of 1000 seeds (g)
Control	Every 1 week	70.50 ± 3.42b	11.8 ± 1.24ab	2.10 ± 0.150b	13.3 ± 1.05b	6.5 ± 0.32b	6.5 ± 0.32b	1.53 ± 0.045b	38.3 ± 1.74b
	Every 2 weeks	45.30 ± 2.18e	11.2 ± 0.85b	1.82 ± 0.100b	12.3 ± 1.00c	4.1 ± 0.11c	4.1 ± 0.11c	1.50 ± 0.065b	33.5 ± 1.32c
	Every 3 weeks	39.00 ± 2.06f	10.5 ± 0.74c	0.63 ± 0.032a	10.3 ± 0.58e	3.0 ± 0.08e	3.0 ± 0.08e	0.35 ± 0.027c	30.2 ± 1.54d
2% *S. denticulatum*	Every 1 week	78.50 ± 4.22a	12.5 ± 1.00a	2.59 ± 0.133a	14.67 ± 0.64a	7.2 ± 0.20a	7.2 ± 0.20a	1.75 ± 0.066a	30.2 ± 2.01d
	Every 2 weeks	61.00 ± 3.56c	11.8 ± 0.92b	2.18 ± 0.142b	13.67 ± 0.75b	4.9 ± 0.12d	4.9 ± 0.12d	1.75 ± 0.068a	40.1 ± 2.34a
	Every 3 weeks	47.30 ± 2.44d	11.2 ± 0.76b	0.72 ± 0.042c	11.3 ± 0.82d	3.5 ± 0.10e	3.5 ± 0.10e	0.45 ± 0.042c	33.8 ± 1.33c

Means with the same letter(s) in the same column are not significantly different at $p < 0.05$

Table 7 Changes in pigments, TSS, and starch contents of wheat leaves treated with seaweed (*S. denticulatum*) and grown under normal and water deficit

Treatments		Chl. a (mgg^{-1} fr. wt)	Chl. b	Carotenoids	TSS	Starch
Control	Every 1 week	1.213 ± 0.065c	0.421 ± 0.012c	0.275 ± 0.014b	3.53 ± 0.15c	45.32 ± 2.45b
	Every 2 weeks	0.932 ± 0.055d	0.358 ± 0.010d	0.221 ± 0.016c	4.82 ± 0.22ab	44.53 ± 1.89b
	Every 3 weeks	0.854 ± 0.080d	0.313 ± 0.022d	0.182 ± 0.011d	5.38 ± 0.24a	42.3 ± 3.12c
2% *S. denticulatum*	Every 1 week	1.452 ± 0.101b	0.551 ± 0.034a	0.325 ± 0.021a	3.68 ± 0.13c	49.2 ± 3.56a
	Every 2 weeks	1.263 ± 0.098c	0.483 ± 0.032b	0.265 ± 0.012b	4.32 ± 0.18b	48.3 ± 2.88a
	Every 3 weeks	1.830 ± 0.142a	0.421 ± 0.028c	0.235 ± 0.016c	4.91 ± 0.16a	45.5 ± 3.15b

Means with the same letter(s) in the same column are not significantly different at $p < 0.05$

processes (Kusaka et al. 2005). Water deficit in soybean plants, according to Anjum et al. (2011), lowered plant height, lowest node height, leaf area, stem diameter, number of nodes, and branches by decreasing the soil's water potential.

The effect of 2% extract of *S. denticulatum* gave increase in germination rate and shoot length of *Triticum aestivum L.* as compared to control. The increase in germination percentage might be due to the presence of growth promoting substances such as gibberellins, cytokinins, micronutrients (Fe, Cu, CO, Zn, Mn, Mo, and Ni), vitamins, and amino acids. Our results coincided with those of earlier studies on *Cajanus cajan* (Mohan et al. 1994), *Vigna sinensis* (Sivasankari et al. 2006), and *Zea mays* (Al-Shakankery et al. 2014). The beneficial effects of *Sargassum* extract application could be due to improved root proliferation and

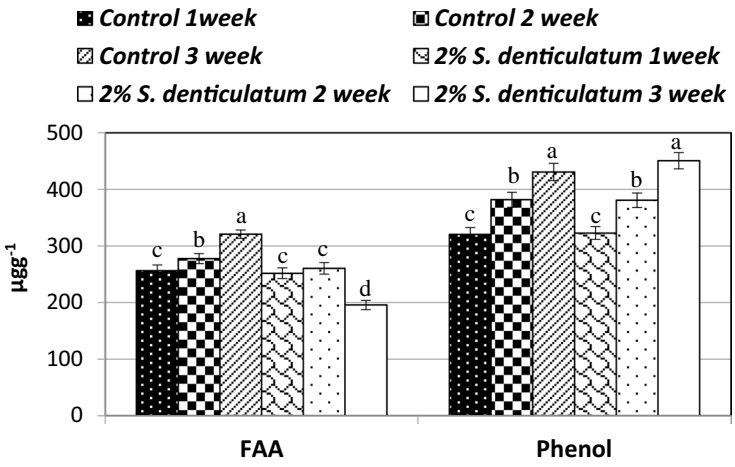

Fig. 1 Changes in FAA and phenol contents (mg/g dry weight) of wheat leaves treated with seaweed (*S. denticulatum*) and grown under normal and drought stress

