

INFLUENCES OF ALPHA - TOCOPHEROL AND POTASSIUM DI-HYDROGEN PHOSPHATE ON GROWTH AND ENDOGENOUS PHYTOHORMONES OF ONION PLANTS GROWN UNDER SALINITY STRESS

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ABSTRACT

Two pot experiments were conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt during 2004 / 2005 and 2005/2006 winter season to evaluate the effect of spraying with alpha - tocopherol and potassium di-hydrogen phosphate on growth, yield, and endogenous hormones of onion plants. There was a negative relationship between salinity and growth characters. The highest depression was detected in whole plants fresh weight irrigated by 5000 ppm whereas the lowest was in top height by 2500 ppm salt level in water of irrigation. The depression in top height and number of green leaves caused by the higher salt level reached about one fold that caused by use of the moderate level of salts, however, the decrement in fresh weight of leaves reached 2-5 fold that caused by the moderate level compared to plants irrigated regularly by fresh water. Top height, number of green leaves and whole plant fresh weight did not show any significant effect neither with alpha-tocopherol or alpha - tocopherol + potassium di-hydrogen phosphate fertilizer. Nevertheless, significant response was detected on dry matter of bulbs.

Increasing in salt stress negatively affected the concentration of IAA, GA and cytokinins in leaves of onion plants. However, subjected plants to salinity at level of 2500 ppm slightly affected but sharply depressed these three growth hormones by the high salt stress used i.e. 5000 ppm. Nevertheless, the concentration of ABA showed a pronounced increase as a result of exposed onion plants to continuous increases in salt concentration.

Neither IAA or cytokinins concentration affected by spraying alpha - tocopherol with or without potassium di-hydrogen phosphate foliar fertilizer, however, GA concentration slightly increased by alpha - tocopherol application in the rate of 100 ppm and tended to decrease to be equal with the concentration of this hormone in the control plants when plants sprayed by alpha - tocopherol plus potassium di-hydrogen phosphate. Furthermore, the concentration of ABA still without changes with alpha - tocopherol treatment and increased by alpha -tocopherol + potassium di-hydrogen phosphate foliar fertilizer compared with the unsprayed plants.

Keywords: Onion – Salinity - Diluted seawater - Potassium and Phosphorus Fertilizer - Alpha – tocopherol - Vegetative growth - Endogenous Hormones.

INTERODUCTION

Onion (*Allium cepa L.*) is one from the main vegetable crops in Egypt and cultivated widely in the most Egyptian regions due its role in to human diets (fresh or after dry consumption) and for exportation. Studies on the irrigation requirements and also the non traditional of water resources for cultivation in new areas are considered one from the main targets for the horizontal expansion beside the nutrients poverty of that soils and take into the consideration the quality and safety of the products.

Salt stress affected adversely on growth and yield of onion were reported by many authors among of them: Malach, *et al* (1989); Mangal *et al* (1989); Badr (2001) and Doss, *et al* (2002).

Alpha-tocopherol (α -tocopherol) is the name of the most active form of vitamin E. It is also a powerful biological antioxidant (Farrell and Roberts 1994 and Traper, 1999). Moreover, fertilizers and amendments also induced a positive effects on ameliorate the bad effects of salt stress (Keya *et al* 2001; Kabir, *et al* 2004; Hussein *et al* 2004; Hussein *et al* 2007).

Foliar fertilization with potassium affected growth, chemical composition and yield of different crops (Oosterhuis, *et al* 1995) and (Chapagain and Wiesman, 2004). The improving affects of phosphorus spraying were reported by Martinaz and lauchi, 1991 and Mosali, *et al* 2006. Moreover, the synergistic effect of combined of potassium and phosphorus or application of compounds contains both elements on productivity and quality of different crops were found by many authors: Ankorion, (1995); Willams and karkafi, (1995) and Sawyer and Barker, (2006).

Tocopherols is a phyto regulator compounds suggested to elevate and tolerate the adverse effects of biotic and abiotic stresses such as moisture and salt stress on plant growth and yield (Demiral and Turkan, 2005 and Raga Babo, *et al* 2005).

