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ENDOGENOUS HORMONES, GROWTH AND YIELD OF BARLEY PLANTS AS AFFECTED BY BENZYL ADINENE UNDER DIFFERENT SALINITY LEVELS

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ABSTRACT

A pot experiment was conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt during the winter season of 2000/2001 to evaluate the effect of 6-benzyl adenine under different salinity levels on growth, yield and the concentration of different endogenous hormones in barley plants cv. Giza 124. Plant height, number of tillers and leaves/plant, dry weight of shoots No. and length of spices and straw, grains and total yield/plant were decreased as the salt concentration increased in water used for irrigation of barley plants. BA (6-benzyl adenine) treatment were slightly affected growth and yield characters. IAA, GA and Cytokinins decreased but ABA concentration was reversely responded by salt stress. Spraying BA at 50 mg/l raised the endogenous concentration of GA, IAA and Cytokinins by 13.76, 12.97 and 22.77 % and lowered the concentration of ABA by 20.97 %, BA treatment contracts the effect of salt stress on the different estimated hormones.

INTRODUCTION

Plants are exposed to many types of environmental stresses among of these stresses osmotic stress in particular that due to salinity and drought, is the most serious problems that limits plant growth and crop productivity in Agriculture (Boyer, 1982 and Yoshiba *et al.*, 1997).

Many authors discuss the relation between growth hormones and salt stress. Popova and Maslenkova (1997) demonstrated that leaf Na^+ and Cl^+ contents in barley were lowered in pretreated plants by jasmine acid Zang–Shigong *et al.* (1999) concluded that BA improved Cl^- absorption in roots and decreased the translocation and content in shoots, this increasing the salt resistance and the adaptability of wheat seedlings to salt stress. Kopier *et al.* (1990) revealed that a stimulatory effect of BA on plant mineral nutrition was also associated with an effect on the level of endogenous cytokinins. Kupier *et al.* (1990) noticed that addition of BA inhibited growth during stress of a salt sensitive variety of barley but over came the decline in growth rates, shoot/root ratio and internal CK content in a salt tolerant variety. Moreover, Hare *et al.* (1997) reported that several studies involved stress – induced decline in cytokinins in root tip and the resulting decrease in CK transport might affect the physiology of the shoots. Other ways and relations had been mentioned such as osmotic adjustment (Sanada *et al.*, 1997) enzymes activity (Popova and Maslenkova, 1997) and gene responsible (Yoshiba *et al.*, 1997 and Hare *et al.*, 1997). This study was conducted to shed more light on the relations of growth hormones and salt stress through its effect on growth, yield and concentration of endogenous hormones in shoots of barley plants.

MATERIALS AND METHODS

Pot experiment was conduced in the greenhouse of the National Research Centre at Dokki, Cairo, Egypt. During the winter season of 2000/2001 to evaluate the effect of salinity and 6-Benzyl adenine on growth, yield and the concentration of endogenous hormones and intern its relation with salt resistant in barley plants. The treatments were as follows :

1- The salt concentration in irrigation water (by diluting Midetrainian sea water with fresh water) : 0 (tap water 200 ppm), 2000, 4000, 6000 and 8000 ppm.
2- Benzyl adenine : 0 (tap water) and 50 mg/l foliar spray.

The experiment included 5 levels of salinity in combination with 2 levels of growth hormone i.e. 10 treatments in 6 replicates arranged in completely randomized design. 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, 2 kg of gravel, (particles about 2-3 cm. In diameter) was used to cover the bottom of the pot. Irrigation water was poured through a vertical tube (2.5 cm. In diameter), so the movement of water was from the base upward.

Grains of barley (*Hordium vulgare*, L.) cv. Giza 124 were sown in 15 November, plants were thinned twice the 1st 20 days after sowing and the 2nd two weeks latter to leave three plants/pot. Calcium superphosphate (16.5 % P_2O_5) and potassium sulfate (48.5 % K_2O) in the rate of 2.29 and 1.14 g/pot were added before sowing. Ammonium sulfate (20.5 % N) in the rate of 6.86 g/pot was added in two equal portion the 1st after two weeks from sowing and the 2nd two seeks latter.

Barley plants were sprayed with Benzyl adenine (BA) at 50 ppm at 75 days after sowing. Spraying was carried out using operated compressed air sprayers, "Nonidet 42" was used as a wetting agent at the rate of 0.10 %. Control plants were sprayed with tap water at the same rate of wetting agent.

