IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 3 Ver. I (Mar. 2015), PP 36-44
www.iosrjournals.org

Salt stress tolerance and antioxidative mechanisms in wheat plants (Triticum durum L.) by seaweed extracts application

Halima Chernane[1], Salma Latique[2], Mounir Mansori[3], Mimoun El Kaoua[4]

[1; 2; 3; 4] *Laboratory of Biotechnology Valorisation and Protection of Agro-resources, FSTG, Cadi Ayyad University, Guéliz- Marrakech, 40000 Morocco.*

Abstract: *In this study we report how do use the Seaweed extract (SWE) obtained from Moroccan macroalgae species Ulva rigida affect salt stress tolerance in wheat plants. Some physiological and biochemical parameter such as, chlorophyll content, carotenoides, total phenolic content and antioxidant enzyme activity of wheat plants (Triticum durum L.) were studied under salt condition at various levels of NaCl (0, 2 and 4 g/l of NaCl). Our result showed that salt stress reduced plant growth and all parameters were affected. The application of SWE enhance vegetative growth in plant with all concentration applied under salt stress condition. Improved leaf pigment (chlorophyll and carotenoid) was observed with all SWE treatment in salt stressed plant compared to control. Also, total phenolic content was increased in wheat plants by SWE treatment under different growth condition and the optimum level was attained with 25% concentration. There was a significant enhancement in Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX) activities. These enzymatic activities increased considerably when plants were sprayed with 25% of Ulva rigida extract under salt stress. Our study showed an enhancement of vegetative growth and improvement of total phenolic, carotenoids content and antioxidant enzymatic activity by Ulva rigida extract in stressed wheat plant. It suggest that Seaweed extract SWE of Ulva rigida can improve salt stress tolerance and contributes to protection of wheat plant against oxidative deterioration*

Keywords: *Seaweed extracts, Wheat; Chlorophyll, Growth, polyphenols, carotenoides, Morocco, Ulva rigida, Antioxidant enzymatic activity: SOD, CAT, APX, Salt stress.*

Abbreviations: *SWE: Seaweed extracts; TPC: total phenolic content. FW: fresh weight; CC: carotenoid content; Chlorophyll a: Chl a; Chlorophyll b: Chl b.*

I. Introduction

Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil. A considerable amount of land in the world is affected by salinity which is increasing day by day. More than 45 million hectares (M ha) of irrigated land which account to 20% of total land have been damaged by salt worldwide and 1.5 M ha are taken out of production each year due to high salinity levels in [1] [2].High salt concentration in the soil or in the irrigation water can have a devastating effect on plant metabolism, disrupting cellular homeostasis and uncoupling major physiological and biochemical processes. [3] [4] [5]. Salt stress like many stress abiotic factor induces oxidative damage to plant cell catalyzed by reactive oxygen species (ROS), such as superoxide radical (O2•−), hydroxy radical (•OH), hydrogen peroxide (H_2O_2) and alkoxy radical (RO•) [6].These species are responsible for damage to membranes and other essential macromolecules such photosynthetic pigment, protein, DNA and lipids [7]. Plants possess several protective mechanisms to cope with ROS in which enzymes as well as reducing metabolites. The enzymatic antioxidant system can be divided in two categories. One reacts with ROS and keeps them at low levels (peroxidase, superoxide dismutase (SOD) and catalase), and the other regenerates the oxidised antioxidants (ascorbate peroxidase and glutathione reductase) [8].The reduction of H_2O_2 by ascorbate can occur directly or it can be catalysed by ascorbate peroxidase. Then, the oxidised from of ascorbate can be reduced enzymatically by deshydroascorbate reductase using glutathione as a substrate, which in turn is reduced by glutathione reductase in the presence of NADPH [9]. The SOD is a metalloenzyme that play a key role in protection against oxidative stress [10]. Catalase (CAT) is a tetramerichromoprotein that exists as multiple isoenzymes encoded by nuclear genes. Ascorbate peroxidase is the most important peroxidase in H_2O_2 detoxification [11]. Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and noenzymatic antioxidant metabolism. These later include β carotene, ascorbic acid, α tocopherol and secondary metabolites such as polyphenolic compounds.

Previous study have shown that exogenous protectants such as osmoregulators (proline, trehalose, etc.), plant hormone (gibberellic acids, salicylic acid, etc.), antioxidants (ascorbic acid, glutathione, tocopherol, etc.), trace elements (selenium, silicon, etc.) have been found effective in mitigating the salt induced damage in plant

[12] [13].These metabolites showed the capacity to enhance the plant's growth, yield as well as stress tolerance under salinity.The beneficial effect of seaweed extract application is as a result of many components that work synergistically at different concentrations, although the mode of action still remains unknown [14].The main objective of this paper is to gain insight into the mechanisms by which seaweeds extract application contributes to protection of plant against salt stress. In our study, we evaluate the impact of the foliar applications of seaweed extract obtained from a macroalgae species Ulva rigida on salt stress tolerance in wheat plants (Triticum durum L.).Some physiological and biochemical parameter such as, growth parameters, chlorophyll content, carotenoides, total phenolic content and antioxidant enzyme activity (SOD, CAT, APX) of wheat plants (Triticum durum L.) were studied under salt condition. Our goal was to gain insight into the mechanisms by which seaweeds extract application contributes to protection of plant against salt stress.

