Plant Archives Volume 20 No. 2, 2020 pp. 8403-8411 e-ISSN:2581-6063 (online), ISSN:0972-5210

WATER SALINITY AND SUPPLEMENTAL NUTRIENTS ENHANCING BIOCHEMICAL CONTENTS AND ANTIOXIDANT ACTIVITY OF *SALICORNIA BIGELOVII* NATURALLY GROWN ON NORTHWEST COAST OF EGYPT

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Abstract

Halophytes became one of the most important candidates for future due to their high salt tolerance and bioactive compounds content as well as their medicinal raw materials, nutraceutical, functional and health properties. Thus, *Salicornia bigelovii* is a promising halophytic crop for seawater and saltwater irrigated agriculture in many countries. This study was conducted to investigate the response of *Salicornia bigelovii* to four different levels of well water and sea water (100%well water), (100% sea water), (50% sea water +50% well water) and (25% sea water + 75% well water) with EC 2.4, 51, 30 and 17dS m^{-1}, respectively combined with four different supplemental nutrients fertilization (NPK), (NPK+ Micronutrients), (Algae) and (NPK+ Micronutrients+ Algae) in addition to control treatment. The highest antioxidant activity and total soluble phenols content were recorded with control treatment (without irrigation) followed by irrigation with 50% sea water + 50% well water (30 dS m^{-1}) which resulted also in the maximum content of flavonoids. Total carbohydrates percentage was increased with 25 % sea water +75% well water (17 dS m^{-1}) and decreased with increasing salinity level up to 30 and 51 dS m^{-1}. Plants irrigated with 50% sea water +50% well water (30dS m^{-1}) and fertilized with NPK+ Micronutrients+ Algaerecorded the highest values of whole plant dry weight, nitrogen and protein percentages as well as total free amino acids content. On the other hand, the addition of 100% sea water (51dS m^{-1}) combined with NPK+ Micronutrients+ Algae recorded the maximum content of proline. Irrigation with 100% sea water (51dS m^{-1}) and addition of NPK only gave the highest values of antioxidant activity and total carbohydrates percentage. Moreover, the irrigation with 50% sea water + 50% well water (30dS m^{-1}) combined with NPK fertilizer resulted in the maximum values of total soluable phenols and flavnoids content. The high salt tolerance and supplemental nutrient fertilization increasedthe biochemical contents and antioxidant activity of *Salicornia bigelovii* naturally grown on Mariut site Northwest Coast of Egypt.

Key words: *Salicornia bigelovii,* seawater, well water, antioxidant activity, flavonoids, phenols and proline.

Introduction

Chenopodiaceae family contains high amounts of protein, sulfur and minerals (Norman *et al.,* 2013) which favor glass wort as an edible plant. *Salicornia*, developed in extremely saline environments, produces antioxidative metabolites, simple and complex sugars, quaternary amino acids derivatives, alcohols, sulfonium and tertiary amines. This plant is used for human consumption and animal feed (Doncato and Costa, 2018), beside that, its oilseeds are potential source for biodiesel and biofuel production (Bailis and Yu, 2012 and Abideen *et al.,* 2015). Halophytes are used for many commercial purposes due to their nutritional value as well as their medicinal raw materials, nutraceutical, functional and health properties (Fan *et al.,* 2013).

Plants have to activate metabolic defense mechanisms which regulate ion flux and provide scavenging systems that can reduce the level of toxic ions which lead to ionic imbalances and induce oxidative stress (Boyer, 1982).

Halophytes have evolved a range of adaptations to tolerate seawater and high salt stress. Thus, *Salicornia* spp. are promising crops for saline water-

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irrigated agriculture in the coastal deserts of many countries (Zerai *et al.,* 2010).

The population of the world is expected to exceed 9 billion people by 2050 which means 34% increase in population and 70% increase in food and energy demand (Nedjimi and Beladel, 2016). Salinity, a major abiotic stress, is a serious limitation to crop productivity, especially in arid and semi-arid zones (Hasegawa *et al.,* 2000). For this reason, several management practices can be adopted in this regard to reduce the adverse effects of using saline water for irrigation (Aziz *et al.,* 2013).