Sampling :

Ten plants were randomly taken from each treatment for growth measurements at harvest after 150 days from sowing). The following data were recorded, plant height (cm) of main stem, number of tillers, dry weight of shoots (g/plant), number of leaves, weight of straws (g/plant), number of leaves, weight of straws (g/plant), grains weight (g/plant), spike length (cm), spikes/straw, grains/straw and grains/spikes per plant.

The data were statistically analyzed as described by Snedecor and Cochran (1982).

Determination of plant growth hormones :

For endogenous growth hormone determinations, one sample from the shoots was taken after 90 days from sowing was taken from the 2nd season. Thirty gram fresh weight of the shoot were extracted with 80 % cold methanol according to the method of Badr *et al.* (1971). The extracts were

treated for HPLC analysis using water 746 data modular. Milipoa apparatus. Samples for extraction were taken after 90 days from planting from treatments; Tap water, 4000 and 8000 ppm salinity levels in combination with 0 and 50 ppm benzyl adenine spraying only.

RESULTS AND DISCUSSION

A. Endogenous hormones :

1. Gibberellins :

Data presented in Table (1) and Fig. (1) show that gibberellins concentration in shoots of barley plants drastically depressed by high level of salinity. Irrigation with water contains 4000 and 8000 ppm salts decreased gibberellins (content) by 33.42 and 46.52 % in combarable with that irrigated by tap water. In this concern, El-Antaby *et al.* (1994) revealed that the increases in inhibitors levels shown to be a function of age and paralleled in most cases the increase in level of salinization.

Roy *et al.* (1995) reported the same trend. However, Moussa and Sallem (1996) noticed that the highest gibberellins activity was observed when plants were grown under mild stress (4000 ppm) used 6000 ppm level diminished gibberellins activity while it disappeared by irrigation with water contains 12000 ppm salts.

Table (1) : Effect of spraying barley plants with benzyl adenine on the amounts of endogenous hormones of the shoots of plants grown under the two levels of salinity (Calculated as ng/g fresh weight).

Endogenous hormones / Treatments	Gibberellins content (equivalent to GA_3)	IAA	ABA	Cytokinins content (equivalent to Zeatin)
1. Tap water	232.20	122.30	40.60	66.18
2. Tap water + 50 ppm BA	264.14	138.16	31.66	81.25
3. 4000 ppm salinity	154.60	64.50	79.30	43.10
4. 4000 ppm salinity + 50 pm BA	172.11	73.80	55.58	59.07
5. 8000 ppm salinity	124.18	50.11	86.25	34.36
6. 8000 ppm salinity + 50 ppm BA	133.08	59.91	65.57	39.87

2. Indole acetic acid :

Data in Table (1) and Fig. (1) clearly indicated that the two levels of salinity a decreased IAA concentrations as compared with control plants. Irrigated plants with water contains 4000 ppm lowered the concentration of IAA to be about one half of that in the control plants. Increased salts up to 8000 ppm sharpley decreased IAA concentration from 122.3 to 50.11 ppm. In this concernes, Sinel-Nikova (1981) and Mousa and Sallem (1996) reached to a negative relation between degree of salinity and auxin activity in tomato and triplex plants.