II. Materials and methods

2.1. Preparation of seaweed extract

Collection of seaweeds

Seaweeds Ulva rigida (Chlorophyceae) used in the present study were collected from coastal area of Sidi Bouzid near El jadida city (Morocco) in spring. Morphologically distinct thallus of algae were placed in polythene bags and transported to the laboratory. Samples were washed thoroughly using tap water to remove the salt.

Seaweeds treatment

Seaweeds were shade dried for four days, followed by oven dry for 12h at 60°C. Then the materials were hand crushed and made as coarse powder, was added with distilled water in a ratio of 1:20 (w/v) and boiled at 121°C for 30 minutes. The hot extracts were filtered through a double-layered cheese cloth and allowed to cool at room temperature [15]. The resulting supernatant was taken as 100% seaweed liquid extracts. Seaweed liquid extracts were prepared with different doses: control (0%); 0.2% ;0.5%; 25% and 50%.

2.2. Plant material preparation and treatments

The crop plant tested for the present study was Triticum durum L. cv Karim, an important plant cultivated throughout Moroccan regions.The seeds were collected from National Company of seeds SONACOS. Seeds were surface sterilized with non ionic detergent 6% for 3 minutes with frequent shaking and thoroughly washed many times with deionized water to remove the detergent solution. The germination was carried on impregnated filter paper. After 6 days, the seedlings were transplanted in plastic pot of 30 cm×30 cm containing peat.

Experiments were carried out in green house lasted 90 days. Salt stress was induced by sodium chloride (NaCl). Plant under study were treated with salt at various concentrations (0,2 and 4 g/l of NaCl) corresponding respectively to the growth conditions: WS, SS1 and SS2. Three lots of plants treated with 0,2 and 4 g/l of NaCl and were considered as control as they don't receive extract of Ulva rigida. Another's lots of plants treated with 0,2 and 4 g/l of NaCl and for each concentration we sprayed with seaweed extracts (SWE) of Ulva rigida separately at for different doses (C1: 0,2% and C2: 0,5%, C3: 25% and C4: 50%).
All the experiments were conducted in triplicates.

2.3. Growth measurement

Plant growth was measured on the basis of two parameters: size of the plant (in cm) and dry weight of plants. The samples of plants (aerial part) were dried in an oven at 80°C until constant dry weight (DW) was obtained. DW was expressed in g/plant.

2.4. Biochemical and physiological parameters determination

Pigment assay

The pigments extracted from 300 mg of leaf fragments with 6 ml of acetone/ distilled water (80:10 v/v) in the darkness condition. The chlorophyll content was determined in three independent aliquots. The content of chlorophyll a and chlorophyll b were estimated using the method suggested by Arnon (1949)**[16]**.
Chlorophyll a (mg/L) =12,7×DO to 664nm – 2,69× DO to 647nm
Chlorophyll b (mg/L) =22,9×DO to 647nm – 4,68× DO to 664nm
The chlorophyll was expressed on a fresh weight basis (mg. g^{-1}FW)
The Carotenoïd content was determined using the formula:
[Cc] = [5*DO 460- (3,19* Ca + 130,3* Cb)/200] Vt/1000* FW
Vt: Volume of total liquid extract in litre, FW: fresh weight in gramme.

Estimation of total phenolic content (TPC)

0,5 g leaves were homogenized with 1mL 95% methanol at 4°C The homogenate was centrifuged at 19,000g for 20 min and supernatant was used for phenol content that estimated by the method of Taga MS and al. 1984 **[17]**. 100 µL aliquot of sample was mixed with 2 mL of 2% Na_2CO_3 and allowed to stand for 2 minutes at room temperature. After incubation, 100µL of 50% Folin-Ciocalteau's phenol reagent was added and then reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using UV-visible spectrophotometer (6305 - UV-visible spectrophotometer (190-1000 nm) – JENWAY).Gallic acid was used as a standard and a calibration curve was prepared with a range of concentration from 10 to 200 mg/L. Phenolic content was expressed as gallic acid equivalent.

Enzyme extractions and assays

Crude enzyme extract was prepared for assay of antioxidant enzymes by the method of Tejera and al., 2004 **[18]**, the extract was prepared by crushing 100 mg of fresh material with 2ml of potassium phosphate buffer (0.1M, pH 6) and 5% of insoluble PVP, centrifuged at 12000g for 30 min. the supernatant was used for estimation of enzyme activity. The Superoxide dismutase (SOD) activity of was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetra-zolium (NBT) following the method of Beyer and Fridovich (1987) **[19]**. One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of NBT. The enzyme activities were expressed as units mg-1 protein.

