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Nutritional and Growth Response of Canola Plants to Salicylic Acid under Salt Stress Conditions

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Abstract: For evaluation the effect of spraying salicylic acid on growth response and nutrients concentration of canola plants grown under salt stress conditions, a pot experiment was conducted in the greenhouse of the National Research Centre,Cairo,Egypt. The treatments were as follows: Salt stress treatments were: 250 (tap water), 2500 and 5000 ppm. Spraying salicylic acid (SA) at the rate of 100 and 200 ppm and tape water as a control.Negative relationship was shown between salt stress degree and plant growth characters which represent by dry weight of leaves and seeds, which decreased as the salt concentration increased in the diluted seawater.Regarding to spraying salicylic acid treatments significant gradual increases were found in leaves and seeds dry weights. The highest increases were obtained at treatment 200 mg/l salicylic acid as compared with control and the corresponding salinity levels. Salinity treatments decreased N, K, Mg, Ca and micronutrients concentration but the reverse was true for Na concentration. N, K, Mg, Ca and micronutrients concentration was increased by spraying salicylic acid in the rate of 200 ppm where P concentration was not affected. On the other hand Na concentration was decreased by spraying salicylic acid. Salinity lowered the K/ Na, Mg/ Na and Ca/ Na ratios by irrigation plants with solution contains 2500 ppm salts and sharply decreased with the high salt solution used (5000 ppm salts), but the reverse was true for Ca /(K+Na) ratio. Salicylic acid affected on the Na ratios with the other macronutrients. Where K/Na, Mg/ Na and Ca/Na ratios increased by salicylic acid spraying and the reverse was only observed with Ca / (K+Na) ratio ,which decreased by 200 ppm salicylic acid application.It could be concluded that spraying canola plants with salicylic acid has effective role for potential growth regulator improving plant resistance to salinity stress.
Keywords : Canola *(Brassica napusL)*, Salt stress, Salicylic acid, Nutrients concentration, Plant growth characters.

1. Introduction

One-third of the world's irrigated lands and half of the lands of semi-arid and coastal regions are estimated to be affected by different degrees of salinity. About 7 percent of total land area and 30 percent of the potentially arable lands in world has been affected by salinity[1,2]. Excess salt in soil or/and irrigation with saline water are an important environmental factor limiting plant growth and the yield of crops. Plants exposed to high salt concentrations must with stand both the water deficit and the ion imbalance imposed by the salt excess. Although, water deficit always has a negative effect, many crop plants are primarily sensitive to the Na excess [3] due to its adverse effects on K nutrition, cytosolic enzyme activities, photosynthesis and metabolism [4].The depression in photosynthetic is the most severely affected processes through salinity stress [5] which is mediated through a stomatal conductance, internal CO_2 partial [6] and stomatal that affect gaseous exchange [7].

Canola (*Brassica nupus* L) is relative tolerant to salinity and sodicity[8], so that it can cultivated in moderate saline soil and can use some saline water in its irrigation. Some investigators advise to use this crop for edible oil production in the new reclaimed areas without competition with the other winter crops as in the old lands.

Salicylic acid (SA), a naturally occurring plant hormone, acts as an endogenous signal molecule responsible for inducing a biotic stress tolerance in plants [9,10]. Exogenous application of SA may participate in the regulation of physiological processes in plants, such as stomata closure, ion uptake and transport [10], membrane permeability and photosynthesis and growth[11]. Salicylic acid treatment affected the nutrient balances in the plant as reported by [12,13,14,15,16,17]emphasized that exogenous application of SA resulted in a significant increase in plant growth both in saline and non-saline conditions. [18,19] have also shown that salicylic acid (SA) plays a role in the response of plants to salt and osmotic stresses. However, [20] stated that the effects of SA on plant resistance to abiotic stress were found contra dictionary, and the actual role of SA in abiotic stress remains unresolved.

Generally, deficiency or very high levels of SA increases the plant susceptibility to abiotic stress. The optimal levels for the highest stress tolerance range from 0.1 mm to 0.5 mm for most plants. But the role of SA at a certain level in moderate and severe abiotic stress may be different. This can be attributed to redox regulations in plant cells. Therefore, this work designed to investigate the response of canola plants grown under salt stress conditions to exogenous application of different concentrations of salicylic acid.