The relation between the antioxidants and fertilization studies still up till now scarce, therefore, this study aimed to shed more light on the response of growth and endogenous hormones to alpha - tocopherol and potassium di-hydrogen phosphate foliar fertilizing treatments of onion plants grown under some salt stress treatments.

MATERIALS AND METHODS

Two pot experiments were conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt during winter seasons of 2004 / 2005 and 2005 /2006 to evaluate the effect of spraying with alpha - tocopherol and potassium di-hydrogen phosphate on growth and endogenous hormones of onion plants . The treatments were as follows:

- A) - Salinity: irrigation by water contains 2500 and 5000 ppm from diluted Seawater and tap water (300 ppm) as a control.
- B) - Spraying alpha – tocopherol at 100 ppm and 100 ppm tocopherol + Potassium di-hydrogen phosphate ($K_2H_2PO_4$) as well as the unsprayed treatment. It means that, 9 treatments which were the interactions within 3 salinity levels and 3 treatments of alpha-tocopherol.

All treatments were designed in split – plots design with 3 replicates. Where the 3 salinity treatments arranged randomized within the main plots, but the hormones treatments were distributed within the sub- plots. Each replicate contained 3 plots.

Metallic tin pots 35 cm. in diameter and 50 cm. in depth were used. Every pot contained 30 Kg. of air dried clay loam soil. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. In this system, 2kg. Of gravel (particles about 2-3 cm. in diameter), so the movement of water from the base upward.

Onion (*Allium cepa L.*) Behary Red variety seedlings transplanting to pots were applied at Dec, 1, 2005 in the 1st season and in Jan, 1, 2006 in the 2nd season. Plants were thinned twice, the 1st days after sowing and the 2nd two weeks latter to leave three plants / pot. Calcium super phosphate (15.5 % P₂O₅) and potassium sulfate (48.5 % K₂O) 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, the 1st after two weeks of transplanting and the 2nd two weeks latter. Irrigation with diluted seawater in different concentrations was started 21 days after transplanting (one irrigation by salt water and the next was by fresh water alternatively). Samples for determination of endogenous hormones i.e. indole acetic acid (IAA) , gibberellins (GA3) , abscisic acid (ABA) , and cytokinins in fresh leaves were taken after 15 days from the last spraying . Identification and determination of acidic hormones (IAA, GA3 and ABA) were carried out by Gas Liquid Chromatography (GLC). Samples were extracted and according to the method adopted by Badr *et al.* (1971). Cytokinins fractions were extracted as previously mentioned for the acidic hormones and were detected by HPLC.

Data collected were subjected to the proper statistical analysis with the methods described by Snedecor and Cochran (1990).

RESULTS AND DISCUSSION

I – Plant growth characteristics:

A- Effect of salinity:

Data recorded in Table (1) indicated that, in the 1st season, there was a negative relationship between salinity and growth characters. Depression was detected of whole plant which the depression in top height and number of green leaves caused by the higher salt level reached about one fold that caused by use of the moderate level of salts, however, the decrement in fresh weight of leaves reached 2,5 fold that caused by the moderate level compared to plants irrigated regularly by fresh water. Furthermore, irrigation onion plants by 2500 or 5000 ppm gave approximately the similar effect on bulb diameter in spite of the non significant of the differences in this criterion. In the 2nd season, generally, increased the salt concentration in the root media by irrigation with water contains 5000 ppm salts decreased the all growth parameters but the differences reached the significant levels in bulb fresh weight only.

In the 1st season dry weight of top, bulb and whole plant as affected by irrigation with diluted seawater were noted in Table (1). The mean values of bulb, leaves and whole dry weight of plants irrigated by diluted solution contains 2500 ppm salts lowered by: 25.86, 39.09 and 40.03 % while when irrigated by 5000 ppm salt solution lowered by: 53.53, 52.99 and 59.42 % compared to that irrigated by fresh water, respectively.

