INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 7, No. 2, p. 173-189, 2015

RESEARCH PAPER OPEN ACCESS

Influence of seaweed extracts on the growth, some metabolic activities and yield of wheat grown under drought stress

Wedad A. Kasim[1*] Elsayed A.M. Hamada[1], Nehal G. Shams El-Din[2], SalwaK.Eskander[1]

[1]*Botany Department, Faculty of Science, Tanta University, Tanta, Egypt*

[2]*National Institute of Oceanography and Fisheries, Alexandria, Egypt*

Article published on August 16, 2015

Key words: Drought stress, growth criteria, peroxidase, photosynthetic pigments, *Sargassumlatifolium*, *Triticumaestivum, Ulvalactuca*.

Abstract

The physiological effect of drought on the 30-days-old *Triticumaestivum* plants was assessed and the alleviating role of seaweed extracts *(Sargassumlatifolium, Ulvalactuca*andtheir mixture) on drought stress was evaluated. Drought treatment (40% and 20% field capacity) resulted in a significant decrease in some growth criteria, photosynthetic pigments and activity. Furthermore, it led to oxidative stress and increased cell membrane leakage in the stressed wheat plants and resulted in the increase of antioxidant (enzymatic and non-enzymatic) defense mechanism. Pretreatment with seaweed extract of *Sargassum* (1.5%) or *Ulva* (1%) led to the alleviation of the above mentioned damaging effects of drought on *Triticumaestivum*duringvegetative stage while a mix of the two types of seaweed extracts resulted in antagonistic effect. Seaweed extractof *Sargassum*or *Ulva* antagonizes the oxidative damaging effects of drought not only directly through activating the antioxidative system, such as catalase, peroxidase and ascorbate, but also through providing hormones and micro nutrients essential for wheat growth.

* **Corresponding Author:** Wedad A. Kasim ✉ wedkasim@yahoo.com

Introduction

Drought stress induces several physiological, biochemical and molecular responses in crop plants, which would help them to adapt to such limiting environmental conditions (Arora *et al.*, 2002; Shehab*et al.*, 2010). Drought impacts include growth, plant structure, membrane integrity, pigment content, tissue osmotic potential and the antioxidant defense mechanism and photosynthetic activity (Benjamin and Nielsen, 2006; Duan*et al.*, 2007; Praba *et al.*, 2009).The susceptibility of plants to drought stress varies depending on thestress degree, different accompanying stress factors, plant species, and their developmental stages (Demirevska*et al.*, 2009). At the physiological and metabolic levels, drought causes inhibition of shoot growth, adjustment of leaf area, stomatal closure and reduction of transpiration, inhibition of photosynthesis, shifts in carbon and nitrogen metabolism, synthesis of compatible solutes, and secondary oxidative stress (Xoconostle-Cázares*et al.*, 2011).

Drought induces oxidative stress in plants by generation of reactive oxygen species (ROS) such as O_2–, H_2O_2 and OH• radicals which can directly attack membrane lipids and increase lipid peroxidation and the content of malondialdehyde (MDA) which is considered as an indicator of oxidative damage (Mittler, 2002; Moller *et al.*, 2007; Farooq *et al.*, 2009).To keep the levels of active oxygen species under control, plants have non-enzymatic and enzymatic antioxidant systems to protect cells from oxidative damage (Mittler, 2002).The non-enzymatic antioxidants include β-carotenes, ascorbic acid (AA), α-tocopherol (α-toc)and reduced glutathione (GSH),while the enzymes include superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathionereductase (GR) (Xu*et al.*, 2008). The balance between ROS production and activities of antioxidative enzymes determines whether oxidative signaling and/or damage will occur (Moller *et al.*, 2007). The capability of scavenging ROS and reducing their damaging effects may correlate with the drought tolerance of plants (Tsugane*et al.*, 1999).

Seaweeds are macroscopic algae, growing in intertidal and subtidal regions of the sea, and serve as an excellent source of food, fodder, fertilizer, and industrial raw material (Parthiban *et al.*, 2013). Recently, bioactive substances extracted from marine algae are used in agricultural and horticultural crops as bio-fertilizers to improve their yield and quality and to reduce the negative environmental impact (Houssien *et al.*,2011). Seaweeds provide an excellent source of bioactive compounds such as essential fatty acids, vitamins, amino acids, minerals, and growth promoting substances. They have alsobeen reported to stimulate the growth and yield of plants (Bhasker and Miyashita, 2005), enhance antioxidant properties, and develop tolerance to drought stress (Spann and Little, 2011).

Although numerous studies have been carried out on the taxonomy, distribution, photochemistry and antibacterial activities of seaweeds, little work has been done on the influence of their extracts on the growth of wheat grown under drought stress. Therefore this study was planned to determine the effect of priming of wheat grains by presoaking in the extract of *Sargassumlatifolium*,*Ulvalactuca*and their mixture and grow under drought stress during the vegetative stage through recording some changes in the photosynthetic pigments and activity and some enzymatic and non- enzymatic antioxidant defense mechanism.