F value	864.52**	124.26**

**significant difference at $p<0.01$

Columns of each parameter with the same letter(s) is not significantly different

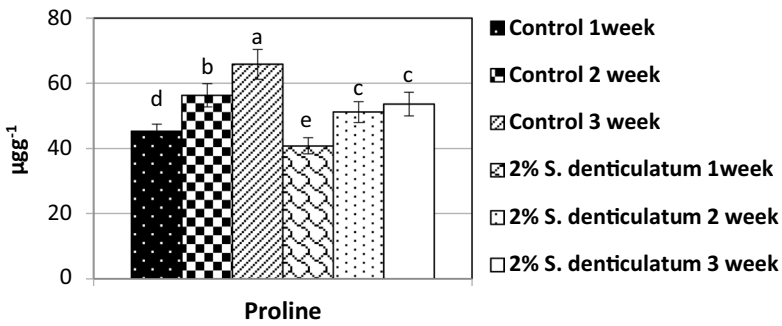

Fig. 2 Changes proline (mg/g dry weight) of wheat leaves treated with seaweed (*S. denticulatum*) and grown under normal and drought stress

F value	956.55**

**significant difference at p<0.01

Columns with the same letter(s) is not significantly different

establishment, allowing plants to mine more nutrients in a balanced proportion from further away and deeper soil layers.

Photosynthesis is required for plant growth and reproduction, and chlorophyll is the green pigment responsible for light absorption and photosynthesis (Nelson and Cox 2004; Marschner 2011). According to Mourey-Bringuier (1986), algal extracts improve pigment concentration by means of phytohormones included in these extracts, mainly cytokinin, which protects chlorophyll from degradation under water stress and increases nitrogen assimilation. Seaweed liquid fertilizer boosted total chlorophyll and carotenoids content in *Cyamopsis tetragonoloba* at a lower dosage (20%) with or without chemical fertilizer (Arumugam et al. 2009), possibly due to the presence of plant growth agents in the seaweed extract used (Mostafa and Zheekh 1999). Our results are also consistent with those reported by Salma et al. (2014), which show that plant tolerance to abiotic stress and particularly water stress is significantly improved by applying algal extracts. Similar observations were reported for the effect of extracts of *Chaetomorpha antennina* and *Rosenvingea intricate* on the chlorophyll contents of *Abelmoschus esculentus* and *Raphanus sativus* plants (Thirumaran et al. 2006 and 2007). The increases in pigments may be due to the presence of high amount of magnesium in the liquid fertilizers of *S. denticulatum*. Marine algae contain cytokinins, gibberellins, auxins, auxin-like, and other growth promoting compounds (Yokoya et al. 2010).

Plants' acquisition of drought resistance is intimately linked to the buildup of soluble carbohydrates (Hoekstra et al. 2001). The increase in sugar concentration could be due to starch degradation (Fischer and Höll 1991). Ketabchi

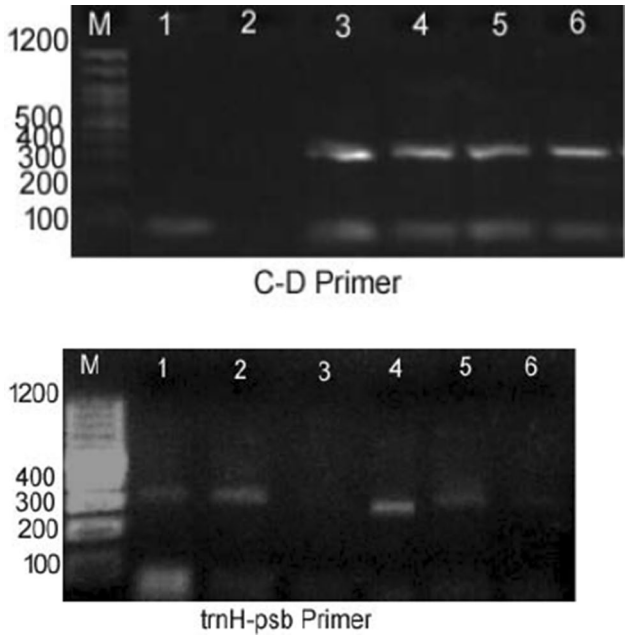

Fig. 3 Agarose gel showing amplified trnL intron (C-D) primer and trnH-psb intergenic region for wheat plants irrigated every 1. one week, 2. two weeks, 3. three weeks, 4. foliar plants with seaweeds extract irrigated every one week, 5. foliar plants with seaweeds extract irrigated every two weeks, 6. foliar plants with seaweeds extract irrigated every three weeks, Lane M: 100 bp. Ladder

and Shahrtash (2011) indicated that soluble sugar concentrations increased at the same time as starch quantities declined. In this study, foliar treatments with algal extract increased starch accumulation in all water intervals, which could be related to seaweed extract's promoter effect on chlorophyll levels.

