COUNTERACTION OF SALT STRESSED BARLEY ON SANDY SOIL BY PLANT GROWTH REGULATORS

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ABSTRACT

Greenhouse experiment were conducted on two barley varieties (Giza 123 (salt tolerance) and Giza 125 (salt sensitive)) . Plants were treated with 25, 50 ppm paclobutrazol (PP_{333}) or 10 ppm abscisic acid (ABA) as foliar spray 3 weeks after sowing date. Four levels of NaCl salinity (0, 2500, 5000, 10000 ppm) were used two days after plant growth regulator treatments. The results indicated that, the harmful effect of salt stress was more clear on variety Giza 125 than that found on variety Giza 123. Where, the decrease of plant height and shoot fresh weight by the highest NaCl level were obvious as compared with control treatment. The reduction of two parameters reached 40% and 69% in Giza 123 as well as 66% and 81% in Giza 125, respectively. However, treatment of plant growth regulators significantly reduced the injury effect of salt stress. The ABA treatment had a better effect than that obtained by paclobutrazol ones. Also, the negative effect of salt stress on grain yield was significantly improved by application of plant growth regulators. However, regression analysis between grain yield and some chemical constituents in barley leaves, illustrated that, the K/Na ratio in barley leaves had the highest R^2 value (0.88) with grain yield whereas R^2 values of chlorophyll and proline contents in leaves gave 0.33 and 0.71 respectively. So, the negative effect of salinity on barley may occurr due to decrease of K uptake and increasing of Na uptake. Consequently K/Na ratio was decreased. So improving of barley growth and yield could be achieved by ABA application. This work aimed to determine the best physiological parameter related to salt stress in barley plants may be due to the rebalance of K/Na ratio in leaves.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the world's fourth of the most important cereal crop after wheat, maize and rice. Barley is known to possess a relative ability to tolerate salinity beside its lower requirements for water and fertilizer compared with other cereal crops. In Egypt the cultivation area of barley is reached 105,977 feddans in 2006 which yielded 136,211 tons (according to IAS, 2006). This production doesn't meet our domestic needs. Therefore, bringing a new land under cultivation is a fundamental goal to face the great needs of fast population increment. Most of uncultivated land in Egypt is sand and irrigated with ground water which in some cases contains different levels of salinity.

It is argued that, for plants exposed to salinity stress, the primary cause of reduction in growth rate is not the direct inhibiting effect of Na^+ or Cl^- ions on metabolic processes. The decrease in growth rate is rather due to a change in hormonal balance of the plant, which originates from a modulation of hormonal production by root, the organ sensing the soil environment. Therefore, metabolic perturbations, considered in the mechanistic approach as the cause of growth reduction, are consequent to hormonal changes induced by the presence NaCl. The enhancement of growth of salinized plants following hormonal treatments has been observed by many authors (Davies & Zhang, 1991; Itai & Birnbaum, 1991; Kefu *et al* 1991 and Poljakoff-

Mayber & Lerner, 1993). Finally, Amzallag (1997). It concluded that when increasing the level of salinity, the hormonal balance becomes less and less compatible with maintenance of harmonized communications such as control of sink activity, ion uptake, cell expansion, stomatal movement, and growth of the youngest organs. All these factors induce many secondary physiological perturbations, which eventually affect the physiology of the whole plant. This should be the reason why an external supply of hormones is able to restore the growth rate, at least partially under saline conditions.

MATERIAL AND METHODS

Ten barley grains from each variety (Giza 123 and Giza 125) (obtained from the Wheat Res. Dept., crop Res. Inst., Egypt) were sown per pot (35 cm diameter with bottom drainage) on November 20th for season 2002-2003. The pots were filled with washed sandy soil (15 Kg sand per pot). The pots irrigated with tap water for the first three weeks, then seedling were thinned to two plant per pot. Three weeks after sowing, aqueous solutions of 25 and 50 ppm paclobutrazol (PP_{333}), 10 ppm Abscisic acid (ABA) and tap water (Control) were foliar sprayed on plants using hand air compressed atomizer. Two days after growth regulators treatments, the salt treatments were added to water irrigation. Pots were arranged were complete randomized design with ten replicates. Each plant growth regulator treatment includes four salinity levels which were, tap water (control), 2500, 5000 and 10000 ppm NaCl irrigation was done to field capacity. Pots were fertilized with Arnon and Hoagland (1940). The irrigation with fertilizer solution started two weeks after sowing and till three weeks before harvest. The plants were harvest 160 days after sowing date.