Materials and methods

Growth conditions and treatments

Grains of wheat (*Triticumaestivum* cv. Gemeza 9) were supplied by the Egyptian Ministry of Agriculture and selected for apparent uniformity of size and shape. *Sargassumlatifolium* was collected from the shore of AlTor City (28° 14'61" N; 33° 37'05" E) during November, 2013; while the seaweed*Ulvalactuca*(sea lettuce) was collected from the National Institute of Oceanography and Fisheries from Suez Bay (29°58'10" N- 27°38'39" N;

32°21'43"E-34° 05'46"E).The collected algal species were identified according to Nasr (1940) and Jha*et al.* (2009).

Wheat grains were washed with distilled water and divided into four groups.Each group was sown in plastic pots (40 cm diameter and 45 cm depth) containing 20 kg untreated clay-sandy soil (2:1 w/w); 5 pots were used for each treatment and 10 grains were sown in each pot. The first group of grains was pre-soaked in water for 3 h and considered as control. The second, third and fourth groups were pre-soaked for 3 h in 1.5% *Sargassumlatifolium* extract, 1% *Ulvalactuca* extract and a mixture of both, respectively.

The grains were left to germinate and grow at the normal environmental conditions of 16/8 h. light/dark, at 25/15 ± 2 °C day/night, respectively and relative humidity of 65% and irrigated with tap water twice a week during their growth season.

The drought stress was applied by calculating 60%, 40% and 20% of the full field capacity (100% soil saturation with water). After 14 days of growth, the pots were irrigated every 5 days with 60% field capacity for the control, 40% field capacityas drought 1 (D1) or with 20% field capacity as drought 2(D2).The 30-day old vegetative were collected for sampling.

Physiological analyses

Growth criteria as (root depth, shoot height, fresh and dry masses of root and shoot, and leaf area) were measured.The photosynthetic pigments, chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids (carot.) were determined in the leaves of the 30-day old plantsaccording to Arnon (1949) for chlorophylls and according to Horvath *et al.* (1972) for carotenoids as adopted by Kissimon (1999). Photosynthetic activity (Fv/Fm) of dark-adapted leaves was measured with OS-30 p chlorophyll fluorometer (Hudson, NH 03051 USA).

Lipid peroxidation level was measured by determining malondialdehyde (MDA) content according to the method of Heath and Packer(1968) and calculated using the extinction coefficient (155 mM^{-1} cm^{-1}). For measurements of electrolyte leakage, fresh leaves were cut into small pieces; one-half g of them was immersed in 20 ml distilled water; after 24 hours immersion, the electrical conductivity (µmohs/cm) was measured by EC meter in the leakage solution. Ascorbic acid was estimated according to Oser (1979) and calculated as mg/g f.m using a calibration curve.

Activities of peroxidase [EC1.11.1.7] and catalase [EC1.11.1.6] were assayed according to Kato and Shimizu (1987) and they were expressed in units of µM / g f.m.

Analysis of seaweed extracts

A. Heavy metals

The mixed acid–digestion method was used for element determination according to Allen *et al* (1974). The measurements were carried out using the Atomic Absorption flame emission Spectrophotometer (Model Perkin Elmer 2380 Atomic Absorption Spectrophotometer).

B. Hormones

According to Shindy and Smith (1975) the different aqueous phases were prepared for GLC determination of the acidic hormones asauxin (IAA), abscisic acid (ABA), gibberellins(GAs) and cytokinins. Computer-controlled GLC-MS analyses of TMS (trimethylsilyl) derivatives of authentic standards or extract fractions were carried out with a Systems 150 output control module on a Finnigan mass spectrometer (Model 1015C) interfaced to a Varian Aerograph GLC (Model 1400) fitted with a Goelke all-glass separator. Retention time and temperature for each peak were recorded and compared to those of TMS derivatives of authentic standards. Chromatography of unknowns and standards was also done to facilitate identification.

C. Glycinebetaine

Concentrations of Glycinebetaine (GB) were

estimated in the seaweed extracts using a standard curve developed with different known concentrations of GB as described by Grieve and Grattan (1983).

3.Statistical analyses

The results were statistically analyzed using one way Analysis of Variance (ANOVA) to determine the degree of significance for the obtained variations by the used treatments. The analysis was carried out by COSTAT statistical program.

Results

Exposure of wheat plant during the vegetative stage to drought stress (40% and 20% filed capacity) resulted in a general reduction in growth. Data shown in Table 1 indicated that, in the 30-day-old seedlings, treatment of D1 caused highly significant decrease in each of root depth, shoot height and leaf area, where the percentages of decrease were 10%, 18% and 37% relative to the control, respectively; while in case of the treatment of D2, the percentages of decrease were 24%, 35% and 66 %, respectively.

Table 1. Effect of drought stress on the root depth, shoot height and leaf area of 30-day-old *Triticumaestivum*(L.) grown in clay sandy soil (2:1w/w) and irrigated with 60% of water field capacity as a control (cont.), with 40% of water field capacity as drought 1 (D1), with 20% of water field capacity as drought 2 (D2)) after soaking the grains for 3 hours in 1.5% *Sargassum* extract (Ext.1), 1% *Ulva* extract (Ext.2) and 1:1 mixture of them (Ext.1+Ext.2).