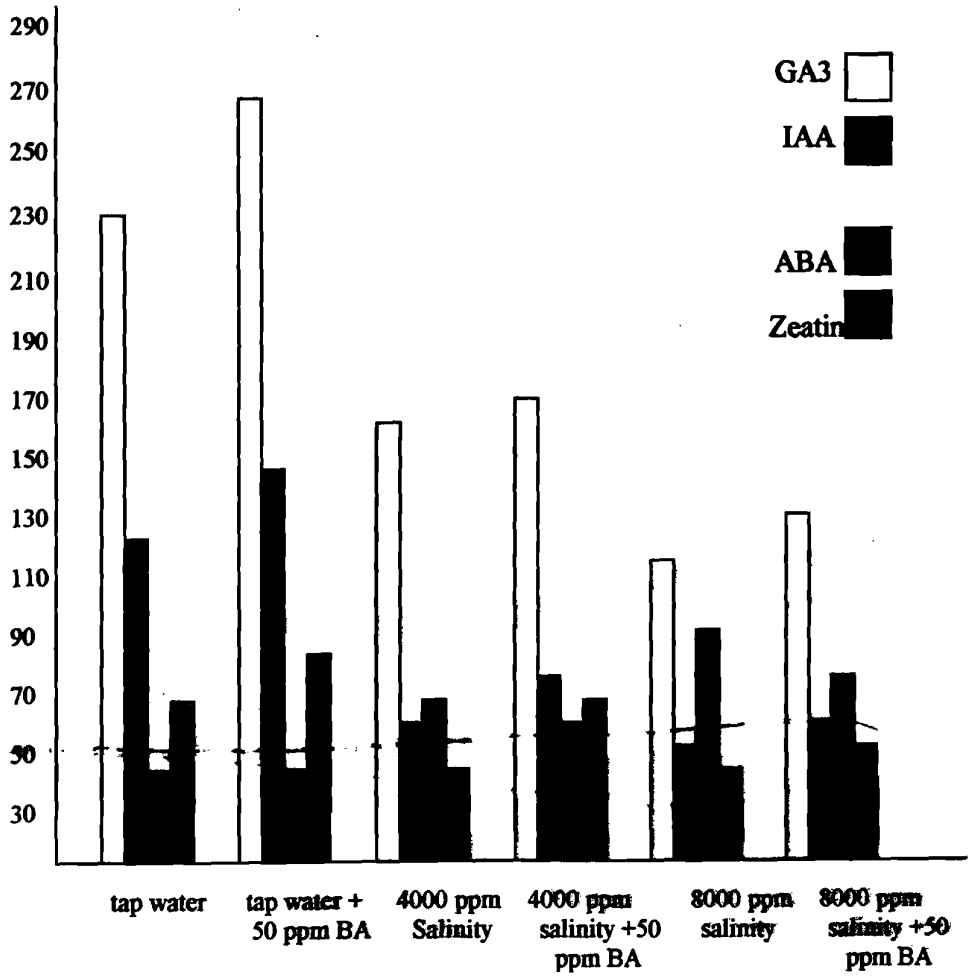

Fig. (1) : endonegous hormones of the shoots of barley plants sprayed With benzyl adenine under the two level of salinity.

1- tap water
2- tap water +50 ppm BA
3- 4000 ppm salinity
4- 4000 ppm salinity +50 ppm BA
5- 8000 ppm salinity
6- 8000 ppm salinity +50 ppm BA

3. Abscisic acid :

Data recorded the effects of salts on ABA are in Table (1) and Fig. (1). The data show that using the two levels of salinity caused marked increases in the concentration of ABA in the extract of barley shoots. The increment in ABA conc. was 97.95% by salinity level 4000 ppm, however, it raised to be 115.30% when salts increased to 8000 ppm. These data are in agreement with those obtained by Roy *et al.* (1995) on rice and El-Antably *et al.* (1994) on tomato.

Concerning the relation between ABA and salts stress, El-Antably *et al.* (1994) on tomato, related the increase in ABA under saline conditions to an enzyme conversion of precursor to inhibitor B during prolonging wilting or salinity exposure. Thomas *et al.* (1992) noticed that ABA level increased 8 to 10 fold in leaves during salt stress. These conclusions are in line with those previously maintained by Koshimizu *et al.* (1995). Also, El-Antably *et al.* (1994) supported the findings of Eder and Harber (1977) that such elevated ABA levels induced by salinity are important for rapid osmotic adjustment via stomata closure and also favor the accumulation of amino acids in general and proline in particular and improve the adaptation to salinity.

4. Cytokinins :

Data in Table (1) and Fig. (1) revealed that the two levels of salinity caused a depressive effect on cytokinins content especially when salinity increased to 8000 ppm. Promotive effect on cytokinins content in barley were induced by BA application. This may be attributed to less synthesis of cytokinins in plant roots and intern less quantity of this hormone which translocated to the shoots. Furthermore, salinity may affect the movement of cytokinins from roots twards the top and intern lowering the levels of cytokinins in the leaves. Moussa and Sallem (1996) reported the influence of spraying BA, the highest cytokinins content attained when BA was sprayed on barley plants which grown under non or salt stress. However, the stimulative effect of kinetin tended to decrease with increasing salinity levels, whereas, cytokinins content increased when BA sprayed. Depending upon the above mentioned data, it is concluded that the dippressive effect of salinity on cytokinins was minimized to some extent by treating with BA.

Spraying BA in the rate of 50 ppm slightly increased the conc. of gibberellins. IAA and cytokinins, Kopier *et al.* (1990) demonstrated that addition of BA inhibited growth to some extent and enhanced the cytokinin conc. On barley in Egypt. Moussa and Sallem (1996) found that kinetin enhanced gibberellins activity. On wheat, Rabie (1996) revealed that foliar application with GA, BA and prolamine increased the level of action and achieved remarkable increase in gibberellins activities in shoots extracts of wheat during flowering and milk stages. On reverse, ABA decreased with BA application. Similar results were obtained by Rabie (1996), Moussa and Sallem (1996) and Bondok *et al.* (1992), Uesutigi *et al.* (1994) indicated that BA application caused a drastic decrease in endogenous ABA levels in peach within 2 hours of application.