Growth and yield parameters:

Two samples were taken 90 and 160 days after sowing. At the first sample, plant hight (cm) and shoot fresh weight (g) were recorded. At harvesting time (the 2nd sample), weight of 100 grains was determined.

Chemical analysis

Chemical analyses were conducted in leaves at the 1st sample to determine the total chlorophyll, proline, Na^+ and K^+ contents.

Potassium and sodium contents in leaves were determined using flam photometer petracount PFP.

The maximum number of expanded leaves on main shoot under the highest salinity level was five leaves. Then Chlorophyll content of these expanded leaves was determined according to Arnon (1949).

Method of Bates *et al* (1973) was followed to determined proline content. Data were statistically analyzed using SAS procedures (2004**).**

RESULTS

Data presented in Tables (1 and 2) showed that, increasing of NaCl levels up to 10000 ppm without plant growth regulator treatments, significantly reduced plant height and shoot fresh weight. However, the harmful effect of salt stress was more clear on variety Giza 125 than that found on variety Giza 123. The decrease of plant height and shoot fresh weight at the highest NaCl level 40% and 69% for variety Giza 123 and were

66% and 81% for the second variety, as compared with control treatment. On the other hand, without salinity application, plant height and shoot fresh weight obviously decreased by supplying plant growth regulators. As for, the interaction between plant growth regulators and salinity treatments, data in Tables (1 and 2) showed a positive effect for plant growth regulator treatments on shoot fresh weight. However, the plant growth regulators treatment significantly reduced the injury effect of salt stress on shoot fresh weight.Concerning the plant height on the other hand the obtained results were contradietory. The plant growth regulator treatments reduced the injury effect in some salinity application especially with 2500 ppm. The ABA had a better effect, than that obtained by paclobutrazol (PP_{333}).

Table 1: Effect of PP_{333} and ABA on plant height of barley plants grown under different salinity levels.

Salinity level	123				125			
	Control	PP_{333}		ABA	Control	PP_{333}		ABA
		25	50			25	50	
Control	66	60	57	59	67	60	56	57
2500	54	55	50	57	49	55	50	52
5000	47	46	41	45	36	39	35	37
10000	40	38	30	40	23	20	21	19

LSD interaction = 10.496

Table 2: Effect of PP_{333} and ABA on shoot fresh weight of barley plants grown under different salinity levels.

Salinity level	123				125			
	Control	PP_{333}		ABA	Control	PP_{333}		ABA
		25	50			25	50	
Control	45	40	39	42	46	43	46	47
2500	30	36	35	35	22	33	33	37
5000	23	30	27	28	16	20	24	26
10000	14	20	19	20	9	12	13	13

As for yield parameter, Figure (1 a & b) clearly showed that, adding the first NaCl level at (2500 ppm) sharply decreased the weight of a hundred grains to be 27% lowers than the control for the Giza 123 and 48% for the Giza 125. This negative effect of salt stress on weight of a hundred grains (g) was significantly improved by application of the plant growth regulators. The highest positive effect of plant growth regulators on weight of 100 grains was found by ABA treatment for both varieties. However, the threshold clearly showed that the weight of 100 grains without plant growth regulator treatments, at 5000 & 10000 ppm NaCl salinity levels were lower than the half of weight of a hundred grains (g) that obtained by control treatment (threshold). Meanwhile, under 5000 ppm NaCl level all plant growth regulator application were increased the weight of a hundred grains (g) over their threshold for variety Giza 123, whereas, ABA only have been successfully increased the weight of a hundred grains (g) over its threshold for variety Giza 125. At 10000 ppm salinity level. The plant growth regulator applications have been failed to increase the weight of a hundred grains (g) to reach its threshold.

Fig. 1a. Effect of PP_{333} and ABA application on weight of hundred grains of variety Giza 123.

Fig. 1b. Effect of PP_{333} and ABA application on weight of hundred grains of variety Giza 125.