The productivity depends on supplemental nutrient fertilization, so during the growing season, plants require a balanced and sufficient supply of macro and micro nutrients for maximizing their growth and yield. There isa potential to use beneficial microorganisms as plant growth promoting. Using algae as a biofertilizer improves the physiological performance of the plant.

This study aims to investigate the effect of water salinity levels and supplemental nutrients fertilization on the biochemical contents and antioxidant activity of *Salicornia bigelovii* naturally grown on Mariut site Northwest Coast of Egypt.

Material and Method

Salicornia bigelovii plant naturally grown on Mariut site, Northwest Coast of Egypt which growing in scattered spots (0.5–1.5 m^2/spot), a homogeneous spots were selected for experiments during May 2018 to January 2019. The aim of this study was conducted to investigate the response of *Salicornia bigelovii* to four different levels of well water and sea water (100% well water), (100% sea water), (50% sea water + 50% well water) and (25% sea water + 75% well water) with EC 2. 4, 51, 30 and 17dS m^{-1}, respectively combined with four different supplemental nutrient fertilization: (NPK (20-20-20)), (NPK+ micronutrients fertilizer (Fe3%+ Mn3%+ Zn3%+ Cu0. 5% in chelated form)), (algae extract) and (NPK + micronutrients fertilizer + algae extract) in addition to control treatment.

The composition of algae extract was as follows: 13.3% N, 2.22% P, 2.13% K, 0.44% Ca, 0.22% Mg, 0.01% Na, 19.3% Fe, 4.5% Zn, 6.8% Mn, 1.8% Cu, 15.89% total amino acids, 3.2 mg g^{-1} indole butyric acid, 13.7 mgg^{-1} indole acetic acid and 1.2 mg g^{-1}gibberellic acid.

The layout of the experiment was factorial arrangement in a completely randomized design of all combination treatments. Each two weeks, the fertilization treatments were applied as soil application in the rate of 3g L^{-1} for each fertilizer during May, June, July in combination with saline water irrigation treatments.

One square meter from each spot were defined and harvested in January 2019 and the dry weights of whole plant (kg m^{-2}) were determined.

Nitrogen percentage of the dry plant was determined by the method described by Cottenie *et al.,* (1982), the concentration of protein in the dryherb had been estimated from nitrogen concentration of dry herb according to following formula: Protein % = Nitrogen % * 6. 25, total free amino acids content in dry herb was determined by using ninhydrin reagent according to Moore and Stein (1954), proline content in dry herb was carried out by using ninhydrin reagent according to Carillo and Gibon (2011).

The antioxidant activity was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical DPPH according to Brand-Williams *et al.,* (1995). Total phenolic content (mg g^{-1}) of each treatment were determined in the dry plant samples according Singleton *et al.,* (1999).

Flavonoids content (mg g^{-1}) was determined in the dry plant samples according to Quettier *et al.,* (2000). Total carbohydrates percentage of each treatment was determined in the dry herb according to a modified version of the DuBois assay (DuBois *et al.,* 1956).

The experimental soil (0-30 depth) was analyzed according to the method described by Jackson (1973), was sandy saline calcareous soil (having the following characteristics: The texture was sandy with EC 11.5 dSm^{-1}, pH 8.5, Calcium carbonate ($CaCO_3$) 26, 2%; Organic Matter (OM) 1.2 %, K 122, Ca 110, Mg 58, Na 2000, Fe 5.3, Mn 3.4, Zn 0.5 and Cu 0.4 ppm). During the cropping season from May 2018 to January 2019, the lowest minimum temperature was 15, maximum temperature reached 40^{o}c and the relative humidity was always more than 50%.

Data were statistically analyzed according to Snedecor and Cochran (1990). The least significant differences (LSD) were used to compare the means.

Results and Discussion

The results illustrated in table 1 showed that irrigation with four different levels of well water and sea water *i.e.* (100% well water), (100% sea water), (50% sea water +50% well water) and (25% sea water + 75% well water) with EC 2.4, 51, 30 and 17dS m^{-1}, respectively combined with four different levels of supplemental nutrient fertilization (NPK), (NPK+Micronutrients), (Algae extract) and (NPK+Micro Micronutrients +Algae) had a significant effect on whole plant dry weight, nitrogen and protein percentages, total free amino acids and proline contents of *Salicornia bigelovii* naturally grown on Mariut site.