Treatments	Length cm/plant		Fresh mass g/plant		Dry mass g/plant		Leaf area cm^2/leaf
	Root	Shoot	Root	Shoot	Root	Shoot	
Cont	26.9 ± 0.2	30.1 ± 0.2	0.29±0.01	1.2±0.01	0.043±0.002	0.19±0.001	5.4 ± 0.1
D1	22.1 ± 0.2	27 ± 0.1	0.14±0.005	0.9±0.05	0.024±0.002	0.15±0.002	3.4 ± 0.1
D2	16.6 ± 0.1	22.8 ± 0.3	0.13±0.002	0.6±0.01	0.023±0.0005	0.11±0.001	1.8 ± 0.2
Ext1	30.7 ± 0.3	33.9 ± 0.6	0.33±0.006	1.3±0.03	0.046±0.0005	0.2±0.002	6.5 ± 0.1
Ext1+D1	22.7 ± 0.5	30.8 ± 0.2	0.19±0.007	1.4±0.05	0.03±0.001	0.23±0.005	5.2 ± 0.2
Ext1+D2	22.9 ± 0.2	26.5 ± 0.2	0.18±0.002	0.8±0.02	0.035±0.001	0.16±0.002	3.8 ± 0.2
Ext2	32.2 ± 0.4	34.2 ± 0.2	0.29±0.002	1.3±0.05	0.049±0.002	0.23±0.003	5.4 ± 0.2
Ext2+D1	25.4 ± 0.4	28.7 ± 0.3	0.29±0.001	1.1±0.01	0.056±0.002	0.19±0.006	4.9 ± 0.1
Ext2+D2	22.1 ± 0.1	26.8 ± 0.2	0.19±0.003	0.7±0.001	0.036±0.001	0.14±0.006	2.8 ± 0.2
Mix	29.7 ± 0.7	28.9 ± 0.4	0.3±0.006	1.3±0.01	0.043±0.002	0.19±0.003	5.7 ± 0.1
Mix+D1	22.4 ± 0.1	26.7 ± 0.6	0.13±0.001	0.9±0.001	0.022±0.001	0.14±0.004	2.6 ± 0.2
Mix+D2	18.3 ± 0.2	24.9 ± 0.2	0.09±0.002	0.5±0.03	0.019±0.001	0.09±0.003	1.5 ± 0.1

The combined treatments of drought with priming by presoaking of wheat grains in the extract1 (*Sargassum*1.5%) or extract 2 (*Ulva* 1%) resulted in a significant recovery from the harmful effects of drought stress where the root depth, shoot height and leaf area were increased compared with the single treatment of drought stress but the values remained lower than those of the control;the mixture resulted in ahigh decrease in the measured criteria.

Priming of wheat grains by presoaking in the mixture of seaweed extracts were not effective where the shoot height was slightly decreased compared with the control. Drought treatment resulted in a highly significant decrease in wheat root and shoot fresh masses, where the percentages of decrease were 51% and 25% with D1; while with D2,they were 55% and 50%, respectively, relative to the control. Similarly, root and shoot dry masses were significantly decreased by drought stress and the percentages of decrease were 44%and 21 % with D1,while with D2,they were 47% and 42% respectively, compared with the control.

The combined treatments of drought with the priming of wheat grains with seaweed extracts completely overcame the inhibitory effects of drought stress, except in case of the mixture of the two extracts which resulted in a decrease in both fresh

and dry masses of root and shoot, compared with the single treatment of drought stress (Table 1).

Drought treatments led to negative effects on photosynthetic pigments and photosynthetic activity in 30-day-old wheat seedlings (Table 2).Both treatments of D1 and D2 caused highly significant reduction in chl a, chl b, and carotenoids, where the percentages of decrease in case of D1 were 20 %, 86 % and 40 %, respectively, and in case of D2 they were 26 % , 43 % and 28 %, respectively compared with the control. The chl (a/b) ratio showed a highly significant increase in case of D1 treatment which was represented by 447 %, relative to the control.The photosynthetic activity (Fv/Fm) was largely inhibited by drought treatments compared with the control, where D2 treatment caused a highly significant decrease which was 15 % it was 9% in case of D1 (Table 2).

Table 2. Effect of drought stress on chlorophyll a (chl a), chlorophyll b (chl b), carotenoids (carot.), Chl(a/b) ratio and photosynthetic activity of 30-day-old *Triticumaestivum*(L.) grown in clay sandy soil (2:1w/w) and irrigated with 60% of water field capacity as a control (cont.), with 40% of water field capacity as drought 1 (D1), with 20% of water field capacity as drought 2 (D2)) after soaking the grains for 3 hours in 1.5% *Sargassum* extract (Ext.1), 1% *Ulva* extract (Ext.2) and 1:1 mixture of them (Ext.1+Ext.2).

