Plant Archives Vol. 18 No. 2, 2018 pp. 2335-2339 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF SALINITY STRESS AND SELENIUM SPRAYING ON BROAD BEAN PLANT *VICIA FABA* L.

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Abstract

The experiment was conducted in the old botanical garden belong to Biology Department/ College of Education for Pure Science - Ibn Al-Haitham/Baghdad University for growing season 2015-2016 to study the effect of irrigation with four concentrations of sodium chloride (0, 50, 100, 150) $mM.L^{-1}$ and spraying with selenium in three concentrations (0, 10, 20) mgL^{-1} on growth of broad bean plant using clay pots. The experiment was design according to completely randomized design (CRD) with three replications.

Results indicated that broad bean plant irrigated with saline water and increasing concentrations of sodium chloride in growth medium caused a significant decreased in the plant growth parameters (plant height, no. of compound leaves. $Plant^{-1}$, leaf area, proline acid content, concentration of nitrogen, phosphorus and potassium) whereas spraying with selenium showed significant increase in growth parameters studied, as for interference for both factors, the best values was at the concentrations 10, 20 $mg.L^{-1}$ selenium which elevate the adverse effect of high sodium chloride concentration 150 $mM.L^{-1}$.

Key words: Salinity stress, broad bean, selenium, oxidative stress.

Introduction

Broad bean plant *Vicia faba* L. is one of Fabaceae family, it is a stiffly erect plant, the leaves are compound with distinct grey-green color, the flowers are clusters with five white petals, the fruit is a broad leathery pod contain 3-8 seeds. The food is very low in saturated fat, cholesterol and sodium. It is also a good source of dietary fiber, protein, phosphorus and manganese and a very good source of folate (Ali *et al*., 1990).

The world is subjected to great challenges because of decrease of arable land comparison with the increase of world population, that´s on account of increasing environmental constraints particularly to salinity which limited cultivation of agricultural crops, plant can't tolerance the excessive amount of salt that's accumulate in their tissues the most commonly NaCl (Reynolds *et al*., 2005).

Salinity has detrimental effects, the excess of salts leads to both ionic and osmotic stresses, and causing changes in dietary and enzymatic balance (Türkan and Demiral, 2009). Oxidative stress is a factor in a biotic stress induced by salinity and occurs when there is imbalance between the production of reactive oxygen species (ROS) and antioxidant defense for example O_2^- and H_2O_2, OH^- (Jaspers and Kangasjärvi, 2010), under normal growth conditions ROS production is low as a normal product of plant metabolism, however, environmental stresses for example salinity disrupt the cellular homoeostasis lead to extravagant production of ROS causing oxidative damage to cellular apparatus lead to lipids, proteins, and DNA damage, convert membrane properties such as fluidity, ion transport, loss of enzyme activity and ultimately cell death (Sharma *et al*., 2012), in order to avoid the oxidative damage, plants raise the level of endogenous antioxidant defense such as superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD) that play important role in scavenging stress-induced ROS generated in plants, when these defences fail to stand up the self-increase auto oxidation with ROS, cell death ultimately occurs (Li, 2009). Synthesis and accumulation of organic osmolytes as compatible solutes is on of the mechanisms for adaptation to stress such as free amino acid particularly proline which increasing the capacity for osmotic adjustment to combine productivity with salt

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tolerance. (Verbruggen and Hermans, 2008).

The study of (Amirijan, 2010) explored the effect of salinity stress with four concentrations (0, 50, 100, 200) mM NaCl on soybean plant, increasing salinity concentrations caused a reduction of plant height, fresh weight, dry weight, the activity of nitrogenase and increasing in proline content, sodium content, but the content of potassium and calcium decreased.