The interaction effect of salt stress and 6 benzyl adenine on endogenous hormones of barley plants are in Table (1). These data show that irrigation with 4000 ppm solution decreased GA_3, IAA and Zeatin by 33.42, 47.26 and 34.88 %, respectively. Meanwhile, spraying plants with 50 ppm (6-benzyl adinene) lowered this decrement to be 25.88, 39.66 and 10.74 %, respectively. Furthermore, raised the conc. of salts around plant roots to 8000 ppm sharply depressed the conc. of the aformentioned endogenous hormones by 46.52, 59.02 and 48.08%, while it be 42.69, 51.01 and 39.76 %, respectively. In addition of BA at 50 ppm in the contr. In the contrary addition of BA in the rate of 50 ppm limited the increment in ABA conc. The level of salt 4000 ppm caused 79.95 % but it be 38.74 % when plants received 50 ppm BA. Moreover, at 8000 ppm salt in the root media increased ABA in barley shoots by 115.30 % Nevertheless the increment could be measured by 63.68 % by BA application.

Concerning the combined effect of benzyl adenine and salt stress. Sinel-Nikova (1981) noticed that, in general, higher significant values of inhibitors than control was shown till the last age with increasing tendency due to increase in salinity level and advancement in age. El-Antably *et al.* (1994) support these findings. Kupier *et al.* (1991) recorded that the addition of 5 X 10^{-9} moll. BA/m^3 retarded the decreases in cytokinins caused by salinity. Moussa and Sallem (1996) demonstrated that auxin activity was adversely correlated with the increase in plant age and salt stress. They added that mild salt stress and proline favoured gibberellins activity. The data showed that BA treatment induced barley plant to tolerate salinity stress.

B. Growth and Yield :

Data presented in Table (2) show that increased the salt concentration in water of irrigation up to 8000 ppm clearly depressed the growth characters. The highest effect was in No. of tillers (69.28 %) and the lowest was (50.51 %) in main stem length compare to the control plants.

Many workers reached to the similar results. Moussa and Sallem (1996) and Nour *et al.* (1982). These effects could be attributed to the effect of salts on water availability, mineral absorption, photosynthesis and protein metabolism (Boyer, 1982, Milteva *et al.* (1991), Yadav (1993) and Moussa and Sallem (1996).

Regardless the effect of salinity, one spray in the rate of 50 ppm of Benzyladenine slightly affected growth and yield characters of barley plants (Table 2). Rabie (1996) found that both BA and prolamine increased plant height, fresh and dry weight and area of flag leaf at flowering of wheat plants. She also added that yield and yield characters responses are in line with that of growth criteria.

The effect of interaction between salinity and BA on growth characteristics are in Table (2). These effects on no. of tillers/plant, weight of stems and dry weight of shoots were significant. Addition of BA depressed slightly the decrement in growth parameter. In addition, total yield, spike and straw yields showed the same response of growth criteria.

The role of BA on overcoming the depressing effect of salinity on growth may be due to the enhancing effect sof endogenous cytokinins (Table 1) which

Table (2) : Effect of spraying barley plants with benzyl adenine on growth and yield of barley plants as affected by Benzyl adenine in different salinity levels.