In the 2nd season, negative relation was detected between the increase of salt stress and dry weight of bulb, top and whole plant but the differences were not significant between the values of these parameters in plants subjected to 2500 and 5000 ppm were not significant.

The depression in growth traits may be due to the increase in the osmotic pressure of soil solution and mineral accumulation (Backhausen, *et al.* 2005 Hussein *et al.*, 2007), Moisture adjustment in the plant tissues (Heuer and Nadler, 1998; Teixeira and Pereira, 2007), toxicity of Na⁺ and Cl⁻ excess ions in different physiological processes such as protein building (Debouba, *et al.*, 2006 and Hussein, *et al.*, 2004), photosynthesis (Manceau *et al.*, 2004), endogenous hormones and antioxidant activity (Hussein, *et al.*, 2002).

Table (1): Effect of different levels of salinity on growth characters of onion plants during the two successive seasons of 2004-2005 and 2005-2006.

(1st season)

Salinity ppm	Top Height	No. Of leaves	Bulb diameter	Fresh weight (g):			Dry weight (g):		
				Top	bulb	whole	Top	bulb	Whole
T.W.	60.47	7.33	2.41	46.86	32.31	79.17	14.46	9.21	23.67
2500	56.10	6.33	1.97	28.12	26.91	55.03	10.72	5.61	16.33
5000	51.20	5.45	1.86	22.33	19.31	41.64	6.72	4.33	11.05
LSD at 5%	9.22	N.S	N.S	6.29	9.98	27.34	4.99	2.87	7.38

(2nd season)

Salinity ppm	Top Height	No. Of leaves	Bulb diameter	Fresh weight (g):			Dry weight (g):		
				Top	bulb	whole	Top	bulb	Whole
T.W.	56.8	7.22	2.74	26.18	11.09	37.07	4.01	3.92	7.60
2500	51.0	6.77	2.90	21.78	8.67	30.45	3.22	2.58	5.80
5000	51.7	6.67	2.47	19.22	7.21	26.43	2.82	2.38	5.20
LSD at 5%	N.S	N.S	N.S	N.S	0.76	7.45	N.S	1.26	2.05

T.W. = tap water

b) - Effect of alpha-tocopherol:

Table (2) showed the response of different growth parameters as affected by foliar treatments of alpha-tocopherol and potassium di-hydrogen phosphate in two experimental seasons. In the 1st season top height, number of green leaves and whole plant fresh weight did not show any significant effect neither with alpha - tocopherol or alpha- tocopherol+potassium di-hydrogen phosphate fertilizer. Nevertheless, significant response was

detected on dry matter of bulb. This was true for leaves as well as whole plant dry weight. The increment in bulb diameter, leaves number by alpha-tocopherol application solitary from 9.62 in leaves and 12.2 % in bulbs to in leaves , in bulbs and whole plant dry weight from 25.18 to 28.46 % by the combination of tocopherol + potassium di-hydrogen-phosphate foliar spraying. This means that the two plant parts responded similarly to the antioxidant and foliar fertilizer and their combination.

In the 2nd season, treated with alpha- tocopherol gave the highest values of bulb diameter and fresh weight of bulb. While, combined alpha- tocopherol and potassium di-hydrogen phosphate fertilizer spraying gave the highest vales in plant height, number of green leaves, top and whole plant fresh weight, top and whole plant dry weights. Moreover, this data could be concluded the synergistic effect resulting from sprayed the antioxidant and foliar fertilizer of component contains K and P elements. Thalooh, *et al.* (2006) revealed that potassium significantly increased all growth parameters of mung bean plants. They added that potassium superior in the features of area, No. of branches/ plant and stem dry weight/ plant than Mg and Zn. These findings were supported by Basole, *et al.* (2003),

Table (2): Effect of alpha- tocopherol treatments on growth characters of onion plants during the two successive seasons of 2004/2005 and 2005/2006.