The Catalase (CAT) activity was measured according to Chandlee and Scandalios (1984) **[20]** modified by Abdul Jaleel and al. (2007) **[21]**. The assay mixture contained: 2,6ml of 50 mM potassium phosphate buffer (pH 7.0); 0.4ml of 15mM H_2O_2 and 0.04ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in Units mg^{-1} protein (U=1mM of H_2O_2 reduction min^{-1} mg^{-1} protein). The Ascorbate peroxidase (APX) activity was determined according to Asada and Takahashi (1987) **[22]**. The reaction mixture (1ml) contained 50mM potassium phosphate buffer (pH 7.0), 0.5mM ascorbic acid, 0.1mM H2O2 and 200µl of enzyme extract. The absorbance was read as decrease at 290 nm against the blank. The enzyme activity was expressed in Units mg−1 protein (U= change in 0.1 absorbance min^{-1} mg^{-1} protein). Total soluble protein concentration was measured by dye binding assay as described by Bradford (1976) **[23]**.

2.5. Statistical analysis

All data were analyzed using the SPSS statistical package (version20.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA, followed by the Student Newman Keuils post hoc test, was used to compare differences in the data (P<0.05). Values are expressed as the mean ± SD.

III. Results and discussion

3.1.Growth parameters

Salt stress reduced size and dry weight of wheat plants compared to the control (Table1). The shoot length was reduced by 11,59% and 21,7% under SS1 and SS2 compared to control, moreover the dry weight of plant was decreased by 51,8% and 60% under SS1 and SS2 compared to control.The application of seaweeds extract (SWE) enhanced significantly these growth parameters in controls plants (WS) and in stressed plants. The maximum of vegetative growth was recorded with 25% of Ulva rigida extract. Thus, the shoot length and dry weight of plants stressed by SS2 were reduced only by 10% and 53,15% respectively compared to control plant.

Table 1. Effect of salt stress on shoot length and dry weight of wheat plants (Ttriticum durum L.) sprayed or not with Ulva rigida extract (U). WS: plant without stress, SS1: plant treated with salt stress at 2g/l of NaCl; SS2: plant treated with salt stress at 4g/l of NaCl. Results are means ± S.D (n = 3) Different letters in a single line show statistically significant differences for P< 0.05

			Growth conditions		
			WS	SS1	SS2
Shoot length (cm)	Control	0%	$69{,}33^{e}$ ±1,05	$61{,}23^{g}$±5,72	$54{,}13^{i}$±2,83
	Ulva rigida	0,2%	$69{,}36^{e}$±1,15	$68{,}55^{e}$±6,55	$61{,}47^{g}$±3,77
		0,5%	$72{,}48^{c}$±2,05	$69{,}85^{d}$±9,30	66,52f±6,76
		25%	$76{,}7^{a}$ ±1,35	$74{,}60^{b}$±8,21	$69{,}85^{d}$±5,20
		50%	$74{,}48^{b}$±2,05	67^{e} ±6,55	60^{h} ±3,77
Dry weight (g.plant-1)	Control	0%	$1{,}60^{b}$ ±0,21	0,77 g ±0,2	$0{,}64^{g}$ ±0,04
	Ulva rigida	0,2%	$1{,}70^{b}$ ±0,05	$0{,}97^{f}$ ±0,06	$0{,}83^{f}$ ±0,11
		0,5%	$1{,}87^{b}$ ±0,08	$1{,}31^{d}$ ±0,09	$0{,}90^{f}$ ±0,04
		25%	$2{,}22^{a}$ ±0,06	$1{,}53^{e}$ ±0,05	$1{,}04^{h}$ ±0,01
		50%	$1{,}67^{b}$ ±0,08	$0{,}91^{f}$ ±0,09	$0{,}80^{g}$ ±0,04

Salt stress induces a significant decrease in dry weights and shoot length .Reduction of plant growth under saline conditions is common phenomenon **[24]** but such reduction occurs differently in different plant organs.Salinity can affect plant growth because the high concentration of salts in the soil solution interferes with balanced absorption of essential nutritional ions by plants **[25]**. An increase of vegetative growth was observed when seaweed extract were applied. This benefit effect of algae extract may be due to the presence of some growth promoting substances present in seaweed extract like auxins **[26]**. In addition, the growth enhancing potential of the seaweed extract might be attributed to the presence of macro and micronutrients **[27]**. Alternately the beneficial antistress effects of seaweed extracts may be related to cytokinin activity **[28]**.

3.2. Leaf pigment

The chlorophyll **a** was reduced by 28,9% and 30,7% under salt condition SS1 and SS2 respectively compared to the control plant (**Fig. 1 A**). While the chlorophyll **b** was reduced by 28,80% and 38,98% under the same conditions (**Fig. 1 B**). The treatment of stressed plants with seaweed extract reduces significantly the stress effect on pigment content. The all concentration of SWE improves the pigment content of stressed and unstressed plant. Maximum values of chlorophyll **a** were found in unstressed plants that sprayed with 0,5 % and of 25% Ulva rigida extract (234×10^{-2} mg g^{-1} FW and 236×10^{-2} mg g^{-1} FW respectively). The highest values of chlorophyll **b** were recorded with 25% treatment (90×10^{-2} mg g^{-1} FW).

