Interactive effect of salinity and cobalt on tomato plants II- Some Physiological Parameters As Affected By Cobalt And Salinity

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Abstract: The study was carried out at the greenhouse, of Faculty of Agriculture Ain Shams University, during 2003 August 15th to study the interactive effect of salinity and cobalt on growth and mineral composition of two tomato varieties differing in their salt tolerance, namely Moneymaker (as salinity sensitive) and Edcawy (as salinity tolerance). A pot experiment was carried using acid washed sand and 10 kg capacity plastic pots. Five weeks old tomatoes seedlings were transferred to each pot. The saline water used was prepared in five concentrations namely 1000, 2000, 3000 and 4000 ppm. Cobalt was added once at the first week in the form of CoSO₄.7 H₂O in 5 levels: 0.0, 7.5, 15.0, 22.5 and 30.0 ppm cobalt. All treatments were triplicated in complete randomized block design. Ten weeks after transplanting, chlorophyll content, leaf water potential, endogenous hormones (IAA, GAS and ABA), proline content and leaf anatomy were determined. The obtained results could be summarized as follows: In the upper leaves, chlorophyll content decreased with the increase in both salt concentration and cobalt levels over 7.5 ppm. In the lower leaves, chlorophyll content showed stable level at all cobalt levels while it decreased with increasing salinity over 1000 ppm. Leaf water potential increased as salinity increased. Introducing cobalt content to the media decreased leaf water potential and the least figure obtained was recorded at cobalt concentration of 7.5 ppm. When cobalt increased leaf water potential was increased. Endogenous Auxins and Gibberelins increased with increasing cobalt concentration except at higher one (30.0 ppm). As salinity levels rises these hormones were reduced in all cobalt concentrations. Abscisic acid showed gradual increase as cobalt addition increase irrespective of salinity levels. Proline content increased in leaves of all treated plants with the increase of salinity and cobalt levels. Cobalt at 7.5 ppm level has a promotive effect in increasing upper epiderm, pleased, spongy, lower epidermis and blade thickness tissues at all salinity levels.

Key words: Cobalt, salinity, proline, hormones, leaf anatomy, chlorophyll, leaf water potential

INTRODUCTION

Salinity became a serious problem for agriculture, all over the world. Egypt is adopting furrow irrigation-systems and is also expanding in cultivating the desert. Salinity, water shortage and low water quality are the main problems for agriculture production under such circumstances. The north coast of Egypt is comprised of marginal land. The available irrigation water has a relatively high salt content. Other areas in Egypt , and many other areas in the arid zones of the world are experiencing similar problems of increased salinity of soils, and/or irrigation water.

The ability of plants to tolerate excess salts in the rhizosphere is of considerable importance in the arid and semi-arid regions where salinization of soils usually prevails.

There is evidence that cobalt has a positive effect on growth and yield of tomato plants. It is suggested that cobalt reduces the peroxdase activity which is known to affect the breakdown of Indole acitic acid (IAA). Plant hormones, especially abcissic acid (ABA) appear to play an important role in plant water relations through their effect on stomata and abcissic acid reduces opening of stomata.

Thus, the present study attempts to increase salt tolerance of plants by adding cobalt, which might minimize the deleterious effects of salinity on plant growth and osmotic pressure in cell sap and anatomical features, as well as yield productivity of tomato plants.

The adverse effect of salt stress on plant growth is attributed to the specific toxic effect of ions excessively salt ions that are observed from the saline soil, to the process of building up the osmotic potential of the plant

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cells, or to the imbalanced of nutritional cations in tissues of the salt affected planted or due to reduction in carbon fixation during photosynthesis and to increasing carbon release in respiration. The retarding action of salinity is much more sever at the late than the early stages of growth, obviously due to the commutative effect of the salt. Root system was more reduced by salinity than shoot system^[1]. Wooley^[2] found that dry weight, minerals content and yield of tomato plants were increased by 12 % following the application of 1 millimole sodium chloride per liter. These results may be due to the effect of sodium on the physiological processes of plant particularly the substitution of inadequate potassium by sodium. He also, stated that if sodium is essential for growth and development of tomato plants, it would be required in amounts less than 0.1 micro mole per gram dry weight of plants.