Proline is important for osmotic adjustment, stability, and protection of enzymes, proteins, and membranes from drought-induced osmotic stress (Ashraf and Foolad 2007). With drought stress, foliar applications with seaweed extract lowered proline and free amino acid levels in wheat plants. Our findings are consistent with those of previous soybean research (Rathore et al. 2009). In *Vigna sinensis* L., similar findings were reported (Sivasankari et al. 2006).

Many plants have shown an increase in phenol content in various tissues when subjected to osmotic stress (Mansori et al. 2019). These increases could be related to total phenols' role as a key regulator of plant metabolic processes and, as a result, overall plant development (Abdallah et al. 2015). Furthermore, phenols serve as a substrate for numerous antioxidant enzymes, reducing the effects of drought stress (Bakry et al. 2012). Another mechanism behind phenolic compounds' antioxidant benefits is their ability to reduce membrane mobility (Sadak 2016 b). Furthermore, phenolic compounds have an antioxidant effect as free radical scavengers due to their reactivity as electron or hydrogen donors, which helps to stabilize and delocalize unpaired electrons, as well as their role as transition metal ions chelators (Huang et al. 2005).

The increase in phenolic content in treated plants can explain the favorable effect of seaweed extract on wheat drought resistance. Abiotic and biotic stressors, on the other hand, can cause phenolic buildup in plants (Yamasaki et al. 1995). Active oxygen species are produced during drought stress and can cause oxidative damage (Ortega-Villasante et al. 2016). Enzymatic and non-enzymatic antioxidative systems, such as phenolic compounds, which have perfect structural chemistry for free radical scavenging action, mitigate the harmful effects of reactive oxygen species (Ahmed et al. 2010). The polyphenol-derived radical is able to stabilize and delocalize the unpaired electron, as well as the high reactivity of phenolic compounds as hydrogen or electron donors (chain-breaking function). The osmotic adjustment in plants under drought stress is caused by the accumulation of compatible osmolytes at high concentrations.

The non-coding characters trnL-F intergenic spacer and psbA-trnH intergenic area of cpDNA are more changeable than the coding sections. Non-coding portions of cpDNA have been found to have more variants and mutations than coding sections in several studies (Skuza et al. 2020). According to our PCR experiments, the trnL intron was shown to be present in long-term drought-stressed plants for three weeks, as well as all foliar treatments with seaweed extract with or without drought stress and lacking in control plants irrigated for one and two weeks.

The precise loss of an intron has been reported from other chloroplast genes (e.g., Downie and Palmer 1992; Wallace and Cota 1996; Jansen et al. 2008). Such a process has been already reported for the loss of both group I and group II introns in plants, fungi, and animals (e.g., Campagna and Downie 1998; Hu 2006; Jeffares et al. 2006). Other mechanisms of intron loss could be simple genomic deletion and in-frame intron deletion (Niu et al. 2005). The chloroplast psbA-trnH intergenic region gene is losing from the chloroplast genome may be as a response to severe drought stress (3 weeks). These findings suggest that sequencing analysis should be used in more research. The failure of psbA-trnH intergenic region amplification in seedling plants could be due to nucleotide variations as single-nucleotide substitutions that prevent PCR amplification (Bru et al. 2008). This indicates that the foliar treatments with seaweed extract may have reduced the harmful effect of severe drought stress. The results showed that the investigated regions of the chloroplast genome are variable in wheat plant treatments with or without seaweed extract under drought conditions. Thus, it can be assumed the chloroplast psbA-trnH intergenic region gene missing from the chloroplast genome as a response to severe drought stress (Sun and Guo 2016). The psbA gene encodes the D1 reaction center protein of photosystem II.

The D1 protein is the primary target of the damage, and it is sacrificed in order to avoid complete inactivation and disassembly of PSII (Nixon et al. 2010).

The role of chloroplast psbA UTRs in the regulation of gene expression has been investigated intensively for more than twenty years (Zurawski et al. 1982); preservation of its structure influences psbA longevity, processing, and maturation.

Finally, we may conclude that foliar spraying seaweed extract (*S. denticulatum*) with antioxidant components (phenolics), suitable osmolytes, and some osmoprotectant molecules such TSS, proline, and free amino acids improved wheat performance. As a response to water scarcity, seaweed extract increases diversity in the non-coding chloroplast DNA (cpDNA) regions trnL intron and psbA-trnH. Marine algae are one of the forms of biological fertilization that does not contaminate the environment, increases crop yield, and aids in water conservation, which is now the most important aspect of Egyptian agriculture.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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