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ORIGINAL ARTICLES

Barley Response to Salt Stress at Varied Levels of Cobalt II. Some Physiological and Chemical Characteristics

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ABSTRACT

Two field experiments were carried out at El-Hamoul- district Kafr- El-Seikh governorate, Delta Egypt as a saline soil, to study effect of cobalt on barely growth, yield quantity and quality, under salinity condition. Barley seedling at the third truly leaf were irrigated once by cobalt concentrations. Cobalt was added in the form of $CoSO_4$ $7H_2O$ in 8 levels: 0.0, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 ppm. After heading stage, leaf water potential, endogenous hormones (IAA, GAS, cytokinine and ABA) and proline content were determined. The obtained results could be summarized as follows:

- Cobalt at 10.0 ppm decrease leaf water potential and gave the highest IAA, GAS and cytokinine contents.
- Abscisic acid showed gradual increase as cobalt addition increased.
- Proline content increased in leaves at all cobalt levels.
- Cobalt at 10.0 ppm enhanced the status of N, P, K, Mn, Zn and Cu in barley seeds while increasing cobalt level.
- Increasing cobalt concentration in plant media up to 10.0 ppm had a promotive effect on seeds chemical constituents.
- Increasing cobalt levels in plant media above 10.0 ppm decreased the promotive effect.

Key words: Barley - Cobalt- Salinity- Hormons- Proline content- Nutritional status.

Introduction

Barley *(Hordeum vulgare L)* is the main cereal crop grown in salt affected area in Egypt. A considerable decrease in grain yield of cereals following the irrigation with saline water has been observed. The negative effects of salinity on growth were reported by Hamdy *et al.,* (2005).

Generally, barley is considered as salinity tolerant crop. It has been particularly satisfactory as one of the early crops planted in the process of reclamation saline soils. The threshed salinity tolerance of barley was listed as 8 ds/m (Ayers and Westcot, 1985), but Hassan *et al.,* (1970) found no decrease in the production of dry matter by vegetative parts up to an EC of the soil solution at 12 ds/m. The indication of good salinity tolerance at one growth stage such as germination and seedling does not necessarily mean that other stages will also have good salt tolerance. Nadia Gad and Hala Kandil (2011) pointed that cobalt help wheat plants to tolerate high salinity in El-Hamoul location- Delta Egypt and increased plant growth and yield under high salinity (26.6 ds/m) conditions.

All over the world Salinity became a serious problem for agriculture. Egypt still adapting furrow irrigation system and also expanding in cultivating desert. Salinity, as well as water shortage and low water quality are the main problems for agriculture production under such circumstances. The north coast of Egypt comprised of marginal soils ready for agriculture. The available irrigation water there has a relatively high salt content. The ability of plants to tolerate excess in the rhizosphere is of considerable importance in the arid and semi-arid regions where salinization of soils usually prevails (Abdel Gawad *et al.,* 1987).

Saline soils have salt levels high enough that either crop yields begin to suffer or cropping is impractical. Excessive salts injure plants by disturbing the uptake of water into roots and interfering with the uptake of competitive nutrients (David Franzen, 2007).

Soil salinity is a considerable problem adversely affecting physiological and metabolic processes, finally diminishing growth and yield (Ashraf and Harris, 2004). Salinity is known to retard plant growth through its effect on several facts of plants behavior like osmotic adjustment, ion uptake, protein and nucleic acid synthesis, photosynthesis, enzyme activities and hormonal balance (Dumbroff and Cooper, 1974). Under salinity conditions, Nadia Gad (2005 b) pointed out that cobalt was used to reduce the harmful effect of salinity on tomato plants, transpiration rate being reduced. Angelove *et al.,* (1993) and Stewart (2001) found that cobalt

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reduced the salinity and /or ethrel injury on tomato plants, a suggestion being introduced for possible use of cobalt to irrigate transplants with saline water to overcome the salinity hazard. 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.