Treatments	Chl a	Chl b	Carot.	Chl (a/b)	Photosynthetic activity
	mg/g d.m				(µ mol m^{-2} s^{-1})
Cont	8.6±0.07	5.8±0.08	2.50±0.05	1.5 ± 0.02	0.68 ± 0.008
D1	6.9±0.03	0.83±0.03	1.50±0.05	8.2 ± 0.52	0.62 ± 0.006
D2	6.4±0.18	3.3±0.03	1.80±0.09	2.0 ± 0.18	0.58 ± 0.006
Ext1	9.3±0.15	5.3±0.07	2.20±0.04	1.8 ± 0.12	0.72 ± 0.005
Ext1+D1	8.7±0.19	1.7±0.09	1.90±0.12	5.3±0.36	0.64 ± 0.005
Ext1+D2	7.7±0.16	4.7±0.03	2.80±0.04	1.7±0.14	0.61 ± 0.004
Ext2	10.4±0.23	3.7±0.09	5.10±0.03	3.0 ±0.09	0.76 ± 0.006
Ext2+D1	8.6±0.25	1.8±0.14	1.99±0.08	4.9±0.02	0.66 ± 0.004
Ext2+D2	6.5±0.03	3.8±0.15	1.93±0.07	1.9±0.19	0.66 ± 0.006
Mix	7.6±0.19	3.5±0.14	1.30±0.06	2.2±0.6	0.63 ± 0.005
Mix+D1	6.6±0.06	1.1±0.09	2.02±0.16	6.2±0.45	0.61 ± 0.006
Mix+D2	5.4±0.17	3.7±0.16	2.60±0.11	1.4±0.11	0.58 ± 0.010

One way ANOVA analysis ($P \leq 0.01$) (*** highly significant).

Parameter	LSD	F	Significance
Chl a	0.29	212.12	***
Chl b	0.22	455.83	***
Carotenoids	0.13	458.62	***
Chl (a/b)	0.42	248.35	***
Photosynthetic Activity	0.011	234.37	***

Priming of wheat grains by pre-soaking them in *Sargassum* or *Ulva* resulted in alleviation of the negative effects of drought on both the photosynthetic pigments and photosynthetic activity in the vegetative stage(Table 2). The seaweed extracts treatment alone had a significant positive effect on chlorophyll contents; however, priming with *Ulva* caused an increase of carotenoid content.

Fig. 1 shows that drought treatments caused highly

significant increases in the MDA content and electrolyte leakage, where the percentages of these increases were 8 % and 30% with D1, while they were 351 % and 93%with D2, respectively, compared with the control(Figs. 1A and 1B).In case of the single treatment by presoaking of wheat grains in*Sargassum* or *Ulva* extract, the MDAcontent was decreased while the electrolyte leakage was slightly increased relative to the control.

Drought treatments resulted in an increase in peroxidase and catalase activities by 13% and 44% for D1 and 50% and 100% for D2, respectively relative to the control (Table 3). Similarly, ascorbic acid content was increased after drought treatments by 21 % for D1, and 40 % for D2, relative to the control (Table3). However, in case of the combined treatment of drought and priming of wheat grains by presoaking in *Sargassum*or *Ulva* extract, the catalase and peroxidase activities were increased, compared with D1and D2. On the other hand, compared with the control, great reduction in the peroxidase activity was detected in case of single treatment of priming of wheat grains by presoaking in *Ulva* extract, while it increased the catalase activity; however, the single treatment of priming with *Sargassum* resulted in a noticeable increase in the activities of both peroxidase and catalase.

Table 3. Effect of drought stress on peroxidase and catalase activities and ascorbic acid content of 30-day-old *Triticumaestivum* (L.) plants grown in clay sandy soil (2:1w/w) and irrigated with 60% of water field capacity as a control (cont.), with 40% of water field capacity as drought 1 (D1), with 20% of water field capacity as drought 2 (D2) after soaking the grains for 3 hours in 1.5% *Sargassum* extract (Ext.1), 1% *Ulva*extract (Ext.2) and 1:1 mixture of them (Ext.1+Ext.2)

Treatments	Peroxidase (µM / g f.m)	Catalase (µM / g f.m)	Ascorbic acid (mg/g f.m)
Cont	0.032 ± 0.001	0.009 ± 0.0002	116.3±1.7
D1	0.036 ± 0.001	0.013 ± 0.0006	140.5±1.5
D2	0.048 ± 0.001	0.018 ± 0.0004	162.6±2.9
Ext1	0.035 ± 0.001	0.014 ± 0.0001	108.4±4.5
Ext1+D1	0.046 ± 0.001	0.011 ± 0.0005	136.9±4.5
Ext1+D2	0.054 ± 0.001	0.013 ± 0.0006	149.8±6.2
Ext2	0.021 ± 0.001	0.014 ± 0.0006	102.0±1.5
Ext2+D1	0.039 ± 0.001	0.018 ± 0.0005	184.8±1.5
Ext2+D2	0.042 ± 0.001	0.014 ± 0.0004	202.6±4.4
Mix	0.041 ± 0.001	0.015 ± 0.0004	214.4±1.5
Mix+D1	0.055 ± 0.001	0.012 ± 0.0006	230.6±2.9
Mix+D2	0.065 ± 0.001	0.013 ± 0.0005	294.5±4.5

One way ANOVA analysis ($P \leq 0.01$) (*** highly significant).