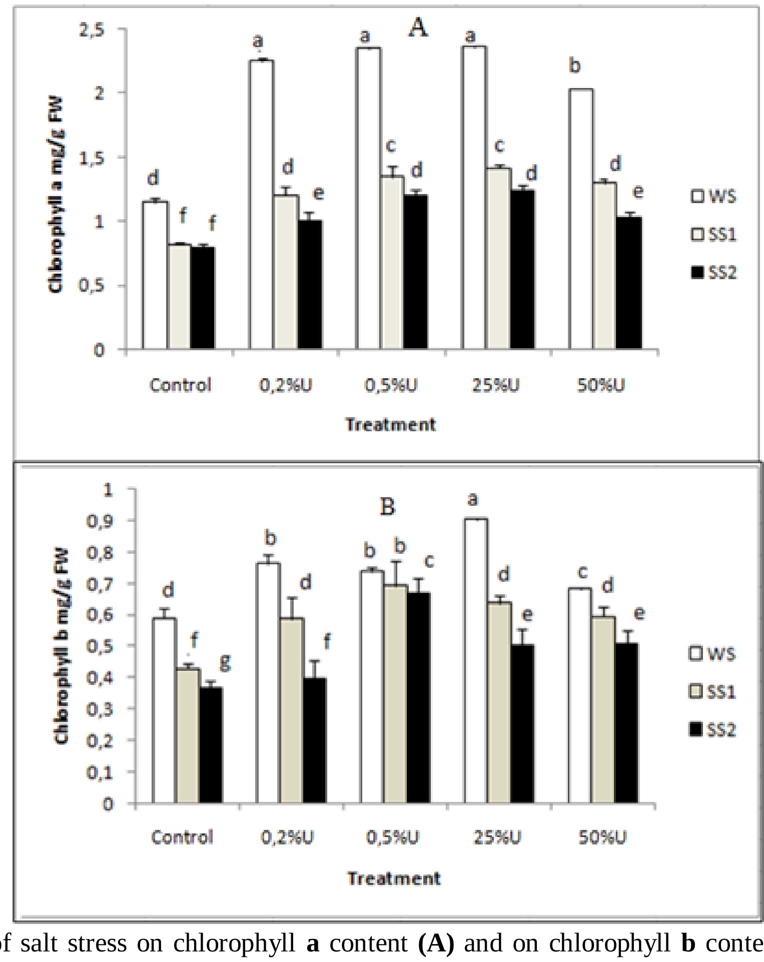

Figure 1. Effect of salt stress on chlorophyll **a** content **(A)** and on chlorophyll **b** content **(B)** in wheat plant leaves sprayed or not with Ulva rigida extract (U); WS: plant without stress, SS1: plant treated with salt stress at 2g/l of NaCl; SS2: plant treated with salt stress at 4g/l of NaCl. Results are means ± S.D (n = 3) Different letters in a single line show statistically significant differences for P<0.05.

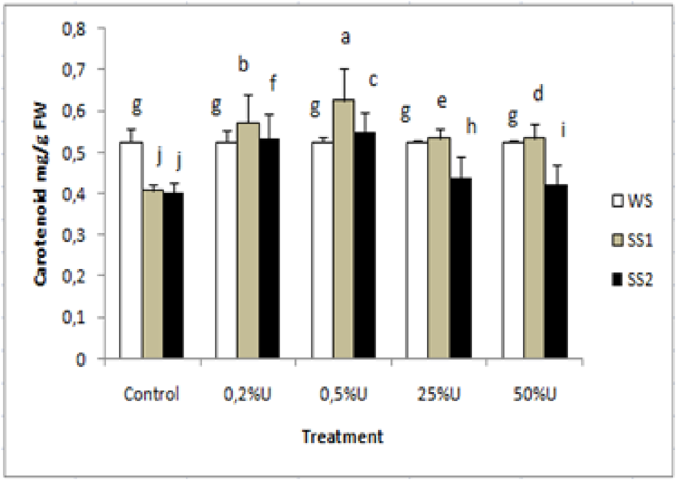

Figure 2. Effect of salt stress on carotenoid content in wheat plant leaves sprayed or not with Ulva rigida extract (U); WS: plant without stress, SS1: plant treated with salt stress at 2g/l of NaCl; SS2: plant treated with salt stress at 4g/l of NaCl. Results are means ± S.D (n = 3) Different letters in a single line show statistically significant differences for P< 0.05.

As shown in figure 2, the carotenoid content (Cc) decreased significantly in wheat plants cultivated under salt conditions (SS1 and SS2). However, treating plants with all concentration Ulva rigida extract can reduces the effect of salt stress and increases the carotenoid content at different growth condition. The maximum of Cc was observed with the application of 0,5% of Ulva rigida extract under salt condition (SS1) (0,64mg/gFW) compared to the control plant (0,4mg/gFW).

Chlorophyll content under control condition was higher in compared to salt stressed treatment. This pigment decreased when plants were subjected to salt stress. Oxidative stress and signs of senescence include loss of chlorophyll and protein and decline in membrane permeability, all of which lead to reduction in photosynthetic capacity. The loss of chlorophyll accompanied by inactivation of photochemical reactions was recorded in plant exposed to salt stress **[27]**. Salt stress leads to an increase in free radicals in chloroplasts and destruction of photosynthetic pigment by ROS **[7]**. Carotenoid are important in harvesting light energy during photosynthesis. Therefore, the decrease in carotenoids , mainly after extend exposure to salt stress , would hinder photosynthesis and, in turn plant growth and development.

