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THE INTERACTION BETWEEN GA_3 AND SALINITY IN VEGETATIVE GROWTH OF TWO BARLEY CULTIVARS (*Wide otpa* AND *Arrayhan*)

Adam, M. A.
University of Misurata, Faculty of Science, Department of Botany, Misurata- Libya.

ABSTRACT

Two different salt sensitive Barley cultivars (*Wide Otpa* and *Arrayhan*) were examined under three different NaCl concentrations, 0.0, 100, and 300 mM. Barley seeds were pretreated with two Gibberillic acid (GA_3) 0.0, And 100 µM concentrations were used. The paper towel (paper roll) method was used to germinate the seeds. Root length, shoot length, root mass, shoot mass and percentage seed germination, and relative water content were measured as indicators of hormone effects at different salt levels.

The results of this study indicate that the use of GA_3 as Barley pretreatment could possibly alleviate the damaging effects of high salt levels on seedlings, particularly in brackish soil. Wide Otpa cultivar was shown more responses to the hormone treatments than the other cultivar, particularly those plants were germinated under 100 mM NaCl.

INTRODUCTION

The effect of plant hormones on plant growth under salinity stress, has been studied in the hope of improving of salt tolerance. The attempt to produce salt tolerant crops was evident in ancient times (Jacobsen & Adams, 1958). Flowers & Yeo (1995) suggested five possible ways, which were appropriate at that time, to develop salt tolerant crops: (1) develop halophytes as alternative crops; (2) use interspecific hybridisation to raise the tolerance of current crops; (3) use the variation already present in existing crops; (4) generate variation within existing crops by using recurrent selection, mutagenesis or tissue culture; and (5) breed for yield rather than tolerance. In addition genetic studies involving gene transfers have had great support from the scientific community.

However, other technical applications such as treatment with plant hormones have led to some exciting results in the improvement of salt tolerance. Gibberellins (GAs) play an essential role in many aspects of plant growth and development, such as seed germination (Jones & Stoddard, 1977; Haba *et al*., 1985; Khafagi *et al*., 1986; Kumar & Neelakandan, 1992; David *et. al*., 1993; Maske *et al*., 1997), stem elongation and flower development (Yamaguchi & Kamiya, 2000). It has been known that plant hormones can regulate plant responses to salt effects, (Camacho *et al*, 1974; Itai *et al*. 1978; Walker & Dumbroff, 1981), increasing wheat and bean seed germination with indol acetic acid (IAA) or gibberellic acid (GA_3) treatment under salt stress (Salama & Ahmed, 1987). Kabar (1990) found the same results in barley and wheat seeds treated with GA_3 and exposed to saline conditions. It reduced Na^+, Ca^{++} and Cl^- accumulation and improved K^+

uptake under salinity and water logging stresses. Increasing of K^+/Na^+ ratio helped the plants to avoid Na^+ toxicity and enhanced shoot growth and grain yield. Kinetin also reduced membrane injury by dehydration and heat stress and improved the water status of plants under both aerobic and anaerobic conditions. Significant growth stimulation under salinity as well as increasing free radicals (Reactive oxygen SP. ROS) while increasing antioxidant material was found in some plants (wheat, barley, rice, broad bean and suaeda sp., etc.) that were treated with IAA or GA_3 (Boucaud & Ungar, 1976a; Parasher & Varma 1988; Kapchina & Foudouli 1991; Ivanova *et al*., 1991; Adam, 1996). A significant improvement in plant height, leaf area, grain size, net CO_2 and photosynthetic capacity of the tested wheat plants was caused by treatment with GA_3 under salt stress as reported by Ashraf *et al*. (2002). Prakash & Prathapasenan (1990) reported a significant increase in rice production in plants that were treated with GA_3 and grown under NaCl treatment. The same researchers reported that GA_3 could enhance the ionic balance in plant cells and reduced the inhibition of growth under saline conditions.

MATERIALS AND METHODS

Plant material: Two Barley cultivars were obtained from Tripoli Seed Improvement Center**.**
Equipment and Supplies: The paper towel (paper roll) method of Hampton & Tekrony (1995) was used to study germination and initial growth of Barley cultivars (Fig 1). Paper towels of the same weigh, thickness, and size (45x23 cm) were used.

Fig(1): Hampton & Tekrony seed germination method.

Twenty five seeds were sown per sheet, then the mean of the twenty five were taken as a one replicate. Three plastic containers (30, 22,12 cm) were used to soak the papers in the various salt solutions. Plastic bags 42x33 cm were used to keep the towels rolls moist. Plastic pots were used to keep rolled towels in an upright position. Then the experiment was carried out under room temperature, and kept for seven days before results were taken.

Seed sterilization: Directly before placing seeds on the paper towels the seeds were surface sterilized in 3.5% sodium hypochlorite solution (NaOCl) for 3 minutes and then immediately gently washed with sterilized, distilled water.

