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Effect of Sulfur and Clean Salt on Antioxidant Enzymes and Proline Content in Improving Salt Tolerance of Two Lettuce Cultivars Grown in Basrah

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Abstract: The experiment was conducted during 2017-2018 and 2018-2019 winter seasons at Abu Al-Khaseeb District at basrah /Iraq on sandy loam soil to study the effect of sulfur at five concentration (0 , 500 , 1000, 1500 and 2000) kg. Ha-1, clean salt at three concentration (0, 0.5 and 1.0) ml. L-1, two cultivars of lettuce local and fajr and interaction among them at electrical conductivity of the irrigation water (7.85 and 9.69) $dS.m^{-1}$. Result showed significant reduction in the activity of catalase (CAT) and peroxidase (POD) enzymes and proline content in all treatments of sulfur and clean salt especially at 2000 Kg. Ha^{-1} sulfur and clean salt at 1.0 ml L-1had significantly decrease in CAT activity (295.80 ، 341.65) U mg–1 FW, POD activity (7.86، 8.98) U mg–1 FW and proline (0.50 ، 0.80) mg g-1DW, comparing with control of CAT activity (663.21, 814.65) U mg–1 FW and POD activity (13.83, 15.52) mg–1 FW and proline (1.19, 2.03) mg g-1DW, respectively for two seasons due to the role of sulfur and clean salt ameliorates the adverse effects of salinity on plants. Fajr lettuce is more salt-tolerant than local due to less antioxidant enzyme levels POD, CAT and proline.

Keywords: *Lactuca sativa* L., Catalase, Peroxidase, Sulfur, Clean salt.

Introduction

Lettuce (*Lactuca sativa* L.) belongs to Asteracae family and considers of favorite winter vegetables crops in Iraq and others countries due to moisture, proteins, oils, fibers, phosphorous, potassium, zinc in addition to thiamine, ribofalfin, niacin, folic acid, B6, C, D vitamins (USDA, 2010). The average of lettuce production in Iraq 7.115Ton. Ha^{-1} and the total production was 22790 tons which is considered low productivity compared with neighboring countries (FAO, 2016).

Salinity is one of the most important abiotic stress factor that affect all vegetable corps, especially in semi-arid and arid regions and about 880 million hectares suffered of salinity because of low rainfall. When plants expose to salinity, it led to produce free

radical species (ROS). Hydrogen peroxide (H_2O_2) (Sairam & Tyagi, 2004), and caused damage to cellular compartment, proteins and lipid membranes and nucleic acid (Foyer *et al*., 1994). Plants can get rid of ROS by using antioxidants enzymatic system which include catalase (CAT) and peroxidase (POD) which damage H_2O_2 to water and oxygen (De Azevedo Neto *et al*., 2006; Chelikan *et al*., 2004). POD locates in cell membrane and it considers one of key enzymes that control plant growth development. The enzymes act on osmotic and ionic homeostasis to face salt stress (Ashraf, 2009). The process of washing sodium from roots zone is necessary to reduce salt accumulation in soil and that can be done by amendments like sulfur and clean salt. The use of sulfur leads to soil salt improvement (Abdelhamid *et al*., 2013). Sulfur is one of essential nutrients for plant growth and takes part in synthesizing amino acid cysteine, methionine, thiamin and vitamin B. It is a strong reductive in electron transporter of light reaction (Brosnan & Brosnan, 2006). Sulfur acts a vital role in reducing pH in high salt soils and increasing nitrogen, phosphorous, potassium and calcium (Beyatee *et al*., 2009). The clean salt consists of calcium and organic materials that salt takes part in replacing calcium instead of sodium (Muhammad & Khattak, 2011) and it acts in hydroxyl and carboxyl property in which they separate sodium element from soil and closes it in order not to attach it by any particles, therefore sodium and chloride elements had washed from soil. Organic material reserves the nutrient elements for plant microorganism and increase the cations exchange capacity like calcium and potassium and also important source for enrichment the microorganism by carbon in the soil, adjust the pH of soil and increasing the amount of nitrogen and phosphorous which have needed by plant (McCauley *et al*., 2017).