The irrigation with 50% sea water + 50% well water (dS m^{-1}) increased the whole plant dry weight and total free amino acid content. Irrigation with 25% sea water + 75% well water (17 dS m^{-1}) gave the greatest increase in nitrogen and protein percentages of the plant. While irrigation with 100% sea water (51dS m^{-1}) recorded the greatest increase in the total free amino acid and proline content of *Salicornia bigelovii* plant. There were no significant differences in the content of amino acids with the irrigation of 100 % sea water or with 50% sea water +50% well water.

This might be due to that plant grown under saline environment activates the metabolic defense mechanisms which regulate ion flux and provide scavenging systems that can reduce the level of toxic ions and this lead to ionic imbalances and induce oxidative stress (Hasegawa *et al.,* 2000). The metabolic shifts to the pentose phosphate pathway lead to an increase in proline synthesis and a decrease in proline degradation, resulting in higher levels of proline that ultimately enhances the level of erythrose-4-phosphate available to the shikimic acid pathway (Al-Amier and Craker. 2007). Susceptibility and

Table 1: Effect of different combinations of irrigation and fertilization treatments on whole plant dry weight, N%, protein %, total free amino acids content, proline content and total carbohydrates % of *Salicornia bigelovii* plants naturally grown on Northwest Coast of Egypt.

Treatments		Whole plant dry weight (Kg m-^{2})	N (%)	Protein (%)	Total free amino acids (mg g^{-1} dry herb)	Proline (mg g^{-1} dry herb)
Irrigation	Fertilization					
Control (without irrigation orfertilization)		1.276	0.62	3.87	206.01	22.16
100% well water	NPK	1.969	1.37	8.56	138.46	13.94
	NPK+ Micro	2.063	1.41	8.81	174.04	19.09
	Algae	1.616	1.28	8.00	172.58	15.84
	NPK+ Micro+ Algae	2.202	1.33	8.31	143.77	19.55
100% sea water	NPK	1.684	1.12	7.00	222.86	39.78
	NPK+ Micro	2.183	1.37	8.56	177.63	18.73
	Algae	1.747	0.78	4.87	263.83	21.47
	NPK+ Micro+ Algae	2.592	1.42	8.87	398.52	46.71
50% sea water + 50% well water	NPK	2.745	1.41	8.81	335.31	28.28
	NPK+ Micro	2.516	1.45	9.06	181.79	21.66
	Algae	2.820	1.18	7.37	138.83	14.85
	NPK+ Micro+ Algae	2.790	1.62	10.12	413.72	12.29
25% sea water + 75%well water	NPK	2.115	1.35	8.43	122.13	22.74
	NPK+ Micro	2.505	1.45	9.06	102.95	35.71
	Algae	1.868	1.33	8.31	232.80	14.95
	NPK+ Micro+ Algae	2.411	1.72	10.75	185.61	19.22
Mean values of Irrigation	Control (without irrigation)	1.28	0.62	3.87	206.01	22.16
	100% well water	1.96	1.35	8.42	157.21	17.11
	100% sea water	2.05	1.17	7.33	265.71	31.67
	50% sea water +50% well water	2.72	1.42	8.84	267.41	19.27
	25% sea water + 75% well water	2.22	1.46	9.14	160.87	23.16
Mean values of Fertilization	Control (without fertilization)	1.28	0.62	3.87	206.01	22.16
	NPK	2.13	1.31	8.20	204.69	26.19
	NPK+ Micro	2.32	1.42	8.87	159.10	23.80
	Algae	2.01	1.14	7.14	202.01	16.78
	NPK+ Micro+ Algae	2.50	1.52	9.51	285.41	24.44
LSD at 5 %	Irrigation (I)	0.09	0.08	0.50	7.08	1.11
	Fertilization (F)	0.11	0.12	0.75	9.12	1.35
	(I) x (F)	0.129	0.20	1.25	11.50	1.76

NPK: Nitrogen, Phosphorus and Potassium fertilizersLSD: Least significant different tolerance to salt stress probably are associated with differences in enzymatic activity in the plant.