Bernstein^[3] suggested that the reduction in growth associated with osmotic stress was attributed to the building up of the osmotic pressure of the root media. It was observed that up to about 30% reduction in seedling fresh weight has occurred. However, there were no changes in shoot dry matter percentage. Mayber and Gale^[4] stated the salinity causes a destruction of chlorophyll which is correlated with the lowering of photosynthetic rate. They added that the decrease in chlorophyll content was due mainly to the destruction of chlorophyll "a". Kalikinskaya[5] found that slight increase of leaves chlorophyll (a + b) content in the salt resistant cv. yusupovskil and decrease in it in cvs. with low salt resistant tomato plants. Rajasekaran and Shonmugavel reported that chlorophyll a and b carotenoids were decreased in all salinity treatments.

Under salinity conditions, [7] found that cobalt increased water content in pea plants. The rates of both and transpiration processes have photosynthesis decreased but stomatal resistance were increased. Stewart^[8] reported that proline oxidation rates were similar in leaves incubated in Abscisic acid (ABA) in water even though the proline level in ABA-treated leaves was 2.5 times the level in the water-treated controls. These results indicated that the metabolic cause of ABAinduced proline accumulation is the stimulation of proline synthesis for glutamic acid. Nadia Gad^[9] has also indicated that ABA prevented the stomatal opening and caused their closure in several plant species. ABA levels in shoots and roots of their tomato or squash increased with increasing cobalt concentration in the growth media. Lipskaya et al [10] pointed out that low level of applied cobalt increased the leaf area as well as the size of chloroplasts in potato plants. They added that application of 2 kg/ha cobalt increased both number and surface area of chloroplasts/unit leaf area, leaf pigment content and leaf area. Walser et al^[11] added, that application of 2.7 kg Co/ha of a field contain 0.81 mg available Co/kg soil increased tomato leaf number as well as surface of chloroplasts/unit leaf area, leaf chlorophyll content, leaf area and rate of photosynthesis, hormonal response were possibly involved.

Sourial et al^[12] on Hindy mango seedlings, found that all tested salinity levels negatively affected the vessel elements and xylem parenchyma of both root and stems while increased their fibers. The leaves of salt-affected seedlings showed thicker transverse section, thicker palisade and spongy tissues as well as higher stomatal frequency than the control. Abd El-Ghani^[13] on peach plants, Abd El-Moteleb^[14] on grape vine and Abd El-Karim^[15] on mango reported similar results. Ponomareva and Kabuzenko^[16] on cotton plant, Porter and Gausman^[17] with tomato plants grown under NaCl salinity, showed that high NaCl salinity and its duration cause reductions in cell expansion of the palisade and spongy paranchyma as well as epidermal cells, laf thickness being increased.

Kaul^[18] grew guva plants cv. Lucknow-49 in nutrient culture with NaCl, Na₂SO₄ or CaCl₂ or All three salts at electrical conductivities of 6, 9, and 12 dS m⁻¹, respectively. The salt stress increased proline content. Also,^[19] reported that inter-cellular concentrations of proline increased with decreasing external water potential from 4 to 28 bars in cultured tomato cv. VF.NT. cherry. Similarly ^[20] irrigated tomato plants with water of 0.9, 1.5, or 4.5 dS m⁻¹ EC. They pointed out that proline contents rose with increasing substrate salinity. Morever,^[21] indicated that proline concentration in guava leaves increased by salinity stress especially at treatment of 4000 and 6000 ppm.

MATERIALS AND METHODS

The experiment was designed to study the interactive effect of concentration cobalt ion and salinity on some physilogical parameters, of two tomato varieties differing in their salt tolerance, namely Moneymaker (as a salinity sensitive variety), and Edcawy (as a salinity tolerant one).

Seeds of tomatoes (*Lycopersicon esculentum Mil, c.v.* Edcawy and Moneymaker) were sown on August 15th 2003 in trays filled with a mixture of sand and peatmoss (1:1 volume basis). Trays were kept under greenhouse condition with all agriculture managements required for production of tomatoe seedlings. Plastic pots 35 cm diameter and 12 kg capacity were filled with 10 kg acid washed sand.