Damaging of H_2O_2 to protein and DNA, causing lipid peroxidation, is removed by POD and CAT (Hossain *et al*., 2007). and it transforms to MDHA (Mono dehydro ascorbate reductase) by the helping of NADPH (Nictamide Adenine Dinucleotide phosphate) and DHAR (Dehydro ascorbate reductase) redducegluthion (GSH) to GSSG and also MDHA reduce (GR) maintaining GSH which catalizes H_2O_2 to water in peroxisomes (Foyer & Noctor, 2005; Ashraf, 2009; Turkan & Demiral, 2009; Rai *et al*., 2011; Eltelib *et al*., 2012).

Proline forms in plant when exposes to biotic and abiotic stress and two genes control on it synthesizing, proline 5 carboxylate synthase (P5C5) which stimulate in the apical dominant of stem in mitochondria. Proline has a function to get reductive potential to mitochondria through the oxidation of proline by proline dehydrogenase (PHD) and proline 5 carboxylate dehydrogenase (P5CDH) and then supply electron to respiration series to continue growth (Hare & Cress, 1997). Kishor *et al*. (2005) showed that the proline acts to protect subcellular compound including amino acids and proteins which consider the basic compound for cell (Parida & Das, 2005).

In an experiment conducted by Bartha *et al*. (2015) in which five cultivars of lettuce where exposed to (50 and 100) mM of NaCl and they showed that free proline depend on the variety of lettuce and there were significant variations in proline content

The objective of the current study was to evaluate the effect of sulfur and clean salt on the activity of enzymes (catalase and peroxidase) and proline content of two lettuce cultivars grown on salty soil in Basrah, Iraq.

Material & Methods

The experiment was carried out during 2017-2018 and 2018-2019 season at Abu Al-Khaseeb district at Basrah, Iraq in sandy loam soil at electrical conductivity of the irrigation water (7.85,9.69 $dS.m^{-1}$). To study the effect of four levels of sulfur and three concentration of clean salt and two cultivars of lettuce (local and Fajr) and interaction between them on the activity of antioxidant enzymes Catalase and Peroxides and Proline concentration. Some physical and chemical properties of the top layer of soil (up to 30 cm) were determined in table (1) and measured irrigation

Table (1): Physico-chemical analysis of soil at two seasons.

Parameter	2017-2018	2018-2019
	Soil value	
EC (dSm^{-1})	16.80	015.9
pH	8.10	07.9
Organic matter (%)	02.8	3.10
Total nitrogen ($mg.Kg^{-1}$)	01.8	1.30
Total phosphor ($mg.Kg^{-1}$)	0.22	0.25
available potassium ($mg.L^{-1}$)	18.6	17.8
Sulfur ($mg.Kg^{-1}$)	0.214	0.153
Dissolved ions (mM)		
Ca++	36	33
Mg++	09.4	9.10
Na+	18.58	18.26
K+	2.42	2.55
Cl-	40	95
CO_3^{--}	-	-
HCO_3^-	2	2
SO_4^{--}	018.3	021.5
Sand (%)	011.8	12.70
Silt (%)	.0067	59.3
Clay (%)	21.20	28.00
Texture Class	Sandy Loam	Sandy Loam

Table (2): Electrical conductivity of irrigation water ($dS.m^{-1}$) at different time of two seasons.

Date	2017-2018	2018-2019
1October	8.12	7.58
15 October	8.55	8.75
1st November	8.53	9.82
15 November	8.35	10.25
1st December	7.80	13.86
15 December	7.40	11.35
1st January	7.15	10.10
15 January	6.81	9.81
1st February	6.55	9.51

15 February	6.12	7.55

water electrical conductivity at two seasons in table (2). Seeds of lettuce Local and Fajr cultivars were sown into 1:1 peat: sandy soil in styropor trays in the nursery on 10/9/2017/2018. lettuce (35 days old) were transplanted in the field in 15/10 during both seasons, at both sides of ridges 2.5 m long. The space between plants 30 cm and between row 0.75 m. The experimental design was factorial in randomized complete blocks with three replications. Sulfur were applied to soil at the rates of 0, 500, 1000, 1500 and 2000) kg .Ha^{-1} and mixed with surface soil 20 days before transplanting while clean salt 12% Ca applied to soil at three concentrations (0, 0.5 and 1) ml. l^{-1}, 10 days before transplanting and repeated twice after transplanting at 15-day intervals between them.