Parameter	LSD	F	Significances
Peroxidase	0.002	331.42	***
Catalase	8.1	87.29	***
Ascorbic acid Content	6.03	771.89	***

It was noticeable that, the combined treatment of drought stress after the presoaking of wheat grains in *Sargassum* extract resulted in the decrease in the ascorbic acid contents, relative to D1 and D2 treatments although they were still higher than that of the control. Compared with the control, the single treatment of only priming of wheat grains by presoaking in *Sargassum* or *Ulva* extract resulted in a decrease in the ascorbic acid, relative to the control. However, a notable increase in the ascorbic acid contents was recorded in case of priming with the mixture of the two extracts of seaweeds (Table 3).

Table 4. Heavy metals content (ppb), hormones content (mg/ 100ml) and glycinebetaine (GB) as µg/g dry weight of seaweed extract for *Sargassum*1.5%(Ext1), *Ulva* 1%(Ext2) and mixture of them.

Treatments	ppb								mg/100ml			(µg/g d.wt)
	Zn	Pb	Cd	Cu	Fe	Cr	Ni	Mn	Cytok.	Gibber.	auxin	GB
Ext1 (*Sargassum*)	1.75	0.16	0.064	0.83	4.42	0.027	0.149	0.17	0.185	12.65	0.103	0.16
Ext2 (*Ulva*)	1.74	0.38	0.063	1.46	7.99	0.025	0.358	0.15	0.130	12.44	0.202	0.428
Mixture Ext1+Ext2	1.55	Undetected	0.073	0.39	5.72	0.021	0.211	0.22	0.511	25.54	0.355	0.641

The analysis of seaweed extracts of *Sargassum*,*Ulva* and their mixture indicated that the seaweed extracts containeddifferent concentrations of micronutrients (Zn, Pb, Cd, Cu, Fe, Cr, Ni, and Mn(Table 4). It was obvious from the resultsthat Pb was not detected in the mixture of seaweed extracts, while high concentrations of Cd and Mnwere detected (Table 4). The results in Table 4 also showed that the extract of *Sargassum* or *Ulva*and their mixture containdifferent concentrations ofphytohormones (cytokinin, gibberellins and auxins), but it was observed that the mixture contained hormones with higher concentrations (about two folds) than that present in the single extract of *Sargassum* or *Ulva*. Table 4 indicated that the extract of *Sargassum* or *Ulva*and their mixture contained glycinebetaine with different concentrations, but the concentration in case of mixture was higher than that present in the individual extract of *Sargassum* or *Ulva*.

Discussion

Drought stress induces several physiological, biochemical and molecular responses in several crop plants, which would help them to adapt to such limiting environmental conditions (Bajaj *et al.*, 1999; Arora *et al.*, 2002).

The data presented herein showed that drought treatments caused significant decrease in the measured growth criteria of wheat as fresh and dry masses, root depth, shoot height, and leaf area during the vegetative stage.These results were in accordance with those of Chartzoulakis*et al.*, (2002);Abedi and Pakniyat(2010) and Fleury*et al.*(2010) for various plant species.Such decline in shoot and root lengths in response to drought might be due to either decrease in cell elongation, cell turgor, cell volume and eventually cell growth (Banon*et al.,* 2006), and/or due to blocking up of xylem and phloem vessels thus hindering any translocation through (Mohamed and Akladious, 2014).

Photosynthesis is one of the most drought-sensitive plant processes; it is harmfully affected by drought stress (Pan *et al.*, 2012). The present results showed that drought treatments reduced the content of chlorophylls *a* and *b*, compared with the control. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Ashraf and Harris,2013).Decreases in photosynthetic pigments were due to instability of protein complexes and destruction of chlorophyll by increased activity of chlorophyll degrading enzymes and chlorophyllase under stress condition (Sayyari *et al.*, 2013). Chlorophyll *a*/*b* ratio was increased in drought-treated wheat seedlings indicating a more negative effect of drought 1 on chl*b* (the main chl in PSII) than on chl *a*. In addition, drought treatments led to a decrease in carotenoid content compared with the control .Generally, many studies have reported drought-induced reductions in the levels of photosynthetic pigments ((Liu *et al.*, 2006; Zlatev, 2009).As a consequence, it was demonstrated from this study that exposure of *Triticumaestivum*to drought stress resulted in a decrease in the photosynthetic activity and this was in harmony with the results of Huseynova(2012)on wheat.

The recorded reduction in photosynthesis, might be arises by a decrease in leaf expansion, impaired

photosynthetic machinery, premature leaf senescence and associated reduction in food production (Wahid and Rasul,2005). However, stomatal closure was generally accepted to be themain determinant for decreased photosynthesis under mild to moderate drought (Cornic and Massacci, 1996; Yokota *et al.*,2002; Flexas*et al.*, 2004). It also reported that drought inhibits the photochemical activities and decreases the activities of the enzymes of Calvin Cycle in photosynthesis (Monakhova and Chernyadev, 2002), decreased leaf area which is considered as one of the most important plant organs due to their role in capturing light and achieving photosynthesis (Xu*et al.*, 2009).

