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ABSCISIC ACID, RELATIVE WATER CONTENT AND WATER USE EFFICIENCY OF WHEAT PLANTS IN RELATION TO GRAIN PRETREATMENT BY PHYTOHORMONES AND IRRIGATION WITH SEA WATER

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تأثير نقع حبوب القمح في بعض الهرمونات النباتية والري بماء البحر على حمض الأبسيسيك والمحتوى المائي النسبي وكفاءة استخدام الماء

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أدى الرى بماء البحر عند تركيزات ١٠٪، ٢٥٪ إلى زيادة معنوية في حمض الأبسيسيك الحر أو المرتبط، ولوحظ أيضاً زيادة في المحتوى المائي النسبي للورقة الثالثة، كما أدت عملية الرى بماء البحر إلى نقص ملحوظ في كفاءة النباتات لاستخدام الماء حيث وجد أن معاملة حبوب القمح قبل زراعتها بالنقع في محاليل من حمض الجبريلليك أو أندول حمض الخليك أو حمض الأبسايسك تؤدي إلى نقص في كميات حمض الأبسايسك التي تنتج عند ريها بماء البحر. كما أدت عملية النقع في الهرمونات المذكورة إلى زيادة في كل من المحتوى المائي النسبي وكفاءة استخدام الماء للنباتات المعاملة بماء البحر.

Key Words : Abscisic acid, Wheet plants, Photohormones, See water

ABSTRACT

Irrigation of wheat plants with sea water (10% and 25%) led to significant increase in free and bound ABA in the leaves, especially at 25%. The relative water content (RWC) of the sea water irrigated plants was either higher (3rd leaf) or lower

(5th leaf) than the control. Water use efficiency was lower in salinized plants than the control.

Grain presoaking in either GA_3, IAA or ABA was able to reduce the levels of accumulated ABA (free and bound) resulting from sea water irrigation. Hormonal treatments caused insignificant or significant increase in RWC. The three hormones were able to increase the lowered values of WUE of the salinized plants.

INTRODUCTION

Developmental responses of the plants grown on saline soils have been discussed by many authors [1-4].

Gibberellic acid (GA_3) and indole-3-acetic acid (IAA) increased plant growth and pigment content of salinized plants [5-6].

Aldesuquy and Gaber [7] demonstrated in *Vicia faba* that seed treatment with IAA, GA_3 or kinetin alleviated either partially or completely the effects induced by two levels of sea water (10% and 25%) on leaf area, pigment content, Hill activity of isolated chloroplast and leaf photosynthetic activity.

Abscisic acid content increases in leaves of plants exposed to water and osmotic stresses [8]. Salinization of sorghum plants [*Sorghum bicolor* (L.) Moench.] decreased water-loss rates and increased water use efficiency under stress in controlled environment conditions [8]. With respect to salt tolerance, wheat is usually rated as only being moderately tolerant [9].

The objective of this study was to determine the effects of hormonal presoaking of grains and irrigation with sea water on the levels of ABA, relative water content (RWC) and water use efficiency (WUE) in wheat plants.

MATERIALS AND METHODS

Plants : Homogenous *Triticum aestivum* L. (cultivar Sakha 69) grains were surface sterilized by soaking in 0.01 M $HgCl_2$ solution for 3 minutes, washed thoroughly with distilled water and divided into four sets which were soaked in distilled water, 50 mM GA_3, 5 mM IAA or 1 mM ABA respectively for about eighteen hours through three days (six hours daily). After soaking, thoroughly washed grains were planted on the 12th of Nov. 1990 (15 per pot) in earthenware pots (30 cm in diameter) filled with 3 kg soil (sand and clay 2/1 v/v). The pots were kept in the greenhouse under a normal day/night regime and irrigated with tap water as required. After two weeks only five uniform seedlings were left in each pot. The plants of each set were divided into three groups, which were irrigated with 0,10 or 25% sea water respectively. The standard sea water contained [kg m^{-3}] Cl^-, 21,6; Na^+, 11.1; SO^{-2}, 2.85; K^+, 0.49; P^{+3}, 16.6; salinity was 38.5 g kg^{-1}, pH = 8.1; its electrical conductivity 62 mS/cm. Following thinning and before heading, the plants received 35 g (N)m^{-54} as ammonium nitrate and 35 g(P) m^{-54} as superphosphate.