(1st season)

Alpha-tocopherol treatments	Top Height (cm)	No.of leaves	Bulb diameter (cm)	Fresh weight (gm/plant)			Dry weight (gm/plant)		
				Top	bulb	whole	Top	bulb	whole
T.W.	51.7	6.45	1.98	28.24	24.95	53.19	9.38	5.72	15.10
Tochopherol	58.3	6.11	2.17	31.88	28.41	60.29	10.47	6.27	16.74
Toch.+KP	57.8	6.56	2.26	37.19	25.17	62.36	12.05	6.17	19.21
LSD at 5%	N.S	N.S	N.S	N.S	N.S	N.S	2.28	1.51	5.59

(2nd season)

Alpha-tocopherol treatments	Top Height (cm)	No.of leaves	Bulb diameter (cm)	Fresh weight (gm/plant)			Dry weight (gm/plant)		
				Top	bulb	Whole	Top	bulb	Whole
T.W.	47.43	6.99	2.66	17.97	7.24	25.21	2.79	2.46	5.25
Tochopherol	54.10	6.56	2.90	22.22	10.30	31.40	3.55	3.75	7.30
Toch.+KP	57.90	7.21	2.68	26.99	9.35	36.43	3.77	3.31	7.03
LSD at 5%	N.S	N.S	N.S	3.28	N.S	9.13	N.S	0.79	N.S

El-Bassiony, *et al.* (2005) stated that the foliar spray with all treatments of antioxidants on faba bean plants induced increments on the number of branches, leaves and leaf area per plant, fresh and dry weights of shoots. Potassium is essential in maintenance of osmotic potential and water uptake and involving in activating a wide range of enzyme systems which regulate photosynthesis, movement of metabolites, minerals uptake and protein building (Marschiner, 1986). Boyhan, *et al.* (1999) mentioned that potassium

is easily leached from soils, particularly sandy soils; therefore, potassium may need to be side dressed in small amounts.

c) – Effect of Salinity X Tocopherols :

Interaction effect of potassium di-hydrogen phosphate fertilization and/or alpha- tocopherol and irrigation by diluted seawater on growth traits were illustrated in Table (3). In the 1st season, the differences in whole plant dry weight only significant with the interaction. Eihther under the saline irrigation by 2500 and 5000 ppm salt level or in plants irrigated by tap water, dry weight of whole plants increased by both treatments i.e. alpha-tocopherol or alpha-tocopherol + potassium di-hydrogen phosphate fertilization but the highest effect was induced by the combination of the two sprayed materials. In the 2nd season, the interaction effect only was significant on plant height, whole plant fresh weight and bulb dry weight. The highest value of plant height in unstressed plant was in plants sprayed by tocopherols while under the medium and higher salt level the highest values was by combined alpha-tocopherol plus foliar spray potassium di-hydrogen phosphate fertilizer. Fresh weight of bulb responded approximately as plant height. However, the dry weight of bulb gave its higher values when plants irrigated 2500 ppm as well as without salt stress. Values were by the combined followed by tocopherols only.

Kabir *et al.* (2004) who concluded that application of higher levels of potassium improves water relations as well as growth and yield of mung bean under Nevertheless, under the higher salt stress treatment (5000 ppm) the highest mild level of saline conditions. This data could be concluded that application of potassium di-hydrogen phosphate fertilizer led to alleviate the tolerance of plants growing under salt stress. Abd El Kader *et al.* (2007) on sorghum supporting this conclusion.

Table (3): Effect of the interaction treatments of different levels of salinity and alpha-tocopherol treatments on growth characters of onion plants during the two successive seasons of 2004/2005 and 2005/2006. (1st season)