Cobalt is border elements; it has a positive effect on higher plants to adapt water stress conditions, which include salinity and drought. Mobility of cobalt was greatest with the saline soils but lowest with alkaline ones (Kumari, 2002). Shanon (2002) pointed out that cobalt was used to reduce the harmful effect of salinity on tomato plants, where the transpiration rate being reduced. Rathsooriya and Nagarajah (2003) attributed the beneficial effect of cobalt on growth of salinized pea plants to an increase in the leaf water potential relative to those untreated plants. The higher leaf water potential could enhance the photosynthesis process. Radin (2004) found that cobalt increased water content in cotton leaves, the rates of both photosynthesis and transpiration have decreased but stomatal resistance was increased. Kaul (2004) stated that, the salt stress increased proline content in guava *(cv. Lucknow-49)* plants especially with 6000 ppm salinity. He also, reported that intercellular concentration of proline increased with decreasing external water potential. Nadia Gad (2005 a) indicated that cuticle tissues of tomato leaves was increased as cobalt addition increased. 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. Stewart (2001) showed 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 (control). These resulted the metabolic cause of ABA induced proline accumulation is the stimulation of proline synthesis for glutamic acid. Nadia Gad (1989) demonstrated that ABA prevented the stomatal opening and caused their closure in several plant species. Abscisic acid (ABA) levels in shoots and roots of both tomato and squash increased with increasing cobalt concentration in the growth media. Jaleel *et al.,* (2009) stated that cobalt is an essential element for humans and animals. The Co- containing vitamin B_{12} doses. It is necessary for normal metabolic functions of the plant.

The Egyptian policy aims to increase the productivity of barley for reducing the gab between Barley production and consumption. Therefore, barley cultivation should be extended in more areas particularly in the newly reclaimed soils and Delta Egypt.

Materials and Methods

Soil Analysis:

Physical and chemical properties of El-Hamoul soil, are shown in Table (1).

Soil sample (Taken from El-Hamoul) was dried and then prepared for analysis using the conventional techniques.

Particle size distribution along with soil moisture parameters of the soil sample was determined as described by Blackmore (1972). Soil organic matter, $CaCO_3$, EC, pH, cations and anions were determined according to Black *et al.,* (1982). Soluble and available micronutrients as well as soluble, available and total cobalt were determined according to Cottenie *et al.,* (1982).

According to the Soil Taxonomy (1996) and Soil Map of Egypt (1982) the selected El-Hamoul location-Kafr El-Sheikh Governorate as saline soil (Typic Salorthids).

Plant Material and Experimental Works:

Two Field experiments were conducted at the agricultural experimental station of Delta Egypt as Kafr El-Sheikh in El- Hamoul to study the effect of different cobalt concentration on growth, yield, yield attributes as well as grain quality of barley grains.

- Preliminary experiment was conducted using soil sample from El-Hamoul- Kafr El- Sheikh Governorate Delta Egypt to define the suitable concentrations of cobalt on barley growth.
- The soil was clayey with plot area will be 21 m^2 (4.2 m wide by 5.0 m long).
- Plot width allowed for six rows as (70 cm rows). Barley grains were sown in constant spaced hills 25 cm on one side of ridge, at approximately 28000 plant fed^{-1}.
- Barley grains *(Hordeum vulgare L cvs. Mill Giza 2000)* were broadcasted on the soil at the rate of 80 kg fed^{-1} on both seasons. The experimental unite (plot) area was 21 m^2.
- The grains were sown at the second week of November in both seasons. The other cultural practices were performed in May in the first and second seasons.
- The seedlings (at third truly leaves) were irrigated only once with different cobalt levels (5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 ppm).

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Determination of Endogenous Hormones:

Auxins (IAA), Gibberelines (GAS), cytokinins and abscisic acid in barley shoots were determined according to Shindy and Smith (1975).

Table 1: Some physical and chemical properties of the used soil at El-Hamoul- Kafr El- Sheikh gavarnorate- Delta Egypt.