Five weeks-old seedlings, with almost the same stem thickness, were transplanted the prepared pots (four seedlings per pot). Irrigation with the prepared saline water was carried out every 5 days to keep the soil almost at its field capacity.

The saline water use was prepared by mixing NaCl, CaCl₂ and MgCl₃ salts according to^[22] as follows:

 $3{NaCl}: 1 { 3 (CaCl_2) + 1 (MgCl_2)}$

This saline mixture were prepared in five concentrations: 0.0, 1000, 2000, 3000 and 4000 ppm. Cobalt was added in the form of CoSO₄. 7 H₂O in 5 levels: 0.0, 7.5, 15.0, 22.5, and 30.0 ppm cobalt. These concentrations were added once at the beginning of the experiment. Moisture content in the pots were kept constant at 70 % of the total holding capacity throughout the experiment by compensating the loss of water due evapotranspiration by distilled water. In addition, 500 cm³/pot of complete Hogland solution^[23] was added at biweekly intervals till the end of the experiment as a nutritive resource. Complete randomized blocks design with three replicates were adapted and the obtained data were statistically analyzed at 5 % level according to the^[24].

Ten weeks after transplanting, leaf water potential was determined in the third mature upper leaf using leaf water potential set (PMS Instrument Company, Covallis, Oregon).

Chlorophyll was determined in fresh plants material of samples representing both third upper and third lower leaves using chlorophyllmeter Spad 502^[25].

Fresh samples of both shoots and roots were taken for analysis of endogenous hormones (Auxins, Gibberlins and Abcisic acid), the modified version of the procedure used by^[26] being adopted using a Perken-Elmer SIGMA 3B Gas Chromotograph apparatus.

Leaf fresh samples (0.5 g) were taken for determination of proline content according to [27].

Thin cross section of fresh new formed leaves were taken and kept in formaline acetic acid (FAA) medium for anatomical study as reported by^[28].

RESULTS AND DISCUSSIONS

Chlorophyll content: Table (1) shows data concerning the effect of applied cobalt on the green pigments in both upper and lower leaves under salinity conditions. The visual observation for tomato plants growing salinity levels, treated with cobalt showed that adverse effect firstly appear with 30.0 ppm cobalt under all salinity levels. Chlorosis being appeared 4 days from transplanting. Increasing salinity levels play an important role in reducing toxicity symptoms. Chlorosis started at 27-28 days at the concentration of 3000 ppm saline and

15.0 ppm cobalt. Necrosis has appeared one week from chlorosis. In plants treated with 2000 ppm saline water and 22.5 ppm cobalt, chlorosis started at 22-23 days, necrosis appeared 5 days later. Data also revealed that Edcawy plants were less affected by high salinity and cobalt levels, than Moneymaker plants. Under 4000 ppm saline water and 30.0 ppm cobalt, Moneymaker plants died. These results reflected that cobalt has a vital role in Moneymaker resistance to salinity and in water balance in tomato plants. Edcawy plants were more resistant to higher salinity concentration and high cobalt levels. The effect of salinized irrigation water on chlorophyll content of tomato plants followed a linear relationship with the increase of cobalt in plant media included its effect in lower leaves. Low cobalt level (7.5 ppm) being with positive effects due to several inducive effects in hormonal synthesis and metabolic activities^[29]. Higher cobalt levels were found to increase the activity of some enzymes such as peroxidases, catalases in plant and hence increasing the catabolism rather the anabolism. Cobalt concentration over than 7.5 ppm hazardous effect of iron status (Tables 2 and 3). Bisht[30] reported that cobalt and iron are competitive elements in the nutrition of tomato plants. Cobalt seemed to have efficient role in iron translocted from lower old leaves to the upper young upper leaves. These results are in harmony with those reported by[31].