Solutions preparation: Three NaCl solutions (0.0, 100, and 300mM) were used as germination media, and for a control, distilled water (0.0 mM) was used. Gibberillic acid (GA_3) was dissolved in distilled water and mixed for 15 min. on a magnetic stirrer. Concentration of 100 µM were prepared from the hormone just before use, and kept in dark bottles.

Hormone and salt treatments: papers towels were soaked in several solutions as follow: (0/0, 0/100, 0/300, 100/0, 100/100, 100/300) hormone, salinity respectively, that for 24 hours before seeds were sown.

Parameters measurement: After seven days of germination the percentage seed germination, coleoptile length, root length, fresh root mass, fresh shoot mass, dry shoot mass, dry root mass, relative water content of shoots and roots were measured.

Results analysis: The results were subjected to variance analysis (ANOVA).

The aim of this study was to try to alleviate the harmful effect of salinity on two barley varieties by using the treatment of plant hormones (Gibberillic acid GA_3).

RESULTS

The effect of increasing of NaCl concentration led to significant decreases of all parameters that were investigated.

The effect of increasing gibberellin concentration on the percentage of germination of two barley cultivars (Fig. 2) was in general not consistent for the different cultivars investigated. The shoot length significantly increased with gibberellin treatments of the two barley cultivars that were treated with non and moderate salt concentrations (Fig. 2).

Root length growth of Wadi Otpa cultivar as shown in Fig. 2 was promoted by GA_3 treatment particularly the plants were grown under 100 mM NaCl.

The effects of increasing GA_3 and salt treatments on the root mass of the six investigated wheat cultivars, are presented in Fig. 2. The treatment of 100 µM GA_3 led to significant increasing in fresh shoot mass of the two barley cultivars, except those plants of Arryhan cultivar were grown under 300 mM NaCl were the treatment caused a decrease in fresh shoot mass.

The effects of increasing GA_3 and salt treatments on the dry shoot mass of the two investigated barley cultivars, are presented in Fig.2 . The figure shows that the treatment with GA_3 had a positive effect on dry shoot mass of Wadi Otpa plants that were grown under 100 mM salinity and Arrayhan plants that were grown under 0.0 and 300 mM NaCl, and in general The dry shoot mass of seedlings of both cultivars germinated under non-saline and slightly saline conditions, tended to increase with an increase in GA_3 concentration.

Fresh root mass was significantly increased in plants of both cultivars that were grown under 100 mM NaCl and treated with 100 µM GA_3 , other treatment didn't show significant results. (Fig. 3)

F2

F3

Significant increases in dry root mass were found in plants that were planted under 100 mM and did not treated with plant hormone, while the treatment with GA_3 led to significant decreases in this parameters. (Fig. 3)
Relative water content of shoots significantly increased in those Wadi Otpa plans that were grown under 100 mM NaCl and treated with GA_3. While hormone treatment led to decreases in relative water content in Arrayhan plants that were planted in high concentration of salinity.
The same result was recorded in relative water content of root in Wadi Otpa plants and that were grown under 100 mM NaCl, while the treatment led to decrease in water content in those plants that were planted under 300 mM salinity. (Fig 3)

DISCUSSION

It is well known that the germination stage is the most sensitive stage of plant life cycle, However, to evaluate that a cultivar been salt tolerant more complicated during deferent plant growth stages (Adam, 2004). Salt tolerance during germination stage could be an indicator to the salt tolerance (Khatun and Flowers , 1995). The percentage of germination of Wadi Otpa plants were decreased under salt treatments, while Arrayhan plants didn't effect by increasing in salt concentration, and according to Khatun and Flowers and further study, the Arrayhan cultivar is a salt tolerant cultivar. (Table 1)

Table (1): ANOVA analysis of percentage of germination

Arrayhan					**Wadi Otpa**			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	0.0001	.9915	4.75	1	2.71	0.125	4.74
Salinity	2	3.22	0.0757	3.89	2	7.60	0.007	3.88
Interaction	2	0.0001	0.999	3.89	2	0.172	0.843	3.88

The individual salt tolerance is varying between plant cultivars, that is supported by many studies such as Adam (2004); Troech & Thompson (1993) ; Epstin , (1977), that variance due to the differences in osmotic pressure of cell sap (Rashid *et al*, 1999), and Na^+ and CL^- concentrations, and the differences in morphological characteristics of different plants. (Baalbaki , *et al* .1999).

The decreases in seed germination under salt effects were recorded in several studies that exposed to the salt effect in plant growth. (Ashraf and Abushakra, 1978). GA_3 is an important factor in enhancing the α-amylase activities in germinating seeds (Palmiano & Juliano, 1972). Salinity has three major effects; increase of osmotic pressure of cell sap, toxic effect, and causes a mineral deficiency. Increases in shoot and root length with GA_3 treatments as shown in Fig.1 and Table (2, 3) could be due to an inherent attribute of the hormone in increasing cell division and cell elongation (Scott, 1984).