2. Materials and Methods

For evaluation of canola growth and its minerals content response to irrigation with diluted seawater and foliar spraying by salicylic acid, a pot experiment was conducted in the greenhouse of the National Research Centre, Cairo, Egypt. The salinity treatments were irrigated by: tap water, 250 ppm (S_0), diluted sea water 2500 ppm (S_1) and diluted seawater 5000 ppm (S_2). The foliar treatments were sprayed with: tap water (Control), 100 ppm SA and200 ppm SA.The experiment included 3 levels of salinity in combination with 3 foliar treatments i.e. 9 treatments in 6 replicates. Every pot contained 30 Kg of air dried soil.The soilis clay loam in texture and has pH 7.9, EC 0.65 dSm^{-1}, organic matter 1.3%, $CaCO_3$ 2.53%. Seeds of canola (*Brassica napus* L.) were sown; plants were thinned twice, after four and six weeks from sowing to three plants per pot. Calcium super phosphate (15.5 % P_2O_5) and potassium sulfate (48.5 % K_2O) in the rate of 3.0 and 1.50 g/pot were added before sowing. Ammonium sulfate (20.5 % N) in the rate of 6.86 g/pot was added in two equal portions, after three and five weeks of sowing .Irrigation with diluted sea water in different concentrations were started 30 days after sowing one irrigation by salt water followed by an irrigation by tap water , alternatively. Salicylic acid were sprayed twice, 60 and 90 days after sowing. The control plants were sprayed by tap water. were determined for each pot at maturity stage. In order to determine the dry weight of leaves and seeds and minerals concentration of leaves of canola plants, plants were harvested at maturity from each pot , divided to leaves and seeds, cleaned, dried in oven at 70 ^{0}C and weighted, leaves grounded in stainless steel mill. Digestion and determination of minerals were done using the methods described by [21]. Data collected were subjected to the proper statistical analysis with the methods described by [22].

3. Results and Discussion

3.1.Growth Parameters

3.1.1. Effect of salinity

Negative relationship was shown between salt stress degree and plant growth characters i.e. dry weight of leaves and seeds which decreased as the salt concentration increased in the diluted sea water. Dry weight of leaves was more affected by salinity than seeds especially at high level of salinity (Table 1). Where,dry weight decreased significantly by a percent of 24% in both leaves and seeds at S_1 treatment, but it highly decreased in leaves by 56 % and only by 28 % in seeds as the salinity of irrigated diluted sea water (S_2) increased 20 times compared with control (S_0).[23], confirmed these results on Moringa. In which the growth and mineral uptake are altering extensively in plants grown under salinity stress. The depression in photosynthesis under saline conditions is considered as one of the most important factor responsible for reduction of plant growth [17,24,25].In addition ,inhibited plant growth may be caused by decreased turgidity from high concentrations of salts in the soil under water deficit conditions [26] and by osmosis [27].

3.1.2 Effect of foliar salicylic acid

Table (1): Effect of salinity and foliar salicylic acid and their interactions on leaves and seeds dry weights of canola plants.

Salinity (S)ppm	Salicylic acid (SA) ppm	Leaves Dry weight (g / plant)	Relative variation (%)	Seeds Dry weight (g / plant)	Relative variation (%)
250	0	3.97	100	4.86	100
	100	4.32	109	5.64	116
	200	4.57	115	5.83	120
2500	0	2.99	100	3.63	100
	100	3.32	111	4.28	118
	200	3.50	117	4.47	123
5000	0	1.67	100	3.35	100
	100	1.90	114	4.05	121
	200	2.04	122	4.32	129
Mean values of salinity	250 (S_0)	4.29	100	5.44	100
	2500 (S_1)	3.27	76	4.13	76
	5000 (S_2)	1.88	44	3.91	72
Mean values of salicylic acid	0 (SA_0)	2.88	100	3.95	100
	100 (SA_1)	3.18	111	4.66	118
	200 (SA_2)	3.37	118	4.87	123
LSD at 5 %	S	0.16		0.18	
	SA	0.11		0.16	
	S x SA	0.23		0.15	

Data in Table (1) clearly demonstrate that salicylic acid foliar treatment caused obvious increases in dry weight of leaves and seeds as compared with the untreated control plants and the corresponding salinity levels. Maximum increments in leaves and seeds dry weights were obtained by application of 200 mg/l salicylic acid (SA2) at all salinity levels (S0,S1 and S2). On the other hand, seeds dry weight was more affected by spraying with both 100 ppm and 200 ppm SA than leaves in comparing with control, which the percent increase in seeds ranged from 16 to 29 % and in leaves were from 9 to 22 %. These results are confirmed by the results of [11,17,28,29,30] on different plant species. Accordingly, these increases in leaves and seeds dry weights might be due to that SA stimulate physiological processes which was reflected on improving vegetative growth that followed by active translocation of the photosynthetic products from source to sink in canola plants.