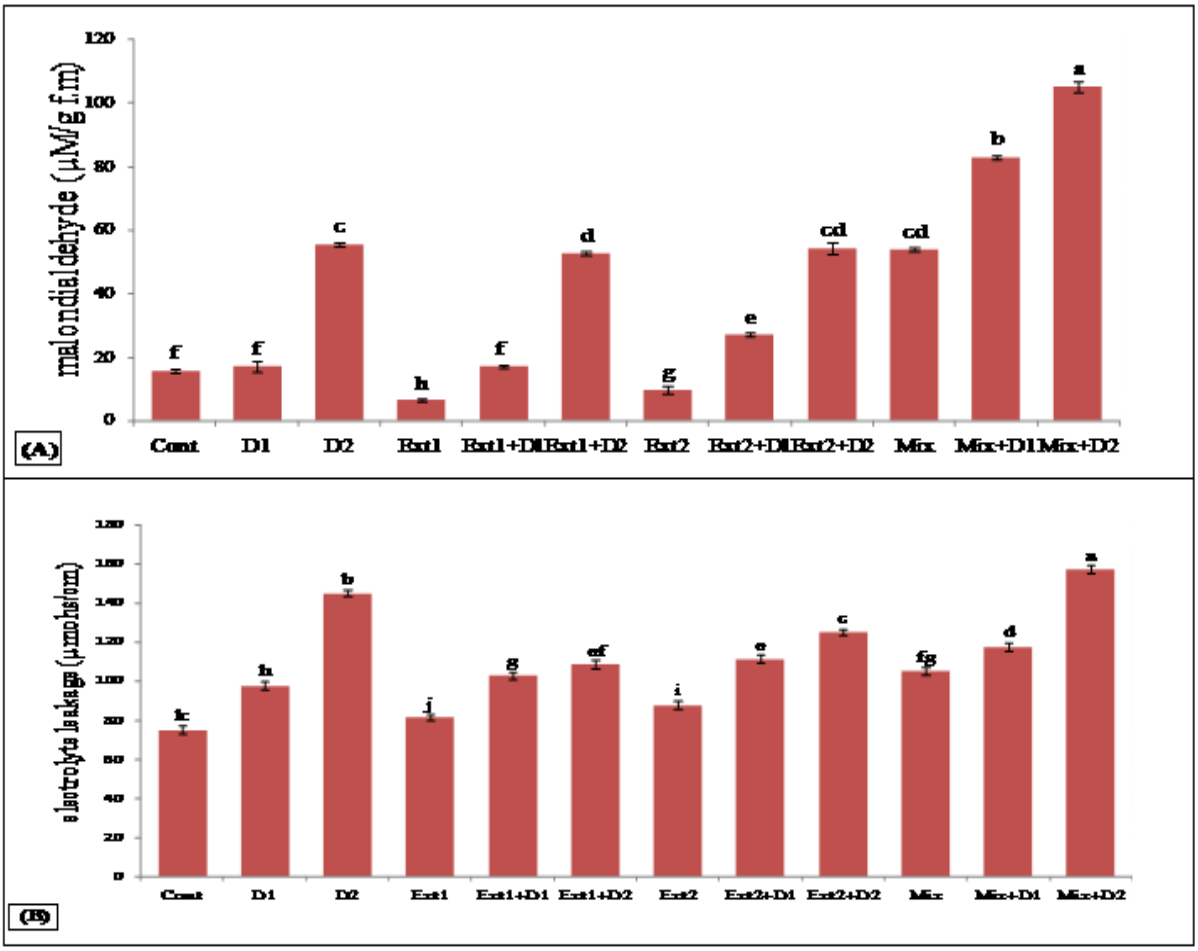

Fig. 1. Effect of drought stress on malondialdehyde (MDA) content (A) ,and electrolyte leakage (B), of 30-day-old *Triticumaestivum*(L.) grown in clay sandy soil (2:1w/w) and irrigated with 60% of water field capacity as a control (cont.), with 40% of water field capacity as drought 1 (D1), with 20% of water field capacity as drought 2 (D2)) after soaking the grains for 3 hours in 1.5% *Sargassum*extract (Ext.1), 1% *Ulva* extract (Ext.2) and 1:1 mixture of them (Ext.1+Ext.2). (Different letters indicate significancy ,similar letters indicate non significancy).