Although selenium is not classified as essential for plant (Terry *et al*., 2000), several studies demonstrate that selenium supply may promote growth activities and enhanced resistance to certain a biotic stresses such as salinity (Djanaguiraman, 2005). The normal concentration of selenium in the soil is low, causing selenium deficiency in human diet, it's average daily intake by adult is between 26-32 µg (Chen *et al*., 2002). Foliar spray technology has been used to increase the selenium contend in plant tissues (Pezzarossa *et al*., 2012). Selenium at low doses can stimulate the growth of plants and counteract environmental stresses whereas at high dosages it causes an oxidative stress, so it play a dual effect (Hartikainen *et al*., 2000). Both its low and high concentrations and chemical form maybe important for protective mechanism (Babula *et al*., 2008). Selenium works on linking between amino acids particularly seleno-methionine, seleno-cystine which have the ability to connect with another amino acid and increases the activity of both nucleic acids DNA, RNA and growth and cellular differentiation. (Castillo-Godine *et al*., 2016). The addition of 1.5 µM selenium to *Vicia faba* L. plant exposed to oxidative stress, found to influence the activity of antioxidants and thus regulate the ROS levels, balance, the levels of GSH ,thiols (-SH), ferredoxin (Fd_{red}) and NADPH ,whereas a higher level of Se 6 µM enhance O_2^- levels and decreased cell viability (Mroczek-Zdyrska and Wójcik, 2011).

Materials and methods

This project was done at botanical garden belong to Biology Department College of Education for pure science-Ibn Al- Haitham/ Baghdad university, during the growing season (2015- 2016), factorial experiment with four concentrations of salinity (0, 50, 100, 150) m $M.L^{-1}$ sodium chloride and three concentrations of selenium (0, 10, 20) mg.L-1 ($NaHSeO_3$), were used to explore the effect of the two factors on broad bean plant. The treatment combinations were represented by three replicates pots and arranged as a completely randomized design (CRD). Seeds were grown in pots (with capacity 10 Kg Soil) in 2-11-2015 and irrigated with water and kept approximately 50% of field capacity, two weeks after plantation the plants were slash to five plants in each pot. Salt stress was initiated 34 days after seed plantation, NaCl was added three times the first was in 6-12-2015 ,the second was in 8-12-2015 the third in 10-12-2015. Selenium was sprayed in 13-12-2015 in the morning ,one plant for each treatment was harvest 57 days after planting , plant height and no. compound leaves. $Plant^{-1}$ were measured ,plants samples were oven dried (75^0C to constant mass) and pulverized, a known weight was digested according to (Agiza *et al*., 1960), for determination of mineral composition, nitrogen (Chapman and Pratt, 1961), phosphorus (Matt, 1970) and potassium (Page *et al*., 1982). Free amino acid proline content in young plant of fully expand leaves of each treatment was quantified using the ninhydrin reagent (Bates *et al*., 1973). Plant leaf area was calculated as method (Abo EL-Zahaba *et al*.,1980). Statistical analyses were carried out, differences between the means were detected according to (<0.05) using test (SAS, 2010).

Results and Discussion

The effect of salt stress on some morphological parameters of the treated broad bean plant were evaluated, plant height and no. compound leaves. plant-1 (table 1) were significantly reduced by increasing salinity levels from 0 to 150 $mM.L^{-1}$ by about (16.49 ,41. 20)% respectively, in contrast selenium spray treatment from 0 to 20 $mg.L^{-1}$ caused a significant increase for both parameters by about (23.48 ,21.71)% .For the interactions between the two factors, 20 mg $.L^{-1}$ selenium diminished the negative effect of 150 mM $.L^{-1}$ sodium chloride and gave the best value 32.00 cm for plant height in comparison with the value 22.50 cm at same concentration Sodium chloride and without selenium spraying. Also for no. Compound leaves. $Plant^{-1}$, 10 $mg.L^{-1}$ selenium minimized the adverse effect of height salinity 150 $mM.L^{-1}$ and gave value 17.00 in comparison with 9.50 at the same sodium chloride and without selenium spraying.

In fact salinity has an adverse effect on leaf area of the plant, from (table 2) it can be seen reduction in plant area by about 43.45% by increasing concentration of sodium chloride from 0 to 150 m $M.L^{-1}$, and a positive response to selenium spraying by increasing the concentration from 0 to 20 $mg.L^{-2}$, and the best increase percentage was at 10$mg.L^{-1}$ selenium by about 17.38% , the result for dual interaction between 10$mg.L^{-1}$ selenium spraying was positive in decreasing the unfavourable effects of 150$mM.L^{-1}$ sodium chloride and gave the value 905.14 cm^2 in comparison with 76.93 cm^2 at the same sodium chloride concentration and without spraying with selenium .