3.1.3 The interaction effects between salinity and foliar salicylic acid

Salicylic acid foliar treatment increased dry weights of leaves and seeds under S_o , S_1 and S_2 treatments, as illustrated in Table (1). The increase percentage of leaves and seeds dry weight enhanced by addition of salicylic acid with raising salinity of irrigation water. The highest values of leaves and seeds dry weight were observed by addition of 200 ppm salicylic acid (SA_2) to plants which irrigated by tap water (S_0) . In contrast, the lowest values of dry weight of both leaves and seeds were recorded in tap water spraying treatment (SA_0) under high salt stress (S_2).In the same manner, [31] showed that salinity caused a marked reduction in growth parameters of Indian mustard (*Brassica juncea* L.) and plants generated by salt stress were completely overcome by the application of SA treatment, whereas, the effect of high concentration of NaCl was reduced partially by SA.

3.2. Mineral Status

3.2.1 Effect of salinity

Data in Table (2) indicated that salinity treatments decreased N, K, Mg, Ca and Zn,Mn, Fe concentration in leaves of canola plants, but the reverse was true for Na concentration. However, P concentration seemed to be without effect with these treatments. Na concentration responded positively to the increase in salt concentration in the root media .The results clearly illustrate that canola plants grown with the high level of salinity (5000 ppm) exhibited an increase of 38% in sodium content and decrease of 18% , 17% , 21 % , 19 % in N,K,Mg and Ca content, respectively , compared to the control .[11] pointed out that plants grown with 50 mM NaCl exhibited also an increase inNa and Cl content, and decrease in N, P, K, and Ca content. Crop performance may be adversely affected by salinity induced nutrients disorder.

Table (2): Effect of salicylic acid foliar spray and salinity on mineral concentration in leaves of Canola plants

Salinity (S)ppm	Salicylic acid (SA)ppm	Macronutrients (%)						Micronutrients (ppm)		
		N	P	K	Mg	Na	Ca	Zn	Mn	Fe
250	0	2.30	0.29	2.60	1.18	2.87	3.83	43	69	85
	100	2.86	0.34	2.70	1.30	2.77	3.93	45	73	90
	200	2.96	0.30	3.20	1.39	2.80	4.06	56	74	98
2500	0	2.18	0.33	2.43	1.07	3.33	3.65	39	57	83
	100	2.72	0.29	2.47	1.12	3.17	3.87	43	58	83
	200	2.60	0.34	2.47	1.13	3.10	3.80	43	59	89
5000	0	1.88	0.22	2.33	0.91	3.97	2.93	35	54	76
	100	2.48	0.25	2.36	1.06	3.77	3.26	38	56	79
	200	2.32	0.30	2.39	1.09	3.57	3.37	40	55	80
Mean values of salinity	250	2.71	0.31	2.83	1.29	2.81	3.94	48	72	91
	2500	2.50	0.32	2.46	1.11	3.20	3.77	41	58	85
	5000	2.23	0.26	2.36	1.02	3.77	3.19	38	55	78
Mean values of salicylic acid	0	2.12	0.28	2.45	1.05	3.39	3.47	39	60	81
	100	2.69	0.29	2.51	1.16	3.24	3.69	42	62	84
	200	2.63	0.32	2.69	1.20	3.16	3.74	46	63	89
LSD at 5 %	S	0.07	N.S.	0.11	0.07	0.18	0.18	1.0	1.1	0.5
	SA	0.10	N.S.	0.11	0.06	0.06	0.10	1.3	0.5	1.1
	S x SA	0.17	N.S.	0.20	0.10	0.11	0.18	2.2	0.9	1.9

However, [32] reported that the relation between salinity and minerals nutrition of crops are very complex. Numerous studies were conducted and reported the disorder in nutrients as a result of salt stress [23,33,34] .

3.2.2. Effect of foliar salicylic acid

Regarding to SA application on macro- and micro-element concentration in leaves of canola plants, data in Table (2) clearly show that, SA treatments decreases the concentration of Na element while increasing the concentration of N,K,Mg and Ca significantly as compared with control plants and the corresponding salinity levels.