Leaf water potential: Table (2) clearly indicated that increasing salinity levels in plant media increased tomato leaf water potential. Results demonstrating that 7.5 ppm treatment was associated with lowest leaf water potential of both tomato varieties. The greatest effect of cobalt on leaf waters potential in Edcawy (salt-tolerant) plants than in Moneymaker (salt-sensitive) plants at all salinity levels. In spite of continuity of beneficial effects sometimes obtained for 15.0 ppm cobalt compared with control addition of more than 15.0 ppm cobalt confirmed the hazardous effect of leaf water potential. These results agree with those of (16) who found that the observed beneficial effects of cobalt on growth of salinized tomato plants could be partially ascribed to an increase in the leaf water potential relative to those untreated with cobalt. The higher leaf water potential could enhance the photosynthesis process directly by influencing the photosynthesis system or indirectly by decreasing the total leaf resistance to the diffusion of CO₂ into the leaf.

Finally, the results pointed out that cobalt level (7.5 ppm) help tomato plants to resist stresses caused by high salinity and increase the water absorbance capacity and strongly bounds H₂O.in the leaves of tomato plants were growing in the new reclaimed soils.

Table 1: Effect of various levels of cobalt on tomato chlor	ophyll content (spa	d) as affected b	y salinity application.
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Co (ppm)	Zero	Zero		7.5			22.5		30.0				
Salinity levels (ppm)	ity levels (ppm) Edcawy Moneymaker Edcawy Moneymaker		Edcawy Moneymaker		Edcawy Moneymaker		Edcawy Moneymaker						
	In upper leaves												
Control	52.6	49.5	54.2	50.2	50.2	46.0	44.5	41.0	39.4	36.0			
1000	51.2	38.6	52.6	43.0	51.0	35.2	50.3	29.0	48.0	24.1			
2000	48.0	27.1	49.6	29.5	46.3	23.1	41.5	18.6	39.3	18.0			
3000	42.4	16.7	43.5	20.7	40.8	14.2	39.2	11.5	33.6	10.3			
4000	35.0	-	37.3	-	31.5	-	26.0	-	21.1				
				In low	er leaves								
Control	54.5	52.2	54.1	52.0	54.2	51.5	54.3	52.2	54.5	51.8			
1000	54.2	50.8	54.1	51.7	54.0	50.0	54.2	50.9	54.2	51.0			
2000	43.0	49.9	50.6	46.8	47.5	43.2	41.3	37.5	36.0	32.3			
3000	48.2	49.5	46.5	42.0	42.5	38.4	39.2	35.4	32.1	28.0			
4000	42.5	40.0	37.7	33.5	33.6	29.7	30.0	26.5	27.2	23.3			

Table 2: Effect of various levels of cobalt on tomato leaves water potential (bar) as affected by salinity application.

Co (ppm)	Zero		7.5		15.0		22.5		30.0	
Salinity levels (ppm)	Edcawy	Moneymaker								
Control	-7.0	-7.2	-3.7	-5.5	-7.5	-9.7	-8.0	-11.3	-12.6	-14.2
1000	-9.5	-10.0	-5.2	-6.9	-10.5	-12.2	-11.4	-14.4	-13.9	-16.5
2000	-12.0	-12.9	-8.1	-9.30	-12.0	-14.2	-14.3	-18.9	-16.6	-21.5
3000	-14.2	-15.0	-10.0	-11.6	-14.3	-16.3	-17.1	-21.1	-19.0	-26.3
4000	-16.5	-17.3	-12.4	-13.8	-16.8	-17.0	-19.5	-24.0	-21.5	-29.0

Table 3: Effect of various levels of cobalt on tomato of ABA, IAA and GAs (ug/fresh tissue) as affected by salinity application.