The effect of salt stress was notably reduced by the foliar application of SWE and was consistent with those obtained when seaweed extract of Ulva rigida were applied to the fenugreek **[28]**. It was reported **[29]** that the seaweed extract applied as foliar spray enhanced the leaf chlorophyll level in plants by inducing its synthesis.

3.3. Total phenolic content

The salt stress increased significantly the phenolic compounds. In fact, total phenolic content (TPC) was two folds higher in plant submitted to SS1and SS2 respectively compared to the controls plants **(Fig 3**). Treatment with SWE at all concentration enhances significantly the Total phenolic content in wheat plants cultivated under different growth conditions (WS, SS1 and SS2). The highest levels of total phenolic were detected in plants treated with 0,5% and 25% of Ulva rigida extract (34,60 µg GAE mg^{-1} DW and 31,20 µg GAE mg^{-1} DW respectively under SS1 and SS2).

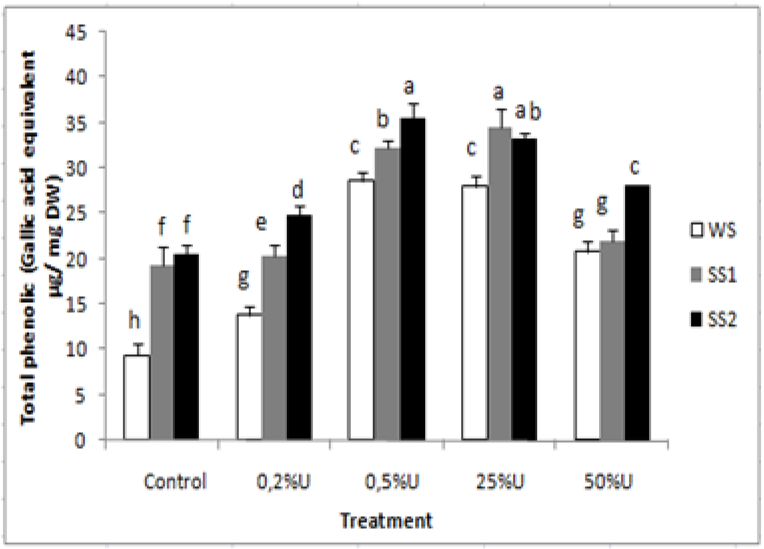

Figure 3. Effect of salt stress on Total phenolic content in wheat plant leaves sprayed or not with Ulva rigida extract (U); WS: plant without stress, SS1: plant treated with salt stress at 2g/l of NaCl; SS2: plant treated with salt stress at 4g/l of NaCl. Results are means ± S.D (n = 3) Different letters in a single line show statistically significant differences for P< 0.05.

Polyphenols represent a large family of plant secondary metabolites. The synthesis of these compounds is induced in response to biotic and abiotic stimuli such and may act as antioxidants to protect the plant against oxidative stress **[30]**. Increase in total phenolic content by application of SWE in wheat plans can be explained by enzyme activation. It was reported that treatment with SWE caused significantly enhanced activities of phenylalanine ammonia lyase (PAL) the most important enzyme responsible for biosynthesis of polyphenols **[31]**.

3.4. SOD activity

The effects of salt stress on the protective enzymes SOD in wheat leaves are shown in Figure 4. The enzymatic activity of SOD declined drastically with salt stress. When the plants stressed were sprayed with different concentrations of seaweed extract, the SOD activity increased considerably. Under salt stress (SS1) this enzymatic activity increased from 3,58 U mg^{-1}protein min^{-1} in control plants to 7, 60 U mg-1protein min-1 in plant sprayed with Ulva rigida extract (25%).While, under salt stress (SS2), the maximum SOD activity was recorded with 0,5% of treatment (6,70 mg-1protein min-1).

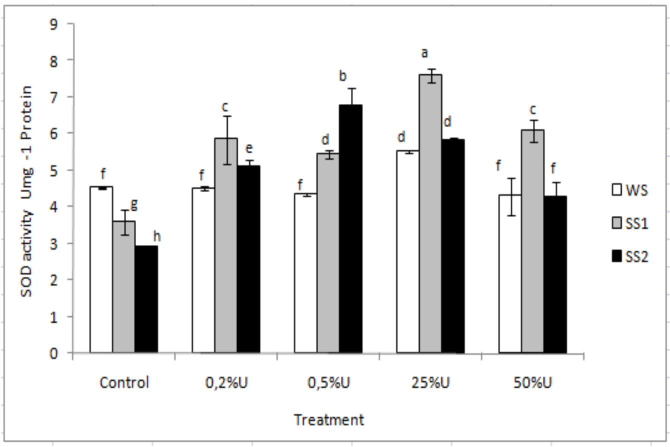

Figure 4. Effect of saline solution on SOD activity in plant leaves of Triticum durum sprayed or not with Ulva rigida extract (U); SW: Without Stress, SS1: Salt Solution at 2 g/l, SS2: Salt Solution at 4g/l. Results are means ± S.D (n = 3) Different letters in a single line show statistically significant differences for P< 0.05.