These results are consistent with those of [35]for cucumber and [36]for strawberry who found that exogenous SA application inhibited Na accumulation, but stimulated N, P, K, Mg, Fe, Mn and Cu uptake. Also, [23]confirmed these results on Moringa. An increase in concentration of K and Ca in plants under salt stress could ameliorate the deleterious effects of salinity on growth and yield [32]. Alteration of mineral uptake from SA applications may be one mechanism for the alleviation of salt stress. In this study, increased nutrient content seems to be involved in stress-tolerance mechanism and play an important role to enhance the activity of enzymes responsible for salinity resistance. Application of SA might improve physiological performance in terms of production of photosynthesis, total oil and dry matter accumulation, which can be related to increased nutrient uptake by SA-treated, plants [36]. In addition, [37] found that P concentration in Cotton plants not affected significantly with spraying by Salicylic acid while other minerals concentrations affected significantly under salt stress .

3.2.3. The interaction effects between salinity and foliar salicylic acid

The interaction effects of salicylic acid application and salinity were illustrated in Tables (2). These data cleared that plants sprayed by SA showed lower percentage of salinity effect on macronutrients and micronutrients when irrigated by 2500 and 5000 ppm salt treatments. When comparing the mean values of nutrients concentrations (table 2) it was found that N, K, Mg and Ca concentrations were significantly decreased by a percent ranged from 17% to 21% and in contrast Na concentration was significantly increased by a percent of 34% as the salinity of irrigated water was increased 20 times; from 250 to 5000 ppm, irrespective of SA treatments. In contrast, the date show that, N,K,Mg and Ca concentrations were significantly increased by a percent ranged from 8% - 24% and Na concentration was significantly decreased by a percent of 7% with spraying by 200 ppm SA, irrespective of salinity treatments. Similar finding was also observed with respect to concentrations of Zn, Mn and Fe. [36] indicated that SA treatments increased almost contents of all nutrients in leaves and roots of strawberry plants under salt stress. The greatest values were often obtained by the 1.00 mM SA treatment. These findings suggest that the SA treatments can ameliorate the negative effect of salinity on the growth of strawberries. In addition, [11] stated that application of salicylic acid resulted in an increase in the content of nutrients and antioxidative metabolism in mung bean and alleviates adverse effectsof salinity stress through maximum decrease in the content of Na, Cl and electrolyte leakage under saline conditions. Moreover, this treatment increased N, P, K, and Ca content under non saline and saline conditions. It was concluded that 0.5 mM SA alleviates salinity-inhibited photosynthesis and yield through a decrease in Na, Cl, H_2O_2 and electrolyte leakage and an increase in N, P, K, and Ca content, activity of antioxidant enzymes, and glutathione content. Under salinity stress, the amounts of Na severely increased and the amount of K, Ca and Mg decreased. Addition of SA in the culture medium inhibited Na and Cl accumulation, but stimulated K, Ca and Mg contents of stressed plants. These results suggest that SA could be used as a potential growth regulator to improve plant resistance to salinity stress. [38] reported that, in stress conditions, while Na content of leaves increased compared with those plants pretreated with salicylic acid, there was a reduction in K content. Salicylic acid pre-treatment alleviated the adverse effects of salt stress based on Na, K measurement.In this concern,foliar application of salicylic acid at 200 and 400 mg/l counteracted the adverse effect of salinity, this accompanied generally by significant increases in plant growth [39,40] found also that foliar-applied of SA improved turgor potential, and leaf and root Ca concentrations.

3. 3. Mineral Ratios

3.3.1. Effect of salinity

Table (3): Effect of salicylic acid foliar spray and salinity on Na ratios concentration in leaves of Canola plants

Salinity (S)ppm	Salicylic acid (SA)ppm	Na ratios			
		K/Na	Mg/Na	Ca/Na	Ca/(Na+K)
250	0	0.91	0.41	1.34	1.43
	100	0.98	0.47	1.42	1.39
	200	1.15	0.50	1.45	1.48
2500	0	0.73	0.32	1.10	1.58
	100	0.78	0.35	1.22	1.46
	200	0.80	0.37	1.23	1.47
5000	0	0.59	0.23	0.74	2.15
	100	0.63	0.28	0.87	1.88
	200	0.67	0.31	0.95	1.77
Mean values of salinity	250	1.00	0.46	1.40	1.43
	2500	0.77	0.35	1.18	1.50
	5000	0.63	0.27	0.85	1.93
Mean values of salicylic acid	0	0.74	0.32	1.06	1.72
	100	0.79	0.37	1.17	1.58
	200	0.87	0.39	1.21	1.57
LSD at 5 %	S	0.07	0.04	0.11	0.10
	SA	0.03	0.02	0.03	0.06
	S x SA	0.06	0.04	0.06	0.10