Co (ppm)		Zero		-7.5		15.0		22.5		30.0	
Salinity	levels (ppm)	Edcawy Moneymaker		Edcawy Moneymaker		Edcawy Moneymaker		Edcawy Moneymaker		Edcawy Moneymak	
					S	hoot					
Zero	ABA	-	-	0.870	0.995	1.506	1.267	2.936	2.789	4.058	3.879
	IAA	1.720	1.661	5.001	4.875	3.490	3.189	2.914	2.860	1.152	1.068
	GA_{S}	1.902	1.840	3.854	3.688	2.762	2.554	1.979	1.951	1.501	1.391
	ABA	1.089	1.287	1.456	2.767	2.038	2.993	3.367	4.122	4.022	5.656
	IAA	1.589	1.503	2.750	2.671	2.670	2.452	2.440	2.201	1.428	1.261
	GA_s	1.841	1.778	3.545	3.336	2.495	2.288	1.977	1.705	1.332	1.86
					F	Root					
Zero	ABA	-	-	1.215	1.871	2.145	3.649	3.576	4.368	4.787	4.223
	IAA	1.844	1.776	3.742	3.645	2.583	2.363	2.196	2.146	1.283	1.187
	GA_s	1.972	1.907	2.046	1.967	2.254	2.085	1.998	1.773	1.386	1.284
4000	ABA	0.789	0.663	2.595	3.945	5.284	2.246	6.632	7.425	6.896	7.521
	IAA	1.485	1.411	3.182	5.032	3.684	3.270	2.023	1.825	1.284	1.135
	GA _s	1.729	1.659	3.592	3.281	2.901	2.663	1.866	1.589	1.403	1.248

Endogenous hormones: Table (3) clearly indicates that content of endogenous Auxins (IAA) and Gibberelins

(GAS) increased with 7.5 , 15.0 and 22.5 ppm cobalt in both varieties unsalinized plants. Increasing salinity levels

Table 4: Effect of various levels of cobalt on tomato of proline (g/100g dry weight) as affected by salinity application.

Co (ppm) Zero			-7.5		15.0		22.5		30.0	
Salinity levels (ppm)	Edcawy	Moneymaker	Edcawy	Moneymaker	Edcawy	Moneymaker	Edcawy	Moneymaker	Edcawy	Moneymaker
				Start	ing date					
Control	0.14	0.15	0.16	0.16	0.15	0.16	0.15	0.16	0.15	0.16
1000	0.14	0.15	0.18	0.18	0.19	0.20	0.22	0.23	0.24	0.25
2000	0.15	0.16	0.19	0.21	0.22	0.23	0.25	0.26	0.27	0.29
3000	0.16	0.17	0.21	0.23	0.25	0.26	0.28	0.29	0.30	0.33
4000	0.16	0.18	0.23	0.25	0.27	0.29	0.31	0.32	0.33	0.36
				End of e	experiment					
Control	0.14	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
1000	0.18	0.19	0.21	0.22	0.24	0.26	0.25	0.27	0.27	0.30
2000	0.20	0.21	0.25	0.26	0.28	0.30	0.29	0.81	0.30	0.34
3000	0.23	0.25	0.29	0.30	0.31	0.34	0.33	0.35	0.34	0.39
4000	0.27	0.29	0.33	0.34	0.35	0.38	0.37	0.35	0.38	0.42
				Proline in	ncrease (%					
Control	100.00	100.00	100.00	100.00	106.67	100.00	106.67	100.00	106.67	100.00
1000	128.57	126.67	116.67	122.22	126.32	130.00	113.64	117.39	112.50	120.00
2000	133.33	131.25	131.58	123.81	127.27	130.43	100.00	311.54	111.11	117.24
3000	143.75	147.06	138.10	130.43	124.00	130.77	103.57	120.69	113.33	118.18
4000	168.75	161.11	143.48	136.00	129.63	131.03	106.45	109.38	115.15	116.67

Table 5: Leaf anatomy of tomato from respective cobalt as affected by salinity application.

			_		40	000 ppm salini	ty							
Cobalt treatment (ppm)		Edcawy							Moneymaker					
	Cuticle	Upper epiderms	Spongy tissue	Paliseede tissue	Lower epiderms	Blade thickness	Cuticle	Upper epiderms	Spongy tissue	Paliseede tissue	Lower epiderms	Blade thickness		
				 (μ)		(μ)								
Zero	0.18	6.2	206.9	321.1	4.4	538.8	0.7	5.9	180.9	220.1	4.1	411.8		
7.5	0.19	6.4	220.6	430.2	4.6	662.3	0.8	6.1	196.1	240.9	4.3	558.2		
15.0	1.50	4.3	130.1	223.1	3.1	362.1	1.1	5.1	170.1	190.1	4.0	369.9		
22.5	1.60	4.3	125.2	220.2	3.2	354.7	1.3	4.9	175.2	191.8	3.9	376.3		
30.0	1.80	4.1	100.9	190.7	3.3	201.0	1.6	4.1	160.9	170.1	3.8	339.5		
LSD 5%	0.11	0.2	5.1	5.0	0.2	8.3	0.1	0.2	5.3	2.1	0.1	7.5		