Salinity ppm	Sprayed compounds	Top Height cm	No. of leaves	Bulb diameter cm	Fresh weight (g/plant)			Dry weight (g/plant)		
					Top	bulb	Whole	Top	bulb	Whole
					Whole			Whole		
T.W.	T.W.	58.0	7.00	2.33	39.97	28.44	68.91	12.88	8.43	21.31
	Tochopherol	59.7	7.33	2.58	43.05	36.45	79.50	65.47	9.09	74.56
	Toch.+KP	63.7	7.67	2.40	57.57	32.53	89.10	15.04	10.11	25.15
2500	T.W.	52.0	6.67	2.03	26.35	25.82	52.17	9.38	4.94	14.32
	Tochopherol	60.0	6.00	1.80	26.49	30.47	56.96	9.62	5.20	14.80
	Toch.+KP	56.3	6.33	1.97	31.51	24.43	55.94	13.15	6.70	19.85
5000-	T.W.	45.0	5.67	1.57	18.40	20.08	38.48	5.88	3.79	9.67
	Tochopherol	55.3	5.00	2.20	26.11	18.30	44.41	6.32	4.53	10.85
	Toch.+KP	53.3	5.67	1.80	22.49	19.55	42.04	7.97	4.66	12.63
LSD at 5%		N.S	N.S	N.S	N.S	N.S.	N.S	N.S	N.S	9.87

(2nd season)

Salinity ppm	Sprayed compounds	Top Height cm	No. of leaves	Bulb diameter cm	Fresh weight (g/plant)			Dry weight (g/plant)		
					Top	bulb	Whole	Top	bulb	Whole
T.W.	T.W.	51.0	7.33	2.57	19.84	8.65	28.49	3.22	2.63	5.85
	Tochopherol	60.3	7.00	2.57	26.36	13.43	39.79	4.60	4.84	9.44
	Toch.+KP	59.0	7.33	2.93	32.33	10.94	43.27	4.20	4.30	8.50
2500	T.W.	46.3	6.00	2.73	16.21	6.93	23.14	2.38	2.37	4.75
	Tochopherol	49.0	6.67	3.03	21.40	10.05	31.45	2.50	2.50	6.09
	Toch.+KP	57.7	7.63	3.23	27.73	9.04	36.77	2.88	2.88	6.57
5000	T.W.	45.0	7.33	2.80	17.86	6.15	24.01	2.38	2.38	5.15
	Tochopherol	53.0	6.00	2.53	18.90	7.41	26.31	2.00	2.00	4.42
	Toch.+KP	57.0	6.67	2.50	20.91	8.06	28.97	2.76	2.76	6.04
LSD at 5%		8.60	N.S	N.S	N.S	6.51	N.S	N.S	1.36	N.S

KP = potassium di-hydrogen phosphate

II - Endogenous hormones:

a)- Effect of Salinity levels

Data in Table (4) showed that increasing in salt stress negatively affected the concentration of IAA, GA and cytokinins in leaves of onion plants. Subjected plants to salinity at level of 2500 ppm slightly affected but sharply depressed by the high salt stress used i.e. 5000 ppm these three growth hormones nevertheless, the concentration of ABA showed a pronounced increase as a result of exposed onion plants to continuous increases in salt concentration amounted by 83.3 and 255.17 % by the 1st and 2nd level of salinity. The decrements in IAA and GA in plants irrigated by 5000 ppm solution reached to be one half that irrigated by tap water, while in cytokines the decrement was 38.49 % compare to the control plants. Hare *et al.* (1997) mentioned that Salinity affected endogenous hormones which decrease IAA, gibberellins and cytokinins and the reverse for ABA. However, Barry *et al.* (1993) noticed that increasing salinity increased ABA content in tomato fruit. Al-Dossouky and Ibrahim (2001) revealed that increasing the concentration of salt by irrigation with diluted seawater increasing the free as well as ABA in the 5th leaf of wheat plants.

b) – Effect of Alpha- tocopherol treatments:

Neither IAA or cytokinins concentration affected by spraying with alpha-tocopherol with or without potassium di-hydrogen phosphate foliar fertilizer (Table 4), However, GA concentration slightly increased by alpha-tocopherol application in the rate of 100 ppm and tended to decrease to be equal with the concentration of this hormone in the control plants when plants sprayed by alpha- tocopherol plus potassium di-hydrogen phosphate. Furthermore, the concentration of ABA still without changes with alpha- tocopherol treatment and increased by alpha- tocopherol + Potassium di-hydrogen phosphate foliar fertilizer compared with the none sprayed plants.

Table (4): Effect of different salinity levels and alpha-tocopherol treatments on the endogenous phytohormones (ng/g fw) in leaves of onion Plants.