Regression analysis between weight of 100 grains yield and some chemical constituents in barley leaves, such as K/Na ratio, chlorophyll and proline content illustrated in Figure (2, 3 & 4). It is obvious that the K/Na ratio in barley leaves had the highest R^2 value (0.88) related to the weight of 100 grains. Meanwhile the regression analysis between the weights of 100 grain and chlorophyll or proline contents of leaves recorded 0.33 and 0.71 respectively. However, among all the plant leaves (5 leaves) in this stage (90 days after sowing), the chlorophyll content of the second full expanded leaf gave the highest R^2 value (Fig. 3) and (Table 3).

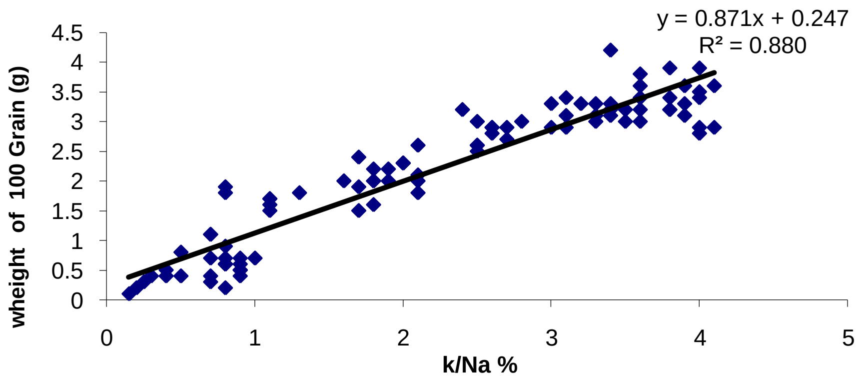

Fig. 2 Regression analysis between K/Na ratio and weight of hundred grains

Table 3. R^2 values of chlorophyll content in expanded leaves on main shoot

Number of leaf	R^2 value
First leaf	0.19
Second leaf	0.33
Third leaf	0.24
Fourth leaf	0.10
Fifth leaf	0.30

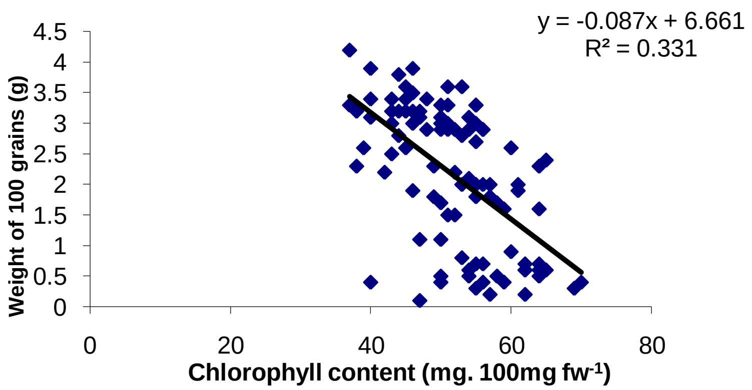

Fig. 3: Regression analysis between chlorophyll content in the second leaf and weight of hundred grains

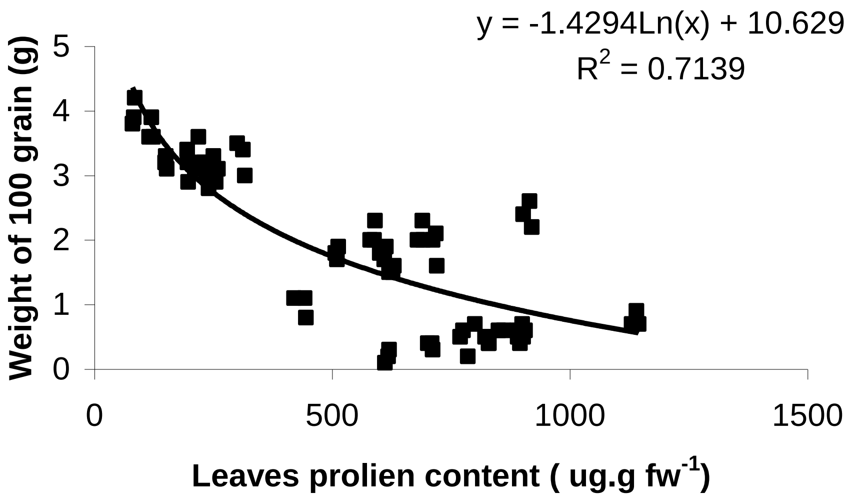

Fig. 4: Regression analysis between proline content in leaves and weight of hundred grains