reduced IAA and GAS of both varieties shoot and root. Auxins and Gibberelins concentration of both varieties roots higher than in shoots. Concentration of Auxins and Giberelins was much higher for Edcawy than for Monymaker plants. This was true for both shoots and roots of the unsalinized plants or plants grown under salinity condition. On the other hand, the abscisic acid (ABA) showed gradual increase as cobalt concentration incraese irrespective of salinity levels of both varieties tomato plants. However, the ABA concentration in roots

was higher than in shoots at all levels of salinity. The content of ABA was much higher in salt sensitive plants (Moneymaker) than in salt-tolerance plants (Edcawy). Cobalt help tomato plants to resist stresses caused by high salinity. The vital role of ABA in adjusting plant water balance under salinity levels and could modify the plant water economy before the leaves became stressed. These results agree with those of^[29]. Responses of salinized tomato plants receiving cobalt were suggested to be attributed to effects of cobalt on transpiration

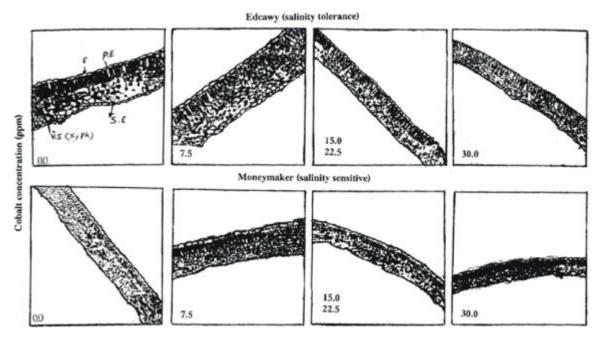


Fig. 1: Leaf anatomy of tomato grown under saline condition 4000ppm as affected by cobalt application

process. When plant roots absorb water, soil solution moves from the non-rhizosphere soil towards roots by mass flow. If these salts are transported to the root surfaces at a greater rate than they are usually absorbed, they tend to accumulate. These results are in harmony with those reported by^[32] and ^[31].

Proline content: Data in Table (4) noticed that proline content increased in leaves of all treated plants with the increase of salinity level. At the beginning of the experiment, no significant differences in leaves proline content was noticed. On the other hand, at the end of the experiment period, leaves proline concentration markedly increased with the increase in salinity level. Besides, cobalt increases proline content at all salinity levels. Increasing salt levels from the control (tap water) to 4000 ppm, the increase in proline concentration of tomato leaves was significant generally when salinity level was raised to 2000 ppm or more. The obtained results confirm those previously discussed by[19] and [21] on guava,[20] and on tomato. They found that the salt stress increased proline content. Cobalt help tomato plant to resist stresses caused with high salinity. The vital role of cobalt in proline biosynthesis, in modifying the plant water economy in tomato leaves of both varieties was confirmed. Proline content was much higher in Moneymaker (salt-sensitive) than in salt-tolerant (Edcawy).

Leaf anatomy: Data in Table (5) and Fig. (1) illustrate the

effect of different concentrations of cobalt on two tomato varieties (Edcawy and Moneymaker) under salinity condition zero and 4000 ppm.

Firstly, they showed that there no considerable differences in histological features of leaves transplants treated with cobalt at 15.0 and 22.5 ppm, these two treatments were presented by one Figure.

The obtained results showed that, Edcawy tomato plants greatly affected with cobalt levels compared to Moneymaker one. Data clearly indicated that cuticle tissues was increased as cobalt addition increased of two varieties. This indicates that 7.5 ppm cobalt has a promotive effect in increasing upper epiderm, plaseed, spongy, lower epidermis and blade thickness tissues. In fact, cobalt help tomato plants to resist stresses caused with high salinity.

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