Salinity ppm	Spraying compoids	IAA	GA3	ABA	CK
T.W.	0	89.87	69.10	50.12	60.12
	Tocopherol	96.07	84.94	43.11	68.61
	Tochoph.+KP	119.76	97.76	40.07	76.11
	Mean	101.90	83.93	44.53	68.28
2500	0	99.11	88.44	47.36	64.17
	Tocopherol	105.77	90.83	45.43	68.01
	Tochoph.+KP	80.73	51.17	130.16	47.17
	Mean	95.20	76.81	74.32	59.78
5000	0	77.11	47.12	149.76	49.13
	Tocopherol	70.65	41.80	157.13	39.12
	Tochoph.+KP	61.18	37.16	166.50	37.76
	Mean	69.65	42.03	157.80	42.00
Means of	0	88.70	68.22	45.85	57.81
Chemical treatments	Tocopherol	90.83	72.52	81.89	58.58
	Tochoph.+KP	87.22	62.03	112.24	53.68

c) – Effect of the interaction between salinity X alpha-tocopherol:

The interaction effect of spraying alpha- tocopherol with and without potassium di-hydrogen phosphate fertilizer and salinity on endogenous phytohormones in leaves of onion plants was recorded in Table (4). Regardless the effect of alpha- tocopherol, and potassium di-hydrogen fertilizer, the concentration of IAA and cytokinins slightly increased in plants irrigated by 2500 ppm solution and tended to decrease by raising the level of salt to be 5000 ppm. ABA concentration in leaves of plants irrigated by moderate salt level treatment and in that irrigation by fresh water (250 ppm) seemed to be equal, but, this hormone markedly increased with the highest salt level used. Furthermore, irrigation onion plants by diluted seawater in the rate of 2500 ppm salt induced clear increase in the GA3 concentration, nevertheless, use of the high concentration of salt in water showed the opposite response.

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تأثير الفاتوكوفيرول وبوتاسيوم داى هيدروجين فوسفات على النمو ومحتوى نبات البصل من الهرمونات الداخلية تحت ظروف الملوحة

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اجريت تجربتان فى صوبة المركز القومى للبحوث خلال عامى 2005/2004 و 2006/2005 لتقييم تأثير الرش بمادة الالفاتوكوفيرول وبوتاسيوم داى هيدروجين فوسفات (الرش بالماء العادى, الرش بمادة الالفاتوكوفيرول (100 جزء فى المليون), الرش بمادة الالفاتوكوفيرول مع مادة بوتاسيوم داى هيدروجين فوسفات) – تحت ظروف ملوحة ماء الرى (الرى بماء الصنبور, الرى بماء يحتوى 2500 جزء فى المليون , الرى بماء يحتوى على 5000 جزء فى المليون).

وكانت اهم النتائج كما يلى:

- استعمال ماء رى يحتوى على 5000 جزء فى المليون ادى الى نقص فى الوزن الغض الكلى للنبات وطول النبات ومتوسط عدد الاوراق.
- لم يكن هناك تأثير واضح للرش بمادة الالفاتوكوفيرول بمفردها أو باضافة بوتاسيوم داى هيدروجين فوسفات اليها على طول النبات او متوسط عدد الاوراق/نبات أو الوزن الغض الكلى للنبات .
- زيادة ملوحة ماء الرى أدت الى نقص فى محتوى أوراق نبات البصل من الأندول أسيتك أسيد ، حمض الجبريليك ، السيتوكينين – و على العكس من ذلك زاد محتوى حمض الأبيسيسيك.
- الرش بمادة الالفا تو كوفيرول بمفردها أو مخلوطة بمادة البوتاسيوم داى هيدروجين فوسفات لم يكن لها تأثير واضح على محتوى السيتوكينين أو محتوى حمض الأبيسيسيك فى أوراق نبات البصل – بينما كان هناك زيادة طفيفة لحمض الجبريلين بالرش بمادة الالفا تو كوفيرول بتركيز 100 جزء فى المليون.