DISCUSSION

The capacity of plant growth of different plants or varieties respond unequally under NaCl saline. These variation in plant growth could be used to elucidate the mechanisms of salinity tolerance. All these studies yielded general concepts related to the physiological basis of salinity tolerance, which were clearly summarized in the review article of Greenway and Munns (1980) and further investigation were developed, Flowers 1985; Gorham *et al*., 1985; Shannon *et al*., 1994; Marschner 1995; Koyro and Huchzermeyer, 2004 and Eisa 2006. It postulates that the reduction in plant growth is consequence of a toxic effect of Na^{-1} and Cl^{-} accumulated in cells and tissues; nutrient imbalance by depression in uptake and/or shoot transport and impaired internal distribution of mineral nutrients especially K/Na ratio and a reduction of water availability resulted in decreasing osmotic potential in soil, which reduced the transpiration stream and photosynthesis assimilation. All the above mentioned assumptions are still today considered to be the basic underlaying cause for growth reduction of plants grown under salt stress. Therefore the physiological basis for salinity tolerance depends on the capacity of the plant to overcome these disturbing effects. However, many observations related to salinity tolerance in plants do not fit this point of view. Termaat *et al*.,1985; Cramer *et al*., 1989 and Gorham *et al*., 1990 reported that the decrease in growth of barley plants can not always be related to toxic effect induced by NaCl accumulation in plant tissues. In the present work, data presented in Table 1,2 and Figure (3) showed obviously reduction in growth characters by application of ABA or PP_{333} under non saline condition.

Consequently lead to significant decreases of weight of 100 grains. These reductions of growth and weight of 100 grains seen to be similar to the salt stress at the first NaCl level (2500 ppm). Therefore, it may be supposed that a perturbation in hormone metabolism is the limiting factor for growth under salt stress. This hypothesis have been supported by the study carried out on the halophyte *Sueda maritima* by Clipson *et al*., 1988. This author found that the level of ABA accumulated in shoots was maximal for plants grown in absence of salinity, and was minimal for plants exposed to 200 mM NaCl, where the growth was obtained. Then it was suggested that the influence of sublethal salinity on phytohormone production depends on the hormonal setting of the plant, and is not limited to a NaCl-induced disorder in the capacity for hormone production. Data in Table (2) and Figure (1) showed clearly enhancement of shoot fresh weight and weight of 100 grains of salinized barley plants affected by plant growth regulator treatment. When increasing the level of salinity, the enhancement of growth and weight of 100 grains decrease but the yield still better than the untreated plants. In this respect Amzallag (1997) reported that, when increasing the level of salinity, the hormonal balance becomes less and less compatible with maintenance of harmonized communications such as control of sink activity, ion uptake, cell expansion, stomatal movement, and growth of the youngest organs, all these factors induce many secondary physiological perturbations, which eventually affect the physiology of the whole plant. This should be the reason why an external supply of hormones is able to restore the growth rate, at least partially.

Data illustrated in Fig. 2, 3 & 4 showed clearly that, the weight of 100 grains was highly related to K^+/Na^+ ratio more than other chemical constituents such as chlorophyll and proline contents in leaves. Therefore the harmfull effect of salinity in barley may be related to the nutrient imbalance as a result of more Na^+ uptake and consequently K^+/Na^+ ratio was decreased. The stimulation of shoot fresh weight and weight of 100 grains by hormonal treatments may be due to the reduction of Na^+ uptake. In this regards Hadi *et al* (2007), studied NaCl effects on accumulation of minerals (Na^+ & K^+) and proline in wheat. They indicated that among the several genotypes studied, the better resistance genotype to salinity had minimum Na^+ content and had more K^+ and K^+/Na ratio and also highly proline contents in shoots than that found in low resistance genotype. The physiological role of ABA on Na^+ uptake under salt stress has been reported by Wang and Haschke (1991), who reported that, the activity of tonoplast ATPase in barley roots was inhibited by Na^+, but the degree of inhibition was decreased by KCl. ABA decreased tonoplast ATPase activity in roots treated or not treated with NaCl. Moreover, Din and Flowers (2002) reported that, ABA seed pre-treatment significantly increased the plant growth of salinity sensitive wheat variety; Punjab-85, under NaCl salinity. Also, it reduced the Na^+ in the third leaf and the fluxes of Na^+ (38%) and Cl^- (40%) and increased those of K^+ (12%) in the plants of the same wheat variety. Furthermore, K/Na ratio calculated in the xylem sap was also enhanced with ABA pre-treatement in Punjab-85 variety under saline conditions. In addition, it was found that ABA regulated the membrane potential of root cells and that this regulation is consistent with the hypothesis that ABA-induced K^+ accumulation in roots is mediated by K^+ channels (Roberts and Snowman, 2000). ABA is produced to high levels in response to water deficit, including salinity (Ingram and Bartels, 1996 and Leung & Giaudat, 1998). Stomatal closure is one obvious result of the chain of events but, in the long run, these interactive effects eventually lead to leaf-surface reduction through limitation of nitrogen uptake, bringing about reduced plant growth under drought (Bahrun *et al* 2002).