Physical	Particle size distribution (%)			Soil texture class	Saturation	Field capacity	Welting point	Available water
	Sand	Silt	Clay		%			
	19.25	15.64	65.11	clayey	64.8	46.2	15.9	30.3

Chemical	pH^a	EC^b	Soluble cations (meq/l)				Soluble anions (meq/L)			
			Ca^{++}	Mg^{++}	K^+	Na^+	HCO_3^-	CO_3	Cl^-	$SO_4^=$
	8.6	26.6	47.5	23.7	0.15	167	0.80	-	178	69.5

Total	Available macronutrients		Available micronutrients				CEC	ESP	Cobalt (ppm)			$CaCO_3$	$O.M^c$
(mg/100g)			(ppm)				meq/100g soil		Soluble	Available	Total		
N	P	K	Fe	Mn	Zn	Cu						%	
3.2	3.0	8.1	7.5	2.0	1.0	5.0	58.3	14.2	0.34	4.46	16.9	2.71	1.20

a) Soil pH was measured in 1 : 2.5 soil-water suspension.
b) EC was measured as dSm^{-1}in soil paste.
c) organic matter.

Determination of Proline Content:

Fresh leaves sample (5 g) were taken for this determination according to Bates *et al.,* (1973).

Measurement of Nutritional Contents:

- Macronutrients (N, P and K) and micronutrients (Fe, Mn, Zn and Cu) of barley seed as well as cobalt were determined according to Cottenie *et al.,* (1982).

Measurement of Chemical Constituents:

- The concentrations of the studied chemical contents percentages (total carbohydrates, total sugars, protein and lipids) in barley seeds were determined according to A.O.A.C. (1970).
- Combine data of the two seasons was statistically analyzed according to Snedcor and Cochran (1982).

Results and Discussion

Endogenous Hormones:

Data presented in Table (2) showed that, all cobalt treatments had increased shoots endogenous hormones such as Auxins (IAA), Gibberelines (GAS) and Cytokinines in barley plants grown under salinity condition compared with control. Cobalt at 10.0 ppm recorded the significant of barley phytohormones. Increasing cobalt levels up to 10.0 ppm significantly increased these hormones. However, higher cobalt levels above 10.0 ppm has depressive effect and caused reduction in endogenous barley hormones due to the effect of soil salinity. Endogenous plant hormones such as Auxins, Gibberelines and cytokinines are known to be involved in the regulation of plant response to salinity stress and counteract the adverse effect of stress conditions. Plant hormones as natural products, can stimulate physiological response to high soil salinity. Different strategic are being employed to maximize plant growth under saline conditions. In fact, the levels of Auxins, Gibberelines and cytokinines under salt stress are help plants to tolerate the high soil salinity. These results are in harmony with those obtained by Cassan *et al.,* (2001) who found that the effect of applied IAA and GAS in alleviation of salt stress might be through the activation of a specific enzyme, which participates RNA and protein synthesis. Results also, was in agreement with those obtained by Shaddad *et al.,* (2008) who showed that, in wheat, while seed germination decreased with increasing salinity levels, the adverse effect of salinity was alleviated by soaking seed with Auxins (IAA or NAA). Confirm these results Hamdia and Saddad (2010) stated that, improving plant hormones synthesis, enhanced plant tolerance to salinity injury.

The presented data in Table (2) also indicated that Abscisic acid (ABA) showed a gradual increase as cobalt concentration increased irrespective to high salinity of the soil. The obtained results indicated that, under saline conditions, abscisic acid (ABA) prevented the stomatal opening and caused their closure in barley plants and reduce the transpiration rate. These results are in harmony with those obtained by Nadia Gad (1989 and 2005a) who demonstrated that ABA prevented the stomatal opening and caused their closure in both tomato and squash plants. Abscisic acid levels in both shoots and roots of the studied plants increased with increasing cobalt concentration in plant media. Abscisic acid help barley plants to tolerate high soil salinity. Confirm these results Tuna *et al.,* (2007) who stated that treatment wheat seedlings grown under saline condition with ABA development of antistress reactions.