Drought lead to excessive production of ROS causing progressive oxidative damage and ultimately cell death(Sharma *et al.*, 2012).The effect of drought on growth and photosynthesis reported above in wheat during the vegetative stage could be due to the oxidative stress caused by drought treatments.The results presented here showed that drought treatments led to an increase in malondialdehyde (MDA) content (lipid peroxidation product)and membrane leakage in the drought treated wheat leaves during the vegetative stage (Fig.1A&1B) which might be attributed to peroxidation of membrane lipids that could be monitored as increased MDA content. In accordance with these findings, the involvement of ROS in harmful effects including membrane lipid peroxidation in plants treated with

drought was detected by many researchers (EL-Tayeb, 2006;Zlatev*et al.*,2006;Heyno*et al.*, 2011; Sharma *et al.*,2012;Chakraborty and Pradhan,2012). Electrolyte leakagefrom damaged tissues was commonly usedto assess cell membrane stability (Farooq and Azam, 2006; Sikder and Paul, 2010).Membrane damagemight be a result of initiated oxygen stress and the accumulation of reactive oxygen species leading to disturbances in membrane configuration (Hoekstra and Golovina, 1999**;** Foyer and Noctor, 2005)andoxidation of cell membrane fatty acids (Hong *et al.*, 2006**;** Dacosta and Hoang, 2007**).**Such damage can result from various mechanisms including oxidation and cross-linkage of protein thiols, inhibition of key membrane proteins as H+-ATPase, and changes to the composition and fluidity of membrane lipids (Farouk, 2011).

Moreover, drought increased antioxidant metabolism including antioxidant enzymes such as catalases, peroxidases, superoxide dismutases and non-enzymic antioxidants such as reduced glutathione and ascorbate that scavenge the ROS(Li *et al.*, 2012).Such changes were assumed to demonstrate plant tolerance to drought. As presented here, drought treatments increased peroxidase and catalase activities (Tabl 3). Similarly, ascorbic acid content was increased after drought treatments, compared with the control (Table 3). These findings led to the suggestion that the maintenance of a high antioxidant capacity might be essential for tolerance of plants to drought exposure (Miller*et al.*,2010; Xu *et al.*,2010).

Seaweed components such as macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxinsand abscisic acid (ABA)-like growth substances affect cellular metabolism in treated plants leading to enhancedgrowth and crop yield (Stirk*et al.*,2003; Ördög*et al.*,2004). Seaweed extracts are bioactive at low concentrations (diluted as 1:1000 or more)(Crouch and vanStaden,1992).

Therefore, the combined treatments of drought with priming by presoaking of wheat grains in the extract1 (*Sargassum*1.5%) or extract 2 (*Ulva* 1%) resulted in a significant recovery from the harmful effects of drought. The favorable effect of seaweed due to its endogenous auxins as well as other compounds in the extracts(El-Miniawy *et al.*,2014) and its content of a high cytokines activity, which could be responsible for the many effects such as plant growth, flowering and chemical constituents. These cytokines are active at very low concentrations and regulate a number of plant functions including cell division, protein, enzyme formation, leaf aging and senescence, shoot elongation, and fruit set (Abdel Aziz *et al.*,2011). Seaweeds extract also contain glycinebetaine (Ramya*et al.*,2010), which improved growth and yield in *Gossypiumhirsutum*(Gorham *et al.*, 2000)and mitigated the adverse effects of drought stress on wheat (Mahmood*et al.*,2009).Gibberellic acid is one of most important growth stimulating substance used for promoting cell elongation, cell division and thus to promote growth and development of many plant species (Mahmoody and Noori, 2014). So that, seaweeds extract could alleviated the drought stress and also enhance growth under normal conditions.

The present results indicated that treatment with priming by presoaking of wheat grains in*Sargassum* or *Ulva* ameliorated the harmful effect of drought stress on the photosynthetic pigments, where it significantly increased chl a, chl b, and carotenoids; whereas the chla/b ratio was reduced. This result was consistent with those of Khan *et al.* (2009).The improvement of photosynthetic activity in case of priming with sea weed extract may also duo to that these extracts were rich of glycinebetaine which delays the loss of photosynthetic activity by inhibiting chlorophyll degradation (Latique*et al.*, 2013).Also, extract of *Sargassum* or*Ulva* contain iron. It is a key component ofbiosynthesis of chlorophyll; it affects chlorophyll synthesis indirectly by affecting its precursor δaminolevulinicacid(ALA) (Kumawat*et al.*, 2006). In the photosynthetic cell, there is a requirement of iron in photosynthetic and respiratory electron transport, nitrate assimilation and nitrogen fixation (Paerl *et al.*, 2001).It is also required for iron containingcompounds in the electron transport chain, for the biosynthesis of pigments, and for the assembly

of the photosynthetic apparatus (Wang *et al.*, 2010). Also, carotenoids were increased with the priming with *Ulva* extract. These results may be due to the combination of the two metals, cadmium and lead, which increased the carotenoid content (Singh *et al.*, 2012). An increase in carotenoid content may be attributed also to the strategy of plants to overcome the metal induced oxidative stress (Kenneth *et al.*, 2000; Vajpayee *et al.*, 2001).