1. Spectrophotometric determination of enzyme activity

A-catalase activity was measured by hydrogen peroxide assay based on formation of its stable complex with ammonium molybdate (Goth, 1991). 0.2 ml of plant extract was incubated in 1ml reaction mixture containing 65 mM hydrogen peroxide in 60 mM potassium phosphate buffer; pH 7.4 at 25 °C for min. The enzymatic reaction was stopped with 1 ml of 32.4 mM ammonium molybdate and the concentration of the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm. Activity was expressed on a fresh weight basis (unit per mg protein)

Catalase activity was calculated by the following formulae:

Volume activity (Units/ml)=Δ A . 2 . V q/ 2.8 Vs.

Enzyme activity (Units/mg)=(Units/ml)/ mg protein/ml.

Vq= reaction volume into cuvette (in ml)

2.8 = extinction coefficient of ascorbate at 290 nm (per mM cm)

Vs=volume (in ml) of sample used.

Peroxidase activity was measured by using a guaiacol assay (Angelini *et al*., 1993). The reaction mixture (final volume 1.75 ml) contained 0.1 M potassium phosphate buffer : pH 7.5, and 5 mM guaiacol and plant extract. The absorbance at 436 nm was continuously detected for 1 min at 30 ºC after adding 0.2 ml of 1M hydrogen peroxide. Peroxidase activity was calculated by the following formulae:

Peroxidase activity (Unit mg^{-1}Fw)=O.D spectrophotometer/(weight of sample/ volume of sample) × volume into cuvette .

2. Determination of proline

Proline was determined spectrophotometri-cally according to the ninhydrin method described by Bates *et al.* (1973). Using L-proline as standard. Approximately 300 mg of dry tissue was homogenized in 10 ml of 3% (w/v) aqueous sulphosalicylic acid and filtered . In the 2 ml of the filtrate, 2ml of acid ninhydrin was added, followed by the addition of 2 ml of glacial acetic acid and boiled for 60 min . The mixture was extracted with toluene , and the free proline was quantified spectro-photometrically at 520 nm. The proline concentration was determined from standard curve and calculated on dry weight.

Results & Discussion

As shown in tables (3-5) the cultivar fajr gave the lowest CAT activity (398.95, 424.91) U

Table (3): Effect of cultivar, Sulfur and Clean salt and their Interaction on Catalase activity (U mg^{-1} FW) for two seasons.

cultivar	Clean salt (ml^{-1})	Growth season 2017-2018						Growth season 2018-2019					
		Sulfur ($kg.Ha^{-1}$)					cultivar × Clean	Sulfur ($kg.Ha^{-1}$)					cultivar×
		0	500	1000	1500	2000	salt	0	500	1000	1500	2000	Clean salt
local	0	663.21	566.57	448.39	418.55	390.29	429.12	814.65	473.01	454.79	440.13	419.52	520.24
	0.5	476.72	421.45	442.31	385.80	351.16	427.43	511.62	461.68	443.75	429.30	406.72	450.61
	1	390.05	353.18	327.89	292.71	290.39	394.80	464.01	447.37	422.46	412.06	358.18	420.81
Fajr	0	619.55	510.34	449.51	375.62	369.08	405.15	707.51	504.91	442.21	416.96	383.28	490.97
	0.5	428.96	417.72	403.76	359.17	333.99	397.17	466.73	417.12	397.80	390.15	350.10	404.38
	1	401.04	376.49	333.04	326.19	295.80	384.53	431.67	393.37	372.33	358.61	341.65	379.53
37.47				37.47			16.76			18.10			8.10
Average sulfur effect		483.28	441.13	400.82	365.76	343.55		493.98	449.57	420.95	409.13	376.58	
LSD 0.05				15.30			cultivar effect			5.72			cultivar effect
cultivar × sulfur	local	509.99	477.05	406.19	365.45	343.95	414.87	596.76	460.69	440.33	427.16	394.81	463.95
	Fajr	483.81	390.23	379.21	353.66	332.96	398.95	535.08	438.45	401.56	391.12	356.34	424.91
LSD 0.05				2.63			9.68			10.45			4.67
							Clean salt effect						Clean salt effect
Clean salt × sulfur	0	641.38	538.45	448.95	380.35	375.27	417.13	791.08	489.96	448.50	428.55	401.40	505.70
	0.5	439.22	423.04	398.97	374.73	360.12	412.55	488.85	439.38	416.95	413.55	378.41	427.43
	1	409.52	345.72	342.19	330.46	294.25	391.03	447.85	420.37	397.40	385.34	349.92	400.17
LSD 0.05				26.50			11.85			12.80			5.72