Finally it could be concluded that the harmfull of salt stress on barley plants may be more related to increasing Na^+ uptake and decreasing K^+ and K/Na ratio. The ABA or PP_{333} treatments decreased the Na uptake consequently increased K/Na ration and improved the weight of 100 grains. From this work also indicted that the K/Na ratio is the best physiological parameter related to salt stress in barley plants. Additional clue was found by the threshold of weight of 100 grains whereas the enhancement of weight of 100 grains of salinized plants has been found by ABA treatment.

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الإجهاد الملحى ومقاومته بمنظمات النمو في نباتات الشعير النامية في التربة الرملية

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أجريت التجربة في الصوبة على صنفي شعير (جيزة ١٢٣ (مقاوم)، جيزة ١٢٥ (حساس للملوحة) رشت النباتات بمنظمي نمو باكلو بيوترازول (٢٥، ٥٠ جزء في المليون) وحمض الابسيسك بتركيز ١٠ جزء في المليون وذلك بعد ٣ أسابيع من الزراعة. استخدمت أربع مستويات ملوحة (صفر، ٢٥٠٠، ٥٠٠٠، ١٠٠٠٠ جزء في المليون من كلوريد الصوديوم) كانت إضافة الملح مع وبدأت بعد يومين من الرش بمنظمات النمو.

أظهرت النتائج:

أن التأثير كان أشد على صنف جيزة ١٢٥ عنه في صنف جيزة ١٢٣. عند مقارنة المستوى الأعلى من الملوحة بالنسبة للكنترول وجد أن طول النبات والوزن الغض للمجموع الهوائي قد انخفضا بمعدل ٤٠%، ٦٩% في صنف جيزة ١٢٣ بينما وصل الانخفاض إلى ٦٦%،٨١% بالنسبة لصنف جيزة، ١٢٥. وقد وجد أن المعاملة بمنظمات النمو قد قللت التأثير الضار للملوحة على الوزن الغض للمجموع الهوائي بدرجة معنوية وكان تأثير حمض الابسيسك أفضل من الباكلوربيوترازول. كذلك فإن منظمات النمو حسنت بدرجة من التأثير الضار للملوحة على وزن المائة حبة.

بين وزن المائة حبة وبعض الصفات الكيمائية في أوراق الشعير مثل نسبة البوتاسيوم/ الصوديوم ومحتوى الأوراق من الكلوروفيل والبرولين لتحديد أفضل قياس فسيولوجي مرتبط بوز المائة حبة تحت ظروف الملوحة. أظهرت النتائج أن نسبة البوتاسيوم/ الصوديوم في أوراق الشعير الأعلى ارتباط مع وزن المائة حبة حيث وصلت النسبة إلى ٠,٨٨ بينما كانت ٠,٣٣، ٠,٧١ بالنسبة لمحتوى الأوراق من الكلوروفيل والبرولين بالترتيب.

فيمكن القول أن التأثير السلبي للملوحة على الشعير قد تعود إلى نقص امتصاص البوتاسيوم وزيادة امتصاص الصوديوم أى إنخفاض نسبة البوتاسيوم/ صوديوم. ودور المعاملة بحمض الابسيسك في تحسين وزن المائة حبة قد يعود إلى إعادة اتزان نسبة بوتاسيوم/ صوديوم في الأوراق.