To determine whether proline accumulates in

Table 1: Effect of sodium chloride stress and selenium spraying on some morphological parameters of bean plant .

Sodium chloride concentrations ($mM.L^{-1}$)	Plant height (cm)				No. compound leaves. $Plant^{-1}$			
	Selenium concentrations ($mg.L^{-1}$)				Selenium concentrations($mg.L^{-1}$)			
	0	10	20	Sodium chloride average	0	10	20	Sodium chloride average
0	33.00	34.00	36.00	34.33	21.00	22.00	25.00	22.67
50	33.50	35.00	40.00	36.17	18.00	18.50	21.00	19.17
100	26.00	32.50	34.00	30.83	16.00	18.50	19.00	17.83
150	22.50	31.50	32.00	28.67	9.50	17.00	13.50	13.33
Selenium average	28.75	33.25	35.50		16.12	19.00	19.62	
LSD (0.05)	Selenium concentrations=3.11 Sodium chloride concentrations=3.60 Interaction=6.23				Selenium concentrations=2.04 Sodium chloride conc.=2.35 Interaction=4.08			

Table 2: Effect of sodium chloride stress and selenium spraying on leaf area (cm^2) of bean plant.

Sodium chloride concentrations ($mM.L^{-1}$)	Selenium concentrations ($mg.L^{-1}$)			
	0	10	20	Sodium chloride average
0	136.28	164.54	141.30	147.37
50	109.90	126.23	125.20	120.44
100	103.31	116.50	131.65	117.15
150	76.93	95.14	77.95	83.34
Selenium average	106.61	125.60	119.02	
LSD (0.05)	Selenium concentrations=3.85 Sodium chloride concentrations=4.45 Interaction=7.71			

Table 3: Effect of sodium chloride stress and selenium spraying on proline acid content of bean plant leaves.

Sodium chloride concentrations ($mM.L^{-1}$)	Selenium concentrations ($mg.L^{-1}$)			
	0	10	20	Sodium chloride average
0	69.00	64.00	50.00	61.00
50	80.00	78.00	71.50	76.50
100	84.00	76.50	76.50	79.00
150	98.00	94.00	79.00	90.33
Selenium average	82.75	78.12	69.25	
LSD (0.05)	Selenium concentrations=3.85 Sodium chloride concentrations=4.45 Interaction=7.71			

response to salinity, the content of free proline was measured ,the results of (table 3) showed an increase in proline content by about 48.08% by increasing in sodium chloride concentration from 0 to 150 $mM.L^{-1}$ on the other side a significant decrease in the proline content by increasing selenium from 0 to 20 $mg.L^{-1}$ about 16.31%, the interaction between the factors demonstrated a better tolerance to salinity, the concentration 20$mg.L^{-1}$ selenium could reduce the adverse effect of 150$mM.L^{-1}$ sodium chloride and the value was 79.00 in comparison with the value 98.00 at the same salt level and without selenium spraying.

The result of (table 4) indicated that salinity had pronounced effect on ion absorption nitrogen, phosphorus, potassium, for instance increasing concentrations of NaCl from 0 to 150 $mM.L^{-1}$ depressed–NPK absorption by (48.53, 66.67, 26.94)% respectively, selenium application increase the availability of NPK for plant uptake as a bout (16.50, 28.57, 21.67)%, a significal interaction occurred between salinity levels and selenium supply which resulted in the NPK supply 20$mg.L^{-1}$ Selenium can counter the adverse effect of 150 $mM.L^{-1}$ sodium chloride, and gave best value for nitrogen 1.84 and potassium 2.50, compare with the same concentration of sodium chloride and non sprayed with selenium 1.27 for nitrogen and 1.55 for potassium whereas the concentration 10 $mg.L^{-1}$ selenium resist the high salinity stress 150$mM.L^{-1}$ sodium chloride and gave the best value for phosphorus 0.21 comparison with 0.15 at 150 $mM.L^{-1}$ sodium chloride and without spraying with selenium.