To measure the water content of the leaves, the relative water content was determined according to Weatherly [10].

For extraction of ABA and its metabolites from fresh wheat leaves (5th leaf), the procedures followed were those according to Goldschmidt et al. [11] and measurements of growth inhibitory activity to determine the concentration in the extracts was by the bioassay described by Wright [12].

The water use efficiency was calculated by dividing the grain yield (tons/hectare) or the biomass yield over the amount of water added (gallons). Therefore WUE_G was calculated from the grains yield and WUE_B was estimated from the biomass yield [13]. Measurement of WUE was carried out during both seasons of 1990 and 1991.

The results were first subjected to an analysis of variance (ANOVA). If the ANOVA showed significant ($P < 0.05$) effect, the least significant difference was performed [14].

RESULTS

1. The influence of hormonal pretreatment on abscisic acid metabolism (Table 1)

1.1. Changes in free ABA

The results (Table 1) indicate that increasing the concentration of the sea water in the irrigation medium increased the levels of free ABA in wheat plants significantly. This increase was proportional to the concentration of sea water applied i.e. at 10% there was ~ 2-folds increase and at 25% the increase in free ABA was ~ 4-folds.

Pretreatment of grains with GA_3 alone was able to reduce significantly the levels of free ABA below the control. Gibberellic acid pretreatment was able to eliminate the effect of sea water on the accumulation of ABA.

Pretreatment of grains with IAA alone brought about insignificant decrease in free ABA. The effect of pretreatment with IAA on the levels of free ABA after osmotic stress due to sea water irrigation has shown that IAA was able to decrease significantly the levels of ABA, with less values at 10% sea water than that shown at 25%.

Grain presoaking in ABA caused insignificant increase in free ABA of the resulting plants. The application of sea water to the plants already resulted from grains previously soaked in ABA resulted in an insignificant decrease in free ABA (at 10% sea water) and significant decrease in free ABA (at 25% sea water).

1.2. Changes in bound ABA

The pattern of changes in bound ABA is quite the same as that of free ABA previously described in relation to irrigation by sea water.

The use of GA_3-pretreatment alone as grain presoaking, obviously gave highly significant levels of bound ABA as compared to the control. The effects of GA_{55} presoaking upon irrigation by sea water clearly shows that GA_{55} was able to cause significant decline in bound ABA if compared to sea water alone.

The use of IAA-pretreatment alone as grain presoaking was able to cause significant increase in bound ABA as compared to the control plants. IAA was able to reduce significantly the bound ABA when sea water was applied.

Presoaking grains in ABA insignificantly increased the bound ABA in the resulting plants as compared with control. However, less amounts of bound ABA were obtained in plants originating from presoaked grains in ABA and irrigated by sea water as compared with sea water alone.

2. The influence of hormonal pretreatment on relative water content (RWC) of wheat plants irrigated by sea water (Table 2a)

The results presented here for the 3rd leaf showed that the values of RWC in wheat plants irrigated by 10 and 25% sea water alone were significantly higher than those of the control plants. Grain presoaking in GA_{55} alone had no effect on the RWC as compared to the control plants. The irrigation with sea water of the plants pretreated by GA_{55} caused insignificant increase in RWC compared to the control plants, these values are still less than those for the plants irrigated by either 10 or 25% sea water alone. Pretreatment with IAA caused insignificant decrease in RWC as compared to control. The influence of IAA on RWC of sea water irrigated plants showed that IAA caused insignificant and significant increase in RWC with 10 and 25% concentration respectively. The use of ABA alone led to an insignificant increase in RWC if compared to control plants. The irrigation by 10% sea water of ABA previously treated plants insignificantly decreased the RWC, while with 25% sea water, the RWC was significantly increased.