Data in Table (3) showed that salinity lowered the K/Na, Mg/Na and Ca/Na ratios. In contrast, Ca/ (Na+K) ratio slightly increased by irrigation plants with solution contains 2500 ppm salts and sharply increased with the high salt solution used 5000 ppm salts. Generally, the ratios of macronutrients to Na (K/ Na, Mg/ Na and Ca/ Na in content basis) decreased as the salt stress increased except Ca / (K+Na) ratio. [8] demonstrated that as soil salinity increased, the K/Na and Ca/Na ratios in the tissues decreased markedly but yields and aerial biomass production were not affected. Similarly ,[41] in borage and echinus plants [42] in different canola cultivars found that the increase in soil salinity resulted in an increase in Na and decrease in K and Ca contents and consequently decrease in K/Na ratio . In spite of the difference between two salt treatments,salt stress can disturb growth and photosynthetic processes by causing changes in the accumulation of Na, Cl, and nutrients, and disturbance in water and osmotic potential. The increasing concentration of Na and Cl in the rooting medium suppresses the uptake of essential nutrients N, P, K, and Ca, and alters ionic relationships. In this concern, [43] reported that NaCl salinity may produce extreme ratios of Na/Ca and Na/K in the plants, causing them to be susceptible to osmotic and specific ion injury, as well as to nutritional disorders.

3.3.2. Effect of foliar salicylic acid

Data presented in Table (3) clear the effect of salicylic acid on the Na ratios with the other macronutrients. Data indicated that K/Na, Mg/Na and Ca/Na ratios increased by spraying of both two levels of SA and the reverse was observed with Ca /(K+Na).[28] stated that acetyl salicylic acid pretreatment (especially 1µM) increased Na content and decreased Kand K/Na ratio and alleviated the adverse effects of stresses on potato plants.

3.3.3. The interaction effects between salinity and foliar treatments

The interactive effects of salicylic acid application and salinity on Na ratios were illustrated in Table (3). Data concerning mean values of both treatments cleared that the highest values of each of K/Na, Mg/Na and Ca/Na ratios and the lowest values of Ca/ (K+Na) ratio were obtained by spraying of 200 ppm SA irrespective of salinity treatments. Nevertheless, the lowest values of each of K/ Na, Mg/ Na and Ca/ Na ratios were obtained by irrigation with water contains 5000 ppm salts, meanwhile, for Ca /(K+Na) it was shown with plants irrigated by tap water (250 ppm) . Furthermore, it is also shown that the highest values of K/ Na, Mg/ Na and Ca/ Na were detected with the plants sprayed by 200 SA and irrigated by tap water. On the opposite side, the lowest ones were with the treatment SA_0 and irrigated by water contains 5000 ppm salts (Table 3). Meanwhile, the highest values of Ca / (K+Na) ratio were obtained by irrigation with water contains 5000 ppm salts and without spraying of SA.The lowest ones were by 100 ppm SA and irrigated by tap water. [23] found that all calculated ratios of mineral concentrations affected significantly by the interaction between salinity and foliar solution with Si plus SA. The values of mineral ratios are fluctuated under irrigation with fresh water. While, under 1st salinity level (2000 ppm) the highest values of all ratios obtained by Si plus SA [44] reported that, shoot and root Na/ K ratio though increased with increase in salinity levels, which was not reversed by exogenous supply of SA. The control treatment recorded the second level in all ratios under the highest salinity level, while sprayed materials fluctuated between 1st and 3rd rank. Salt stress of sea water irrigation led to direct increase in sodium content and produced an increase in Na/K ratio in the aerial parts of button wood plant [45].

4. Conclusion

The main problem with agricultural production in saline conditions is reduced plant growth which is a consequence of several physiological responses including modification of plant water status, decreased turgidity, osmosis , photosynthetic efficiency and carbon allocation and utilization .Application of SA alleviate salt stress, gave highest growth parameters, maximum N, K, Mg and Ca concentrations. Finally these results suggest that foliar salicylic acid could be used as potential growth regulator to improving plant resistance to salinity stress and therefore enhance plant growth and mineral status. In brief, the level of 200ppm SA was the most effective level and its application reduced the damaging action of salinity on canola plants.

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