The first indication for salinity stress can lead to stomata closure, higher salinity which reduces carbon dioxide CO_2 assimilation and inhibits carbon fixation which in turn causes exposure of chloroplasts to excessive excitation energy and over reduction of photosynthetic electron transport system which in turn leads to reduced $NADP^+$ regeneration through the Calvin cycle, leads to lack of election receptor and causing to enhanced generation of ROS and induced oxidative stress, the imbalance CO_2/O_2 ratio leading to photoreception and increased production of hydrogen peroxide ultimately resulting in reducing plant growth (Hernández *et al.*, 2000). Elevated CO_2 mitigates the oxidative stress, involving lower ROS generation and maintenance of redox balance as result of higher CO_2 assimilation rates and lower photoreception (Perez-Lopez *et al.*, 2009). Selenium induced recovery by increasing CO_2 availability for biochemical reactions and higher stomata conductance thus reducing generation of excess excitation energy

Table 4: Effect of sodium chloride stress and selenium spraying on nitrogen, phosphorus and potassium concentration(%) in vegetative part of bean plant.

Sodium chloride concentrations ($mM.L^{-1}$)	Nitrogen				Phosphorus			
	Selenium concentrations ($mg.L^{-1}$)				Selenium concentrations($mg.L^{-1}$)			
	0	10	20	Sodium chloride average	0	10	20	Sodium chloride average
0	2.98	3.10	3.13	3.07	0.45	0.57	0.59	0.54
50	2.30	3.11	2.45	2.62	0.30	0.32	0.32	0.31
100	1.67	2.10	2.19	1.99	0.23	0.34	0.35	0.31
150	1.27	1.62	1.84	1.58	0.15	0.21	0.19	0.18
Selenium average	2.06	2.48	2.40		0.28	0.36	0.36	
LSD (0.05)	Selenium concentrations=0.08 Sodium chloride concentrations=0.10 Interaction=0.17				Selenium concentrations=0.04 Sodium chloride concentrations=0.05 Interaction=0.08			

Sodium chloride concentrations ($mM.L^{-1}$)	Phosphorus			Sodium chloride average
	Selenium concentrations ($mg.L^{-1}$)			
	0	10	20	
0	2.89	2.95	3.08	2.97
50	2.72	2.89	3.00	2.87
100	2.42	3.05	3.10	2.86
150	1.55	2.47	2.50	2.17
Selenium average		2.40	2.84	2.92
LSD (0.05)	Selenium concentrations=0.07 Sodium chloride concentrations=0.08 Interaction=0.14			

further than increase the chlorophyll content and protect chloroplast from damage (Hajiboland, 2014). Selenium regulates the production and quenching of ROS through three pathways the first stimulating the spontaneous dismutation of $O_2^{\cdot-}$ in to H_2O_2 (Carts *et al*., 2010), second mechanism is direct reaction between selenium compound and ROS ,third mechanism is regulation of anti oxidative enzymes (Xue *et al*., 1993). Selenium application resulted in higher concentrations of some free amino acids and accumulation of some osmoslytes under stress conditions such as proline which play important roles in the maintenance of water uptake capacity, (Hajiboland *et al*., 2014).

Proline can suggested as a metabolic marker and encounter salinity stress and proposed to act as a compatible solute that adjusts the osmotic potential in cytoplasm of tissues plants and protect plasma membrane and proteins from damage so it gave manipulation for tolerance to stresses (Kaviani, 2008). Foliar application of selenium stimulate nitrogen assimilation and activate protein synthesis so its able to promote growth and development of plants (Aslam *et al*., 1990), there is activation to nitrate reductase that was accompanied by higher amino acid and protein synthesis (Hajiboland and Sadeghzadeh, 2014), selenium increase the concentrations of organic material and non organic ions, osmoprotectants may enhanced water retention (Emam *et al*., 2014).

On the basis of presented investigation have shown higher concentrations of sodium chloride could be attributed to inhibit plant growth particularly in the cause of 150 m $M.L^{-1}$, foliar application of selenium had a positive role manifested by increasing tolerance of plant to the stress and improved plant growth particularly at the concentration 20 $mg.L^{-1}$.

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