ROS is produced by the photosynthetic electron transport chain in chloroplasts **[32]**. It was reported that SOD enhance salt stress tolerance to plants **[33]**. When SOD activity was high, ROS especially Superoxide radical scavenging was done properly and thus, damage to membranes and oxidative stress decreases, leading to the increase to oxidative stress.

The SWE applied as foliar spray increased the antioxidant potential in stressed plants when compared to untreated control plant. Our result can be explicated by the effect of seaweed extract on the reduction of the cells damage caused by ROS **[34]**.. The application of seaweed extract increase of tolerance to oxidative stress using increased the activity of the antioxidant enzyme superoxide dismutase (SOD), which scavenges superoxide **[35]**.

3.5. APX activity in plants

APX activity decreased with salt stress conditions compared to control (30.06 U mg^{-1} protein min^{-1}) (Figure 5). This enzymatic activity increased when the plants were treated with Ulva rigida extract . The beneficial effect of SWE varied with extract concentrations and the maximum was attained with 25% treatments. Thus, under salt stress (SS1) APX activity increased from 36,40 U mg^{-1}protein min^{-1} in control plants to 56.17 U mg-1protein min-1 in plant sprayed with Ulva rigida extract (25%).

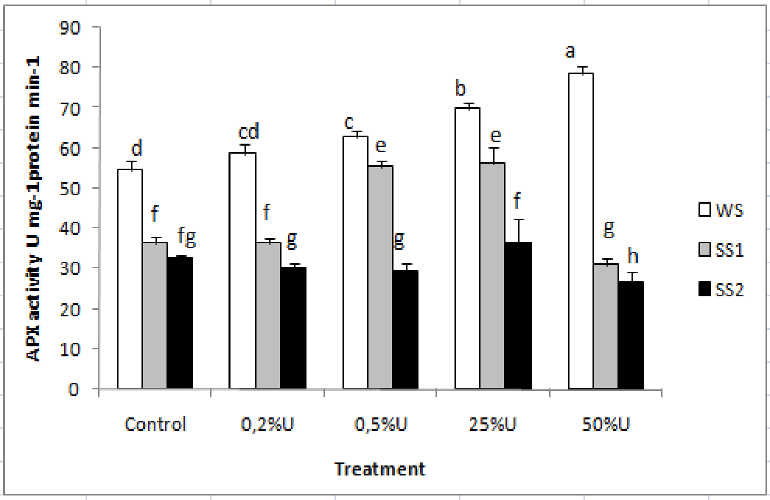

Figure 5. Effect of saline solution on APX activity in plant leaves of Triticum durum sprayed or not with Ulva rigida extract (U); SW: Without Stress, SS1: Salt Solution at 2 g/l, SS2: Salt Solution at 4g/l. Results are means ± S.D (n = 3) Different letters in a single line show statistically significant differences for P< 0.05.

APX is the member of the ascorbic acid–glutathione cycle, and plays a crucial role in eliminating poisonous H_2O_2 from plant cells **[36]**. Ascorbate peroxidase (APX) scavenges peroxidase by converting ascorbic acid to dehydroascorbate (Ozkur et al., 2009) **[37]**. The application of seaweeds extracts enhance the ascorbate peroxidase activity and play a protective role against ROS that are formed during salt stress. This leads to decrease in oxidative stress.

3.6. CAT activity

As shown in Figure 6, CAT activity was constant at all salt levels. But, increased significantly and particularly with application of Ulva rigida 25% under different growth conditions (WS, SS1 and SS2).Thus, under salt condition SS2, CAT activity increased in plant treated with SWE to 18.60 U mg^{-1} protein min^{-1} compared to control (13.55 U mg^{-1} protein min^{-1}).

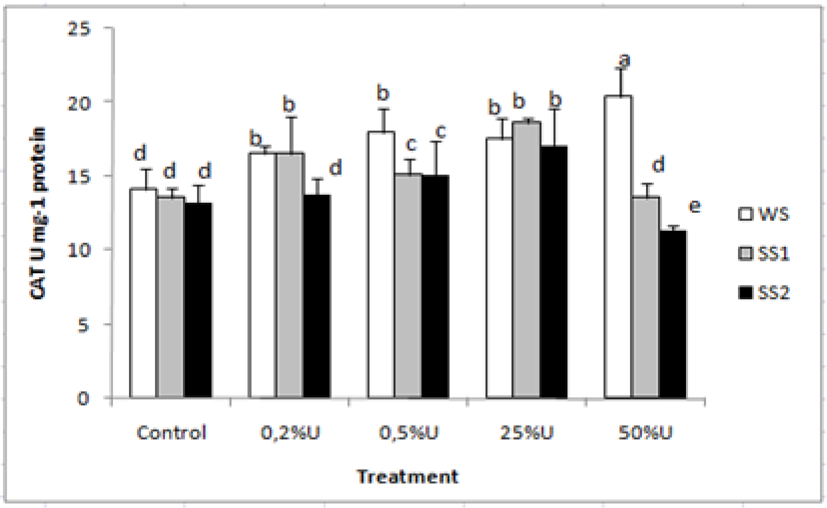

Figure 6. Effect of saline solution on CAT activity in plant leaves of Triticum durum sprayed or not with Ulva rigida extract (U); SW: Without Stress, SS1: Salt Solution at 2 g/l, SS2: Salt Solution at 4g/l. Results are means ± S.D (n = 3) Different letters in a single line show statistically significant differences for P< 0.05.