Salinity ppm	BA mg/l	Plant height (cm)	No. of tillers	D.W. of shoots	No. of leaves	Wt. of spikes	Wt. of straws	Grains weight	Spike length (cm)	Spikes/ straw	Grains / straw	Grains/ spikes
Tap water	0	68.7	4.33	9.63	17.0	9.18	6.23	2.43	8.3	1.47	39.0	26.5
	50	70.0	4.67	10.52	18.0	9.46	6.68	2.55	8.7	1.42	38.2	27.0
Mean		69.4	4.50	10.10	17.5	9.32	6.46	2.49	8.5	1.45	38.4	26.8
2000	0	55.3	3.67	8.67	14.0	7.37	5.48	2.00	6.2	1.35	36.5	27.1
	50	56.7	4.00	8.83	14.7	7.87	5.88	2.00	6.5	1.34	34.0	25.4
Mean		56.0	3.84	8.75	14.4	7.57	5.68	2.00	6.4	1.35	35.3	26.3
4000	0	48.3	2.67	6.83	10.7	6.62	3.15	1.89	6.0	2.10	60.0	26.6
	50	49.0	3.00	7.00	11.0	6.86	3.26	1.93	6.2	2.10	59.2	28.1
Mean		48.7	2.84	6.92	10.9	6.74	3.21	1.91	6.1	2.10	59.6	28.3
6000	0	36.7	2.00	5.77	8.3	4.63	2.37	1.73	5.2	1.95	73.0	37.4
	50	39.7	2.33	5.83	8.7	4.81	2.42	1.79	6.3	1.99	71.9	36.2
Mean		38.2	2.17	5.80	8.5	4.72	2.40	1.74	6.3	1.97	72.5	36.8
8000	0	34.0	1.33	4.27	7.0	3.53	1.83	1.52	4.0	1.93	83.1	43.1
	50	35.3	1.67	4.40	7.0	3.69	1.90	1.61	4.8	1.94	84.7	43.6
Mean		34.7	1.50	4.34	7.0	3.61	1.87	1.57	4.4	1.94	83.9	43.4
Mean values of BA	0	48.6	2.80	7.03	11.4	6.27	3.81	1.93	5.94	1.76	58.3	32.5
	50	50.1	3.13	10.32	11.9	6.24	4.03	1.47	6.30	1.76	57.6	32.1
L.S.D. at 5%	S	1.23	0.50	0.35	0.50	0.20	0.09	0.04	0.57	-	-	-
	BA	0.88	0.29	0.13	N.S	0.09	0.068	0.16	0.35	-	-	-
	SXBA	N.S	0.66	N.S	N.S	0.19	0.15	0.36	0.78	-	-	-

affect plant water balance (McRobic, 1981) and/or decreasing root resistance to water flow (El-Banna, 1985 and Sakr, 1996).

In this concern, Mumtaz *et al.* (1997) revealed that chlorophyll degradation and reduction of root growth resulting from NaCl treatment were counteract by BA treatment. Kupier *et al.* (1991) used salt resistant barley varieties ceathe indose which showed a bad response to salt stress and the shoot to root ratio decreased. However, the addition of BA temporarily overcome this salt induced response.

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هرمونات النمو الداخلية ونمو ومحصول نباتات الشعير وتأثرها بالبنزايل ادنين تحت مستويات الملوحة المختلفة من مياه البحر المخففة

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اجريت تجربة قصارى بصوب المركز القومى للبحوث بالدقى – القاهرة – جمهورية مصر العربية خلال الموسم الشتوى (٢٠٠٠ ، ٢٠٠١) لتقييم تأثير الرش بالبنزايل ادنين (صفر ، ٥٠ جزء/مليون) مع المستويات المختلفة للاجهاد الملحى (ماء الصنبور و ٢٠٠٠ و ٤٠٠٠ و ٦٠٠٠ و ٨٠٠٠ جزء فى المليون من مياه البحر الابيض المتوسط المخففه) على نمو ومحصول ومحتوى هرمونات النمو الداخلية لنباتات الشعير صنف جيزة ١٢٤ .

انخفضت اطوال النباتات وعدد الخلفات والاوراق الخضراء والوزن الجاف للسيقان وطول السنبلة ومحصول القش والحبوب والمحصول الكلى للنبات – كما انخفض تركيز انسجة الاوراق من الجبريلينات والاندولات والسيتوكينينات وزيادة محتواها من حمض الابسيسيك بزيادة تركيز الملوحة فىمياه الرى .

كان للرش بالبنزايل ادنين تأثيرا طفيفا على صفات النمو والمحصول-كما ادى الى زيادة تركيز كل من حمض الاندول اسيتيك والجبريلينات والستيوكينينات ، انخفاض تركيز حمض الابسيسيك بزيادة تركيز الملوحة حول جذور نباتات الشعير .

ادى الرش بالبنزايل ادنين بتركيز ٥٠ جزء فى المليون الى رفع تركيز كل من الجبريلينات واندول حمض الخليك والسيتوكينينات بـ ١٣,٧٦ ، ١٢,٩٧ ، ٢٢,٧٧ % كما ادى الرش الى خفض تركيز الابسيسك اسيد بـ ٢٠,٩٧ % مقارنا بالنباتات التى لم ترش. ومن ذلك يمكن الاستنتاج بان الرش بالبنزايل ادنين ساعد على تقليل آثار الاجهاد الملحى على هرمونات النمو الداخلية المقدرة مما انعكس ذلك على صفات النمو والمحصول .