Interestingly, seaweed extract of *Sargassum* or *Ulva* pretreatment led to decreased levels of MDA contents in the drought stressed *Triticumaestivum*. These results were in agreement with those of Mansori*et al.* (2014) who reported that spraying of bean plants with seaweed extract could alleviate the inhibitory effect of drought stress.These positive anti-stress effects of seaweed extract may be related to the cytokinin activity of seaweed extract as reported by Zhang and Ervin (2004). Cytokinins mitigate stress-induced free radicals by direct scavenging and by preventing reactive oxygen species (ROS) formation by inhibiting xanthine oxidation (Fike *et al.*, 2001).Auxins diminished lipid peroxidation through the stimulation of non-enzymatic (ascorbate, glutathione) and enzymatic (SOD, CAT, APX) antioxidants tightly regulating ROS homeostasis(Niczyporuk and Bajguz, 2013).The present results indicated also thatseaweed extracts contain glycinebetaine (GB) with different concentrations. This GB is considered as one of the compatible soluteswhich contributes to stress tolerance by acting as osmoregulators, since their high solubility in water acts as a substitute for water molecules released from leaves; and in some cases, they also act as active oxygen scavengers or thermo stabilizers (Akashi *et al.*, 2001; Kaushik and Bhat, 2003;Mahmood*et al.*, 2009). The defensive role of glycinebetaine (GB) may either have a positive impact on enzymes and integrity of membranes or may act as an osmoprotectant that helps in protecting against environmental stress indirectly through the mechanism of signal transduction (Subbarao *et al.*, 2001; Chen and Murata, 2011); and protects proteins against the destabilizing effects of dehydration during abiotic stress (Ashraf and Foolad, 2007).

The present data indicated that both POX and CAT activities were significantly suppressed by pretreatments with seaweed extract which was in agreement with the results of Gharib *et al.* (2014) for Rosemary. The recorded increment of the enzyme activities of drought stressed seedlings after presoaking indifferent concentrations of algal extract could be attributedto the presence of anti-oxidative compounds such as ascorbicacid , proline, betaine and glutathione in seaweedextract (Deivanai*et al.*,2011; Tuna *et al.*, 2013; Hemida*et al.*,2014). Marschner(1995) suggests that mineral-nutrient status of plants plays a critical role in increasing plant resistance to environmental stress factors and it is known that, seaweed extract contain micronutrients with different concentration (Bhasker and Miyashita, 2005).One of micronutrients detected in seaweed extract is zinc. The positive effect of Zn on the antioxidant enzymes activity were reported by(Hajiboland and Beirmzadeh,2008;Tavallali*et al.*, 2010).It's protective effect has been reported to be due to its ability to inhibit NADPH oxidation and oxygen centered free radical generation (Abd El-Motty and Orabi,2014).This alleviation may be due to the bioactive compounds within the seaweed extracts that lead to activation of the plant phytohormone biosynthetic pathways which enhancing stress tolerance in plants that stabilize proteins and cell structures, maintain cell turgor, and scavenge reactive oxygen species (Calvo *et al.*,2014).

Although extract of *Sargassum* or *Ulva* result in lessening the effect of drought,but mixing of them result in antagonistic effect where the mixture inhibit pigment where sea weeds sometime become harmful due to the presence of salt which cause the slightly stress condition that's why stress protein formed and amino acid concentration also abnormally increased to overcome this stressthese results reported by(Akhtar*et al.*, 2014).

The present results showed also that priming of wheat grains with the mixture of *Sargassum* and *Ulva* could not alleviate the harmful effects of drought stress. This result may be due to both of the cadmium and

manganese contents of the mixture of the two seaweeds extract, where the recoded value of Cd was 0.414 ppb and that of the manganese content was 1.302 ppb. which can be considered as high concentration. Cadmium is considered a trace element; and is one of the heavy metals and it is a strong phytotoxic element, which inhibits vegetative plant growth and even causes plant death (Sandalio*et al.,* 2001). Common effects of Cd include; affecting water balance of plants by reducing root growth, limiting water uptake via a reduction in vessel size, and causing partial stomatal closure (Özyigit and Akinci, 2009). It also causes a decrease in tissue biomass, chlorosis, and effects on specific physiological (e.g., xylem transport) or biochemical (e.g., nitrogen fixation) processes (Kosma*et al.,* 2004).

Manganese (Mn) has been considered as one of the immediate toxic effect like other heavy metals in plants **(**Christoffers*et al.*, 2003). It reduces the growth of *Viciafaba* plant (Shashik and Roy, 2011). The recorded reduction in growth may be attributed to interference of Mn with photosynthesis as reported by Henriques (2003). Manganese (Mn) is reported to inhibit synthesis of chlorophyll by blocking a Fe-concerning process and its toxicity, in some species, starts with chlorosis of older leaves moving toward theyounger leaves with time (Nagajyoti*et al.*,2010). Concerning Cd, it was also reported that Cd reduces ATP and chlorophyll concentrations in many species, decreases oxygen production and that significantly reduces transpiration rates (Özyigit and Akinci, 2009).

However, the results showed that the mixture of the two types of seaweed extract contain high concentration of gibberellic acid (25.54mg/100ml) which may be considered higher than the effective concentration. In this regard, Abdel-Kader (2001)stated that the lowest concentration of gibberellins was more effective on alleviating the adverse effect of drought than the highest one.So that, it could be concluded that the mixture of two types of seaweed extracts resulte in antagonistic effect on plant growth.

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