The negative effect of high levels of salinity on growth and dry weight of several plants was previously confirmed by Aziz *et al.,* (2013), Badawy *et al.,* (2017), Hanafy Ahmed *et al.,* (2018), Abdel-Kader *et al.,* (2020) and El-Nwehy *et al.,* (2020).

In addition, data in table 1 revealed that application of macro and micronutrients as well as algae extract significantly affected whole plant dry weight, nitrogen and protein concentrations as well as total free amino acids and proline contents. The treatment of NPK+ Micronutrients+ Algae recorded the maximum values in whole plant dry weight, nitrogen and protein percentages as well as total free amino acids content. While, the addition of NPK alone gave the highest content of proline.

These findings may be attributed to many factors; Nitrogen, phosphorus and potassium have important role in supporting growth and yield of plants. Nitrogen is an important nutrient in plant metabolism due to its function in the synthesis of protein and other organic compounds so it is essential for vigorous vegetative growth and yield (Costa *et al.,* 2016). Phosphorus element is required in energy metabolism, synthesis of nucleic acids, respiration, photosynthesis and enzyme regulation (Raghothama, 1999). Several studies revealed that P and K fertilizers play an active role in modulation of secondary metabolites (De La Rosa *et al.,* 2001).

Moreover, Fe functions as a component of protein in significant cellular events such as respiration and cell division; beside its role in the reduction steps of important biological events, like transpiration and photosynthesis as well as in chlorophyll biosynthesis (Einsenstein and Blemings, 1998 and Zocchi *et al.,* 2007). So, iron positively affects the chlorophyll content and other components of chloroplasts, which increases growth and plant dry weight (De La Guardia and Alcantara, 2002). Concerning the role of zinc, Carbonic anhydrase enzyme is activated by zinc.The main functions of this enzyme are increasing absorption of carbon dioxide per leaf area unit, dehydration of carbon dioxide, increasing in photosynthesis and biomass production. The activity of this enzyme is stopped in the plants with zinc deficiency (Mousavi *et al.,* 2013). Zinc is essential micronutrients for protein production in plants and it is essential for composition of ribosome and their development. Amino acids in plant tissues and protein synthesis declined by zinc deficiency (Pandey *et al.,* 2006). Manganese has an important role in chlorophyll synthesis and it is essential in Photo system II, it also involved in cell division and plant growth (Anderson and Pyliotis, 1996).

The enhancement impact of algae extract on plant growth may be attributed to the auxin content of the algae extract which has an effective role in cell division and enlargement. This increases the shoot growth, leaves number and plant dry weight (Gollan and Wright 2006). In this concern, Nofal *et al.,* (2016) and Aziz *et al.,* (2019) found that using algae extract increased growth characters and plant dry weight.

In regard to the interaction treatments, the irrigation with 50% sea water +50% well water combined with the addition of NPK+ Micronutrients+ Algae resulted in the highest values of whole plant dry weight, nitrogen and protein percentages and total free amino acids content. It was clear that, there were no significant differences in whole plant dry weight as well as nitrogen and protein percentages in this treatment or the higher values in other treatments. On the other hand, the addition of 100% sea water combined with NPK+ Micronutrients+ Algae recorded the maximum value of proline content.

Data in table 2 showed that the irrigation with four different levels of well water and sea water *i.e.* (100% well water), (100% sea water), (50% sea water +50% well water) and (25% sea water + 75% well water) with EC 2.4, 51, 30 and 17dS m^{-1}, respectively combined with four different levels of supplemental nutrient fertilization (NPK), (NPK+ Micronutrients), (Algae extract) and (NPK+ MicroMicronutrients+ Algae) had a significant effect on antioxidant activity, total soluble phenols and flavonoids content as well as total carbohydrates percentage of *Salicornia bigelovii* plant.

The results indicated that control treatment (without irrigation) gave the highest antioxidant activity (68.1%) and total soluble phenols content (2.45 mg g^{-1} dry herb) followed by irrigation with 50% sea water +50% well water which resulted also in the maximum content of flavonoids, while they decreased to 57.08% and 1.98 mg g^{-1} dry herb, respectively with increasing salinity level up to 100% sea water (51dS m^{-1}). Generally, low level of salinity decreased the antioxidant percentage of the plant. Total carbohydrates percentage increased with 25 % sea water +75% well water (17dS m^{-1}) and decreased with increasing salinity levels up to 30 and 51 dS m^{-1}.