The results also demonstrate that, the irrigation by sea water raised the values of relative water content compared to the control plants or the hormonal treatments alone or hormones and sea water irrigated conditions.

The changes in RWC of the 5th leaf showed different and more interesting results than those from the 3rd leaf. Thus in control the original value was ~88%, which subsequently decreased by sea water treatments. Gibberellic acid alone caused significant increase in RWC than did IAA or ABA. All the three hormones caused insignificant increments in the values of RWC, except with GA_3, or IAA at 10% sea water which caused significant increase in RWC.

3. Effect of hormonal pretreatment on water use efficiency (Tble 2b):

It is clear from the results that the value of WUE_G in case of irrigated plants by sea water was significantly lower than those of the control. The reduction of the values was more in case of 25% than in 10% concentration. Hormonal pretreatment in either GA_3 or IAA or ABA clearly improved the WUE_G as compared to the plants untreated with hormones and there was a higher response in case of 10% compared to 25% sea water treatments. Indole-3-acetic acid treatments were the best chosen hormone to manifest in higher WUE_G than did both GA_3 and ABA.

From the estimated values of WUE_B, the salinized plants are showing the same pattern of changes like that of WUE_G. It is important to note that at 10% sea water each of the three used hormones was able to result in insignificant increase in WUE_B, but at 25% sea water in all the treatments there was a significant increase in WUE_B as compared with salinity and non-hormonal treated plants.

DISCUSSION

In the present work, sea water application to cultivated wheat plants exerted some effects on some physiological parameters e.g. the levels of free and bound abscisic acid, the relative water content and the water-use efficiency. These parameters have not yet been investigated by others at least for wheat plants.

It was shown by analysis (Ismail, unpublished data) that the sea water contained the following ions: Cl^-, Na^+, K^+, SO^{-2}, P^{+3} and other salts, so it works as an osmoticum (osmolyte).

The results showed that sea water application resulted in a dramatic increase in free and bound ABA. This hormone was already shown to accumulate rapidly and to high levels in plants subjected to water and osmotic stress [15].

It is likely that this increase in ABA is primarily the result of its increased synthesis by the plant [16-18]. It seems likely that to achieve adequate and rapid stomatal control of water loss a large increase in ABA concentration in response to a decrease in water potential would not be necessary [19].

Hence, the logical explanation of the results obtained in this study is that Presoaking of grains in GA_{3-} or IAA was able to eliminate the stimulatory effects of sea water on the synthesis of ABA. Thus both free and bound ABA content were significantly declined by GA_3 or IAA. It was shown in salinized *Pisum sativum* that the seed pretreatment with GA_3 or IAA was capable to counteract any inhibitory effect shown by salinity and consequently there was a decline in ABA levels [5]. Abscisic acid pretreatment was also able to reduce the endogenous levels of free and bound ABA of the salinized wheat plants.

Results indicate that there was no water deficit in wheat plants irrigated by sea water alone, as the RWC values of the treated plants were higher than the control. Both GA_3 and IAA increased values of the RWC at 10% and 25% sea water, while ABA increased it only at 25%. Such increase in RWC of the salinized plants than the control or the hormonal salinized plants may be presumably due to the accumulation of ABA to levels that cause stomatal closure and thus reducing water loss. Stomatal regulation through ABA prevents tissue damage through excess loss of turgor and improves the water use efficiency of plants, allowing more growth on a given supply of water [20].

To control their water status, plants might have two hormonal mechanisms: one which controls stomatal pore width, and a second which controls hydraulic conductivity of the root [16]. It was shown by Bradford [21] that spraying the shoot of flacca plants (an ABA-deficient tomato mutant) with ABA not only caused stomatal closure, but in addition, raised the hydraulic conductivity of the root system. Besides this role in the control of water uptake in the root, ABA may function as a chemical signal which transfers information on water shortage in the root, or in parts of it to the shoot [22].