CAT is another important antioxidant enzyme that H_2O_2 converts in the peroxysome **[38]**. Higher activity of CAT decrease H_2O_2 level in cell and increase the stability of membranes and CO2 fixation because several enzymes of the calvin cycle within chloroplasts are extremely sensitive to H_2O_2 **[39]**.

CAT activity mostly increased in salt stressed plants, but in our study we can not obtained the significant change in this enzymatic activity under salt condition and demonstrate the importance SWE application in the tolerance to salt stress. In plats cells, the cooperation from antioxidant enzymes is essential for the scavenging ROS. The combined action of CAT and SOD converts the toxic $O_2•^-$, H_2O_2 to water and molecular oxygen, averting the cellular damage under unfavorable conditions like salt stress **[40]**.

IV. Conclusion

This study indicated that seaweed extract of Ulva rigida applied to wheat plants at different concentrations under salt conditions improve vegetative growth and increased leaf pigment. Also, The SWE enhance the tolerance of salt stress by activation of antioxidant enzymatic system of SOD, CAT and APX and by increasing the total phenolic content which contributes to protection of plants against oxidative damage to plant cell catalyzed by reactive oxygen species (ROS). The some correlation was found between resistance to salt stress and enhancement of antioxidant system of wheat plant that was activated by SWE application.

However, the mechanism(s) of actions of seaweed extract elicited physiological responses and stress alleviation is largely unknown, it is plausible that the seaweed components exhibit synergistic activity. It would be beneficial to carry out more research including study of antioxidant and substances active in seaweeds extract that stimulate the enzymatic system and lead to salt stress tolerance in Wheat plant.

References

[1]. MG. Pitman and A Läuchli.. Global impact of salinity and agricultural ecosystems. In: Salinity: Environment – Plants – Molecules, A. Läuchli and U. Lüttge (Eds.). Kluwer Academic Publishers,Dordrecht. 2002, 3–20.
[2]. R. Munns, and M Tester. (). Mechanisms of salinity tolerance. Annual Review of Plant Biology. 59, 2008, 651-681.
[3]. G. Tanou, A. Molassiotis and G. Diamantidis. Induction of reactive oxygen species and necrotic death-like destruction in strawberry leaves by salinity. Environ Exp Bot. 65, 2009, (2–3), 270–281
[4]. P. Ahmad, C.A. Jaleel, M.A. Salem, G. Nabi and S. Sharma (2010a). Roles of Enzymatic and non-enzymatic antioxidants in plants during abiotic stress. Crit Rev Biotechnol . 30(3), 2010, 161–175
[5]. P. Ahmad and S. Umar.Oxidative Stress: Role of Antioxidants in Plants, Studium Press, New Delhi, India. 2011
[6]. R. Munns. Comparative phtsiology of salt and water stress. Plant cell Environ. 25, 2002, 239-250.
[7]. A.S. Fahmy, T.M. Mohamed, S.A Mohamed and M.M Saker. Effect of salt stress on antioxidant activities in cell suspension cultures of cantaloupe (Cucumis melo). Egyptian J Physiol Sci. 22, 1998, 315-326.
[8]. Smirnoff. The role of active oxygen in the response of plants to water deficit and dessication. New Phytol.125,1993, 27-58.
[9]. C.H. Foyer, P. Descourvieresand K.J. Kunert. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. Plant Cell Environ. 17,1994, 507–523.
[10]. D.W. Christianson. Structural chemistry and biology of Manganese metalloenzymes. Prog Biophys Molec Biol. 67,1997, 217–252.
[11]. G. Noctor and C.H. Foyer. Ascorbate and glutathione: Keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol. 49,1998, 249–279.
[12]. F. Azzedine, H. Gherroucha andM. Baka. Improvement of salt tolerance in durum wheat by ascorbic acid application. J Stress Physiol Biochem 7, 2011, 27–37
[13]. M. Yusuf, Q. Fariduddin, P. Varshney and A. Ahmad. Salicylic acid minimizes nickel and/or salinity-induced toxicity in Indian mustard (Brassica juncea) through an improved antioxidant system. Environ Sci Pollut Res .19, 2012, 8–18.

[14]. F. Fornes and M. Sánchez-Perales, J.L. Guadiola .Effect of a seaweed extract on the productivity of 'de Nules' Clementine mandarin and navelina orange.Botanica Marina 45, 2002, 486–489.

[15]. K. Rama rao. Preparation of liquid seaweed fertilizer from sargassum, seaweed Research and utilization association Workshop on algal products and seminar on phaeophyceae in India, Madras. 1990.

[16]. D.I. Arnon. Copper enzymes in isolated chloroplasts.Polyphenoloxidase in Beta Vulgaris. Plant Physiol. 24, 1979,1-15.