Table (4): Effect of cultivar, Sulfur and Clean salt and their Interaction on peroxidase activity (U mg^{-1} FW) for two seasons.

cultivar	Clean salt (ml^{-1})	Growth season 2017-2018						Growth season 2018-2019					
		Sulfur ($kg.Ha^{-1}$)					cultivar × Clean salt	Sulfur ($kg.Ha^{-1}$)					Cultivar × Clean salt
		0	500	1000	1500	2000		0	500	1000	1500	2000	
Local	0	13.83	9.97	9.73	9.48	9.32	10.29	15.52	13.88	12.58	12.00	10.46	12.89
	0.5	10.36	9.23	9.08	8.33	8.25	9.05	12.26	11.21	11.06	10.90	9.85	11.05
	1	9.07	8.68	8.53	8.31	8.15	8.53	10.94	10.88	10.36	10.15	9.62	9.79
Fajr	0	12.74	9.44	8.87	8.66	8.25	9.59	14.73	12.81	11.94	10.02	9.45	11.79
	0.5	9.80	8.93	8.65	8.22	8.04	8.73	11.73	10.85	10.12	9.74	9.27	10.27
	1	8.73	8.66	8.28	8.06	7.86	8.32	10.72	9.75	9.47	9.13	8.98	9.61
LSD 0.05				0.54			0.24			0.56			0.25
Average sulfur effect		10.68	9.15	8.80	8.49	8.30		12.59	11.56	10.92	10.32	9.61	
LSD 0.05				0.22			Cultivar effect			0.23			Cultivar effect
Cultivar × sulfur	local	10.63	9.29	8.99	8.71	8.54	9.23	12.91	11.99	11.33	11.01	9.97	11.44
	Fajr	10.42	9.01	8.60	8.31	8.05	8.88	12.27	11.14	10.51	9.63	9.23	10.56
LSD 0.05				0.31						0.33			0.15
							Clean salt effect						Clean salt effect
Clean salt ×sulfur	0	13.06	9.71	9.12	9.07	8.74	9.94	15.12	13.34	12.26	11.10	9.98	12.36
	0.5	10.08	9.08	8.87	8.28	8.15	8.89	11.82	11.03	10.60	10.32	9.56	10.66
	1	8.90	8.67	8.41	8.20	8.01	8.44	10.83	10.32	9.91	9.64	9.30	10.00
LSD 0.05				0.38			0.17			0.40			0.18

Table (5): Effect of cultivar, Sulfur and Clean salt and their Interaction on on Proline (mg g^{-1}DW) for two seasons.