The water use efficiency of wheat plants was greatly reduced under the effect of sea water irrigation. On the other hand, presoaking in hormones exerted positive effects in antagonizing the negative effects shown by sea water on water use efficiency of the salinized plants.

The effect of the used hormones as already proposed in the first part of this study may be a reduction in water loss rate with a concomitant increase in leaf water potential and carbon gain rates leading to higher WUE values than the values of sea water irrigation alone. The results of Aldesuquy [6] pointed out that wheat shoot growth of salinized treated plants was, in general, stimulated in response to presoaking the grains in kinetin or GA_3. IAA + salinity led to negligible effect on the plant growth. These results might explain the different effects of GA_3 and IAA on WUE values obtained here. Also, Aldesuquy and Gaber [7] showed that when seeds of *Vicia faba* were treated with IAA or GA_3 or kinetin and irrigated by sea water (10% and 25%) these hormones were able to alleviate either partially or completely the effects of salinity on leaf area at all growth stages. The same authors also found that IAA, GA_3 or kinetin were able to counteract the deleterious effects imposed by sea water irrigation on photosynthetic activity.

On the basis of the obtained results we find that, this wheat cultivar is not suited for growth in saline irrigated soils due to the decrease in WUE. When it is necessary to use sea water for irrigation of wheat plants, hormonal presoaking in GA_3 or IAA is advisable to give higher WUE very close to and sometimes exceeds the values of plants under normal practice.

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Table 1

Effect of grain presoaking in GA_3, IAA or ABA on the concentration of free, bound and total ABA of wheat plants irrigated by sea water. The values expressed in $\mu g.g^{-1}$ fresh weight

ABA content	Control	Treatments											L. S. D.	
		Sea water		GA_3 + +			IAA - -			ABA + +				
		10%	25%	Cont.* GA_3	100%	25%	Cont. IAA	10%	25%	Cont. ABA	10%	25%	5%	1%
Free	3.8	6.7	14.6	1.5	3.5	3.8	3.3	2.3	4.9	4.1	5.7	8.5	1.3	1.8
Bound	1.3	3.5	6.7	4.5	2.7	4.6	2.7	1.9	3.3	1.4	2.8	4.4	0.7	1.1
Total	5.1	10.2	21.3	6.0	6.2	8.4	6.0	4.2	8.2	5.5	8.5	12.9	2.0	2.9

* Cont. = Control

Table 2

Effect of grain presoaking in GA_3, IAA or ABA on (a) Relative water content and (b) Water Use Efficiency (WUE) of wheat plants irrigated with sea water

(a) Relative water content

Treatments		3rd leaf	5th leaf
Control		77.2	88.1
Sea Water	10%	87.2	87.6
	25%	91.1	83.8
GA_3	Control	79.7	95.2
	+10% sea water	81.7	93.9
	+25% sea water	82.7	89.4
IAA	Control	75.4	90.6
	+10% sea water	81.2	91.5
	+25% sea water	87.3	85.5
ABA	Control	81.9	89.2
	+10% sea water	74.6	92.6
	+25% sea water	86.5	87.5
LSD	0.05	5.1	3.3
	0.01	7.2	4.7

Relative water content and water use efficiency of wheat plants

(b) Water Use Efficiency (WUE)

Treatments		WUE_G	WUE_B
Control		1.53	3.53
Sea	10%	0.82	1.71
Water	25%	0.06	0.28
GA_3	Control	1.60	3.44
	+10% sea water	1.02	1.77
	+25% sea water	0.52	1.37
IAA	Control	1.40	2.82
	+10% sea water	0.98	1.96
	+25% sea water	0.30	0.63
ABA	Control	1.24	2.56
	+10% sea water	0.75	1.72
	+25% sea water	0.17	0.45