[17]. M.S Taga . Chia seeds as a source of natural lipid antioxidants. J Am Oil ChemSoc; 61,1984, 928-931.

[18]. G.NA. Tejera, M. Olivera, C. Iribarne, C. Lluch. Partial purification and characterization of a non-specific acid phosphatase in leaves and root nodules of Phaseolus vulgaris. Plant Physiol Biochem. 42, 2004, 58-5591.

[19]. W.F. Beyer, I. Fridovich. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Analbiochem 161: 559-566.

[20]. J.M. Chandlee and J.G. Scandalios. Analysis of variants affecting the catalase development program in maize scutellum. Theor Appl Gen. 69, 1987,71–77

[21]. C. Abdul Jaleel, R. Gopi, P. Manivannan and R. Panneerselvam. Responses of antioxidant defense system of Catharanthusroseus (L.) G. Don. Topaclobutrazol treatment under salinity. Acta. Physiol. Plant. 29, 2007, 205-209.

[22]. K. Asada and M. Takahashi. Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond B, Arntzen CJ (ed). Photoinhibition. Elsevier, Amsterdam, 1987, 227–287.

[23]. M.M. Bradford. A rapid and sensitive method for quantification of microgram quantities of protein utilising the principale of protein-dye binding. Analytical Biochemistry. 72, 1976, 248-254.

[24]. M. Ashraf and P.J.C. Harris. Potential biochemical indicators of salinity tolerance in plants. Plant Science. 166, 2004, 13–16.

[25]. M. Tester and R.J. Davenport. Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. (Lond.). 91, 2003, 503–527.

[26]. Moony and Van Staden. Algae andcytokinins. J. Plant Physiol. 123, 1986, 1-2.

[27]. P.k . Sharma and D.O. hall. Changes in carotenoid composition and photosynthesis in Sorghum under high light and salt stress. J. Plant .Physiol. 140, 1992, 661-666.

[28]. N.M. Prise and AB Sabale. Effect of seaweed concentrates on the growth and biochemical constituents of Trigonella foenum-graecum L. Journal of Phytology.2, 2010, 50–56.

[29]. W. Khan, P. Usha, R. Sowmyalakshmi. Seaweed Extracts as Biostimulants of Plant Growth and Development. J Plant Growth Regul. 28, 2009, 386–399.

[30]. P.K. Sharma, D.O. hall. Changes in carotenoif composition and photosynthesis in Sorghum under high light and salt stress. J Plant Physiol. 140, 1992, 661-666.

[31]. C.M. André, R. Schafleitner, S. Legay, I. Lefèvre, C.A. Alvarado, A.G. Nomberto, L. Hoffmann, J.F. Hausman and Y. Larondelle, D. Evers. Gene expression changes related to the production of phenolic compounds in potato tubers grown under drought stress. Phytochemistry 70, 2009, 1107–1116.

[32]. R. Mittler. Oxidative stress, antioxidants and stress tolerance, Trends in Plant Science. 7, 2002, 405-410.

[33]. B.D. McKersie, S.R. Bowley, E. Harjanto and O. Leprince. Water deficit tolerance and field performance of transgenic alfafa overexpressing superoxide dismutase. Plant Physiol. 111, 1996, 1177–81.

[34]. N. Candan and L. Tarhan. The correlation between antioxidant enzyme activities and lipid peroxidation levels in menthe pulegium organs frown in $Ca^{2+,}$ $Mg^{2+,}$ Cu^{2+} and Mn^{2+} stress conditions. Plant Science. 163, 2003, 769-779.

[35]. J.H. Fike, V.G. Allen, R.E Schmidt, X. Zhang, J.P. Fontenot, C.P. Bagley, R.L. Ivy, R.R. Evans, R.W. Coelho, D.B. Wester. Tasco Forage: I. Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. J Anim Sci. 79, 2001, 1011–1021

[36]. V. Rai, P. Vajpayee, S.N. Singh, S. Mehrotra. Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of Ocimum tenuiflorum L. Plant Science. 167, 2004, 1159–1169.

[37]. O. Ozkur, F. Ozdemir, M. Bor and I. Turkan.. Physiochemical and Antioxidant Responses of the Perennial Xerophyte Capparis ovata Desf. to Drought. Environ. Exper. Bot., 66, 2009, 487–492.

[38]. I. Fridovich. Superoxide dismutases: Anadaptation to a paramagnetic gas, the journal of biological Chemistry, 264, 1989, 7761-7764.

[39]. J. Yamazaki, A. Ohashi, Y. Hashimoto, E. Negoshi, S. Kumagai, T. Kubo, T. Oikawa, E. maruta, Y. Kamimura. Effetcs of high light and low temperature during harsh winter on needle photodamage of abies mariesii growing at the forest limit on Mt. norikura in central japan, Plant science. 165, 2003, 257-264.

[40]. C. Abdul Jaleel, R. Gopi, P. Manivannan and R. Panneerselvam.. Responses of antioxidant defense system of Catharanthusroseus (L.) G. Don. Topaclobutrazol treatment under salinity. Acta. Physiol. Plant. 29, 2007, 205-209.