Cultivar	Clean salt (ml^{-1})	Growth season 2017-2018						Growth season 2018-2019					
		Sulfur ($kg.Ha^{-1}$)					cultivar ×Clean salt	Sulfur ($kg.Ha^{-1}$)					Cultivar × Clean salt
		0	500	1000	1500	2000		0	500	1000	1500	2000	
Local	0	1.19	0.97	0.81	0.73	0.68	0.87	2.03	1.49	1.38	1.31	1.21	1.49
	0.5	0.92	0.83	0.76	0.69	0.66	0.77	1.56	1.23	1.24	1.16	1.09	1.26
	1	0.81	0.75	0.71	0.66	0.63	0.71	1.55	1.31	1.11	1.08	1.01	1.17
Fajr	0	1.16	0.80	0.78	0.68	0.57	0.81	1.92	1.41	1.24	1.20	0.98	1.35
	0.5	0.85	0.73	0.69	0.62	0.55	0.69	1.93	1.28	1.08	0.91	0.87	1.11
	1	0.77	0.68	0.58	0.56	0.50	0.61	1.22	1.07	0.97	0.88	0.80	0.98
LSD 0.05				0.05			0.02				0.04		0.02
Average sulfur effect		0.95	0.80	0.72	0.65	0.59		1.61	1.27	1.17	1.09	0.99	
LSD 0.05				0.02			cultivar effect				0.01		cultivar effect
Cultivar	local	0.97	0.85	0.76	0.69	0.65	0.75	1.70	1.29	1.24	1.19	1.10	1.30
×Sulfur	Fajr	0.92	0.76	0.68	0.62	0.54	0.70	1.51	1.25	1.09	0.99	0.88	1.15
LSD 0.05				0.03			0.01				0.02		0.09
							Clean salt effect						Clean salt effect
Clean salt ×Sulfur	0	1.18	0.92	0.80	0.71	0.62	0.85	1.97	1.45	1.31	1.26	1.09	1.42
	0.5	0.88	0.78	0.73	0.66	0.61	0.73	1.48	1.26	1.16	1.04	0.98	1.18
	1	0.79	0.72	0.65	0.61	0.57	0.69	1.37	1.10	1.04	0.98	0.90	1.08
LSD 0.05				0.08			0.02				0.06		0.01

mg–1 FW, POD activity, (8.88 and10.56) U mg–1 FW and proline (0.70 and 1.15) mg g-1DW in two seasons respectively. The treatment 1.0 m1.L-1 clean salt gave a lowest CAT activity (391.63400.17 ،) U mg–1 FW,

POD activity (8.44 and 10.0) U mg^{-1} FW, proline (0.69 and 1.08) $mg.g^{-1}$DW for two seasons, respectively. Treatment with sulfur at 2000 Kg. Ha^{-1} gave the lowest CAT activity (343.55، 376.58) U mg^{-1} FW, POD activity (8.30 and 9.61) U mg^{-1} FW, Proline (0.59 and 0.99) $mg.g^{-1}$DW for two seasons respectively.

The interaction between Fajr cv., clean salt at 1.0m1. l^{-1} and sulfur 2000 Kg. Ha^{-1} gave the lowest CAT activity (295.80 and 341.65) $U.mg^{-1}$ FW, POD activity (7.86 and 8.98) $U.mg^{-1}$ FW and proline (0.50 and 0.80) mg g^{-1}DW for two seasons respectively.

The result indicated that there were increment in CAT, POD enzymes activity and proline content, in each two cultivars in the control treatment and this agreed with Zhang *et al*. (2013), Hela *et al*. (2011) and Younis *et al*. (2008) that high salt led to increase in CAT and Zhang *et al*. (2013), Hela *et al*. (2011) and Younis *et al*. (2008) that high salt led to increase in CAT and POD enzymes and proline supplement of sulfur led to increase the metabolism of *Thiobacillus thioparus* bacteria and led to the pH lower and increasing the availability of macro and micro nutrients which lead to increase the solutes which adjusting the water potential and finally regulate the cellular water and absorption of water by plant (Abdelhamid *et al*., 2013; Kadhim, 2016; Riffat & Ahmad, 2018) and finally reduce CAT, POD and proline. Addition of calicium act on increasing the synthesize of carbohydrates and various solutes that lead to adjusting cellular potential (Tian *et al*., 2015) and this agreed with Maeda (2019) that the use of calcium act to promote growth and decrease proline and CAT, POD enzymes.This result indicate that sulfur and clean salt ameliorates the adverse effects of salinity on lettuce plants that grow on sodic soil.

Conclusion

Sulfer and clean salt have had apossitive effect in decerasing salinity effect on lettuce that grow on salinity soil through their effect on oxidative enzyme and proline content used in this study.

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