Table 2: Effect of cobalt on hormones content and porline acid of barley plants grown in salinity soil (El-Hamoul) mean of two seasons.

Treatments	Auxins	Gibberelines	Cytokinines	Abscisic acid	Porline acid
Cobalt (ppm)	(μg/gm Fresh weight of shoots)				(gm/100gm dry leaves)
Control	1.345	1.270	1.270	0.469	0.96
5.0	1.431	1.321	1.315	1.586	1.17
7.5	1.498	2.146	2.151	2.689	1.69
10.0	2.542	2.381	2.460	3.491	2.23
12.5	2.508	2.358	2.211	3.824	2.51
15.0	2.488	2.332	2.184	3.889	2.76
17.5	2.456	2.327	2.153	3.891	2.83
20.0	2.432	2.321	2.124	3.896	2.88
LSD 5%	0.012	0.002	0.001	0.002	0.001

Porline Content:

Data in Table (2) clearly indicated that, when cobalt concentration increased, proline content in barley leaves also increased. At the beginning of the experiment, no significant differences in leaves proline content were noticed. On the other hand, at the end of the experiment period, leaves proline concentration markedly increased under saline conditions. The obtained results confirmed with those previously discussed by Kaul (2004) and Nadia Gad (2005a) on both guava and tomato. They found that the salt stress effect increased proline content. Cobalt help tomato plant to resist stress caused by high salinity. The vital role of cobalt in porline 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 *Edcawy* (salt- tolerant). Yan et al., (2000) added that, proline can also protect cell membranes from salt-induced oxidative stress by enhancing activities of various antioxidants.

Leaf Water Potential:

Data presented in Fig. (1) indicated that, addition of different cobalt levels (0.0, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0 ppm) in plant growth media (El-Hamoul saline soil) significantly decreased barley leaf water potential. These results are in harmony with those obtained by Nagarajah and Rathsooriya (1977) who found that the observed beneficial effect of cobalt on growth of tomato plants on salinized could be increase in the leaf water potential relative to compared those untreated with cobalt The 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 CO_2 into the leaf.

Finally, the obtained results pointed out that cobalt level (10.0 ppm) help barley plants to resist stresses caused by high soil salinity and increase the water absorbance capacity and strongly bounds H_2O in the leaves of barley plants growing in the high saline soils.

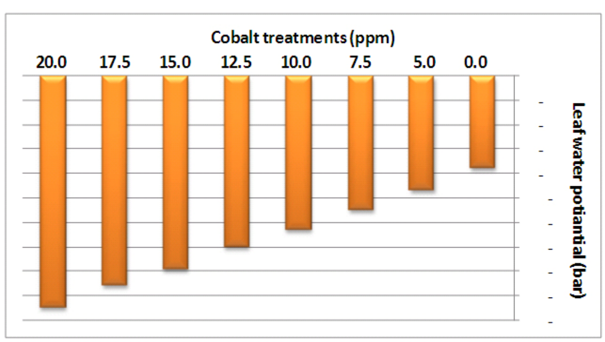

Fig. 1: Effect of cobalt on leaf water potential of barley grown in salinity soil (El-Hamoul) mean of two seasons.

Nutritional Content of Barley Seeds:

Presented data in Table (3) showed the effect of different cobalt levels on mineral composition of barley seeds. Data revealed that, under saline condition, all used levels of cobalt significantly increased the content of macronutrients (N, P and K) as well as micronutrients (Mn, Zn and Cu) of barley seeds compared with control. The highest significant figures of N, P, K, Mn, Zn and Cu were obtained by using cobalt at 10.0 ppm. Increasing cobalt in plant media more than 10.0 ppm decreased this promotive effect.

Table 3: Effect of cobalt treatments on element content of barley seeds grown in salinity soil (El-Hamoul) mean of two seasons.