These results could be attributed to the fact that phenols accumulation is a cellular adaptive mechanism for scavenging oxygen free radicals which formed during stress and could be oxidized for preventing sub cellular damages. In this respect, Wheat plants, subjected to salt stress, showed significantly higher levels of soluble phenols for osmoregulation (Hanafy Ahmed *et al.,* 2002).

This may be due to the assumption that such salt-stressed plants could have less efficiency to convert simple organic compounds into more complex ones. These results are in harmony with Qasim *et al.,* (2003) and Khattab and Afifi (2009) who concluded that salinity stress increased sugars concentration of plants.Salt stress results in accumulation of compounds like sugars termed osmolytes (Hasegawa *et al.,* 2000). These osmolytes prevent macromolecules from denaturation by supporting them to retain their natural configuration (Dubey and Pessarakli, 1995) as well as their role in carbon storage, osmotic adjustment, radical scavenging and osmoprotection (Omami *et al.,* 2006) counteracting the toxic effect of sodium and chloride ions in the shoot of many plant species (Everard *et al.,* (1994).

Irrigation with 50% sea water +50% well water with EC 30 dS m^{-1} produced the highest flavonoids percentage in the plant and decrease with 100% sea water with EC 51dS m^{-1}. These data suggested that the degree of the cellular oxidative damage in plants under abiotic stress is controlled by the ability to protect against oxidative agents (Ksouri *et al.,* 2007). For total polyphenols, salt stimulates the synthesis of flavonoids in these plants, according to Cushine and Lamb (2005), flavonoids are phenolic

Table 2: Effect of different combinations of irrigation and fertilization treatments on antioxidant activity%, total soluble phenols content, flavonoids content and total carbohydrates % of *Salicornia bigelovii* plants naturally grown on Northwest Coast of Egypt.

Treatments		antioxidant activity (%)	Total soluble phenol (mg g^{-1} dry herb)	Flavonoids (mg g^{-1} dry herb)	Total carbohydrates (%)
Irrigation	**Fertilization**				
Control (without irrigation orfertilization)		68.1	2.45	0.52	1.58
100% well water	NPK	60.8	1.76	0.37	1.01
	NPK+ Micro	38.2	1.63	0.45	1.27
	Algae	41.7	2.08	0.53	1.38
	NPK+ Micro+ Algae	59.5	1.90	0.71	2.21
100% sea water	NPK	68.7	2.13	0.55	2.24
	NPK+ Micro	47.5	1.78	0.63	1.58
	Algae	59.5	2.14	0.39	2.20
	NPK+ Micro+ Algae	52.6	1.85	0.49	2.01
50% sea water + 50% well water	NPK	62.8	2.51	0.88	2.06
	NPK+ Micro	59.5	2.31	0.77	2.33
	Algae	57.0	1.51	0.70	1.65
	NPK+ Micro+ Algae	58.2	1.86	0.76	1.71
25% sea water + 75% well water	NPK	53.6	2.16	0.68	1.78
	NPK+ Micro	45.7	1.86	0.55	2.40
	Algae	45.9	2.48	0.51	1.82
	NPK+ Micro+ Algae	67.6	2.66	0.37	2.32
Mean values of Irrigation	Control (without irrigation)	68.1	2.45	0.52	1.58
	100% well water	50.05	1.84	0.52	1.47
	100% sea water	57.08	1.98	0.52	2.01
	50% sea water + 50% well water	59.38	2.05	0.78	1.94
	25% sea water + 75% well water	53.20	2.29	0.53	2.08
Mean values of Fertilization	Control (without fertilization)	68.1	2.45	0.52	1.58
	NPK	61.48	2.14	0.62	1.77
	NPK+ Micro	47.73	1.90	0.60	1.90
	Algae	51.03	2.05	0.53	1.76
	NPK+ Micro+ Algae	59.48	2.07	0.58	2.06
LSD at 5 %	Irrigation (I)	2.3	0.12	0.15	0.12
	Fertilization (F)	3.4	0.15	0.10	0.10
	(I) x (F)	3.8	0.17	0.16	0.14

NPK: Nitrogen, phosphorus and potassium fertilizers
LSD: Least significant differences

compounds have a significant antioxidant anti-tumor and anti-microbial activity and contribute to the prevention of cardiovascular diseases. Moreover, flavonoids are often induced by abiotic stress and have a role in plants protection (Grace and Logan, 2000). Similarly, it is accepted that phenolic compounds give plants a strong antioxidant activity (Jun *et al.,* 2001). Karray *et al.,* (2010) revealed that in *Mentha pulegium*, salt resulted in an increase in polyphenol levelsand their antioxidant activity.

Regarding fertilization treatments, data in table 2 showed that antioxidant activity and total soluble phenols content recorded their highest values with control treatment (without fertilization). While, total carbohydrates reached the maximum percentage in the plants received NPK+ Micronutrients+ Algae. Moreover, it was obvious that there were no significant differences in the content of flavonoids among the different fertilization treatments.

These results might be explained by the fact that deficiency of crucial elements for instance nitrogen was found to enhance accumulation of phenolic compounds in the plants (Ibrahim *et al.,* 2011). Mineral elements' deficiency such as P and K have been reported to up-regulate the amounts of polyphenolic compounds as existing pools or by inducing their de novo synthesis (KováÇcik *et al.,* 2007 and Glynn *et al.,* 2008). Flavonoids content found to be increased as a consequence of phosphorous limitation (Nakabayashi *et al.,* 2014).

On the other hand, the positive impacts of micronutrients fertilizers may be resulted from their role in the formation of many primary and secondary metabolites; iron is essential in the mevalonate-independent pathway of isopentenyl diphosphate and dimethylallyl diphosphate (Rohdich *et al.,* 2004). Cytochrome P 450 enzymes which contain a heme-iron center in their active sites, are also acting in several steps of biochemical pathways of different types of phenolic compounds and flavonoides. Synthesis of dihydro flavonols, a major branch point in flavonoid biosynthesis, requires iron for the activity of the dioxygenase acting on naringenin (Buchanan *et al.,* 2000). Ionic forms of Fe, Zn, Mn and Cu act as co-factors in many antioxidant enzymes.Under micronutrients deficiency the activity of antioxidant enzymes decreases, which leads to increasing plant sensitivity to environmental stresses (Cakmak, 2000). Cu and Mnare involved in the shikimic acid pathway which in hurn leads to the biosynthesis of several phenols, as flavonoids, tannins and lignin (Santiago *et al.,* 2000, Diaz *et al.,* 2001, Loponen *et al.,* 2001, Lin *et al.,* 2005 and Guangqiu *et al.,* 2007). Zinc is one of the most important elements in carbohydrates metabolism, it activates most enzymes in this process such as; Carbonic anhydrase, Fructose-1, 6-bisphosphate and Aldolase enzymes. These enzymes are active in the chloroplasts and cytoplasm and very important in photosynthesis process (Mousavi, 2011).

Concerning the interaction between saline water levels and different fertilizers, plants irrigated with 100% sea water and received NPK only recorded the highest values of antioxidant activity and total carbohydrates percentage. On the other hand, there were no significant differences between the percentage of carbohydrates in this treatment and the higher percentages in other treatments.

Moreover, the irrigation with 50% sea water + 50% well water combines with NPK fertilizer resulted in the maximum values of total soluble phenols and flavnoids. It was clear that, there was no significant difference in this treatment between the content of total soluable phenols and the higher content in another treatment.

Conclusion

The high salt tolerance and supplemental nutrients fertilization enhanced the biochemical contents and antioxidant activity of *Salicornia bigelovii* naturally grown on Mariut site, Northwest Coast of Egypt.

Acknowledgements

This workis a part of the activities of the project entitled: Development the production and quality of *Salicornia* plants on different salty sources of water in the North West Coast of Egypt, principal investigator Prof. Dr. A.I. Rezk. This project was financially supported by the National Research Centre (NRC), Egypt.

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