Treatments Cobalt (ppm)	Macronutrients (%)			Micronutrients (ppm)				Co (ppm)
	N	P	K	Mn	Zn	Cu	Fe	
Control	0.80	0.47	0.83	3.01	1.46	2.91	8.11	0.87
5.0	0.93	0.89	0.97	3.29	1.94	3.44	7.89	1.11
7.5	1.06	1.01	1.41	3.88	2.59	3.89	7.33	1.93
10.0	1.45	1.66	1.94	4.31	2.98	4.35	6.90	2.19
12.5	1.33	1.34	2.42	4.89	3.33	4.91	6.66	3.81
15.0	1.19	1.30	2.36	4.76	228	4.84	6.42	5.46
17.5	1.04	1.26	2.32	4.71	2.21	4.78	6.36	6.70
20.0	0.96	1.23	2.29	4.64	2.17	4.71	6.29	7.92
LSD 5%	0.05	0.03	0.04	0.11	0.20	0.09	0.12	0.06

Data in Table (3) also indicated that, increasing cobalt concentration in saline plant media resulted in a progressive depression effect on iron content in the seeds of barley grown for two seasons. These data are in harmony with those obtained with Blaylock *et al.*, (1993) and Nadia Gad and Abd El-Moez (2011) who found that increasing cobalt levels in plant media being the adverse reduction of Fe content in tomatoes, soybean plant and broccoli heads.

Cobalt Content:

Increasing cobalt concentration in plant media increased cobalt content in barley seeds (Table 3). These results are in agreement with those obtained by Jaleel *et al.*, (2009) who found that cobalt content in soybean increased with increasing cobalt addition in plant media. Stewart (2001) showed that cobalt reduced salinity and/ or ether injury to tomato plants, a suggestion being introduced for possibility of cobalt to overcome the salinity hazard. Kumari (2002) added that mobility of cobalt was higher in acid soils than in alkaline one. When soils were treated with saline solutions such as ($CaSO_4$, Mg SO_4) and others alkali ($NaHCO_4$, Na_2CO_3), the movement of cobalt was greatest with saline salts but lowest with alkaline ones. Confirm Shanon (2002), under salinity condition, cobalt was used to reduce the harmful effect of salinity on tomato plants, where transpiration rate being reduced.

Chemical Constituents of Seeds:

The favorable effect of cobalt on some chemical contents in barley seeds such as total carbohydrates, total sugars, protein and lipids percentages was shown in Table (4). The significant figures resulted with cobalt at 10.0 ppm. Increasing cobalt doses in plant media up to 10.0 ppm increased all chemical contents compared with control and other cobalt concentration. Increasing cobalt above 10.0 ppm decrease the favorable effect of chemical constituents. These data are in harmony with those obtained by Nadia Gad and Hala Kandil (2010) who found that enhancement the content of chemical parameters of tomato fruits such as total soluble solids, total soluble sugar, total protein and vitamin “C”.

Table 4: Effect of cobalt treatments on chemical contents of barley seeds grown in salinity soil (El-Hamoul) mean of two seasons.

Treatments Cobalt (ppm)	%			
	Total carbohydrates	Total sugars	Protein	lipids
Control	59.0	0.81	5.00	0.94
5.0	60.4	0.84	5.81	1.21
7.5	62.5	0.97	6.62	1.90
10.0	66.9	1.22	9.06	2.11
12.5	64.7	1.20	8.31	2.02
15.0	62.6	1.17	7.43	1.96
17.5	62.3	1.12	6.50	1.93
20.0	62.1	1.07	6.00	1.89
LSD 5%	0.32	0.006	-	0.004

Conclusion:

Cobalt help barley plants to tolerate high salinity and increased plant growth and yield under saline conditions. Cobalt levels in barley seeds of 5.46 ppm with the highest cobalt treatment (15.0 ppm) in below the human needs level. Young (1983) reported that the daily cobalt requirements for human nutrition could reach 8 ppm depending on cobalt levels in the local supply of drinking water without health hazard. Cobalt is promosing element under salinity and drought condition as climatic changes.

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