Table (2): ANOVA analysis of shoot length of the two barley cultivars

Arrayhan					**Wadi Otpa**			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	41.34	3.26E-5	4.74	1	8.23	0.014	4.74
Salinity	2	565.05	1.37E-12	3.88	2	76.03	1.53E-7	3.88
Interaction	2	33.03	1.32E-5	3.88	2	3.99	0.046	3.88

Table (3): ANOVA analysis of root length of the two barley cultivars.

Arrayhan					**Wadi Otpa**			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	0.0005	0.9826	4.74	1	0.415	0.531	4.74
Salinity	2	280.5	8.4E-11	3.88	2	74.93	1.66E-7	3.88
Interaction	2	0.0016	0.998	3.88	2	1.823	0.203	3.88

The effects of increasing GA_3 and salt treatments on the root mass of the two investigated barley cultivars, are presented in Fig. 1 and 2. As expected, an increase in high salinity at the 0.0 GA_3 treatments caused a decrease in shoot and root mass except Wadi Otpa cultivar when the salt treatment caused a significant increase, this result support the idea of that cultivar is a salt tolerant cultivar. As presented in Fig 1 and 2 that increasing in fresh shoot mass was due to increasing in water content and not to the dry mass of shoot. Table 4 and 5.

Table (4): ANOVA analysis of fresh shoot mass of the two barley cultivars.

Arrayhan					**Wadi Otpa**			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	0.597	0.454	4.74	1	0.579	0.461	4.74
Salinity	2	3.091	0.0826	3.88	2	2.131	0.161	3.88
Interaction	2	6.920	0.0100	3.88	2	8.339	0.005	3.88

Table (5):ANOVA analysis of dry shoot mass of the two barley cultivars.

Arrayhan					**Wadi Otpa**			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	12.488	0.0041	4.74	1	7.840	0.016	4.74
Salinity	2	145.50	3.86E-9	3.88	2	275.06	9.46E-11	3.88
Interaction	2	4.62	0.0323	3.88	2	3.030	0.086	3.88

The improving of salt tolerance via application of GA_3 could be also due to a decrease in osmotic potential of cell sap (Pallas & Box, 1970, Salama & Awadalla, 1989).

Table (6): ANOVA analysis of fresh root mass of the two barley cultivars.

Arrayhan					Wadi Otpa			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	0.0961	0.7617	4.74	1	1.650	0.223	4.74
Salinity	2	10.072	0.003	3.88	2	12.717	0.0011	3.88
Interaction	2	1.715	0.221	3.88	2	11.852	0.0014	3.88

Table (7): ANOVA analysis of root dry mass of the two barley cultivars.

Arrayhan					Wadi Otpa			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	5.977	0.0308	4.74	1	31.86	0.0001	4.74
Salinity	2	99.19	3.44E-8	3.88	2	60.95	5.18E-7	3.88
Interaction	2	0.612	0.558	3.88	2	39.75	5.09E-6	3.88

Significant increase in was reported in water content of shoot and root of plants of Wadi Otpa barley cultivar that were treated with GA_3 and watered with 100 mM NaCl. This results could due to the osmotic adjustment as mentioned above.

While Arrayhan plants didn't show any effect in water content under 0 and 100 mM salinity and showed decreases with GA_3 treatments for the plants treated with 300mM NaCl. same results was found with Ekanayak *et al* (1993).

Table (8): ANOVA analysis of shoot relative water content of the two barley cultivars.

Arrayhan					Wadi Otpa			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	3.42	0.158	4.74	1	6.22	0.0085	4.74
Salinity	2	4.87	0.0024	3.88	2	56.24	3.27E-7	3.88
Interaction	2	3.98	0.0347	3.88	2	4.51	0.0042	3.88

Table (9): ANOVA analysis of root relative water content of the two barley cultivars.

Arrayhan					Wadi Otpa			
Source of Variation	**df**	**F**	**P-value**	**F crit**	**df**	**F**	**P-value**	**F crit**
Hormone	1	2.354	0.658	4.74	1	2.68	0.1955	4.74
Salinity	2	3.049	0.0787	3.88	2	5.662	0.0084	3.88
Interaction	2	0.394	0.1543	3.88	2	8.347	0.0014	3.88

Dramatically clear that the Wadi Otpa cultivar showed a high responses to the GA_3 treatment than Arrayhan cultivar.

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تأثير التداخل بين حامض الجبريليك والملوحة على النمو الخضري لصنفي الشعير (وادي عتبة والريحان - ليبيا)

مفتاح أحمد عظام
قسم علم النبات - كلية العلوم جامعة مصراتة – ليبيا.

اختصت هذه الدراسة حول تأثير الملوحة على نمو نباتات الشعير ودراسة دور حامض الجبريليك (GA_3) في التأثير على تلك النباتات تحت ظروف الملوحة .
تم اختيار صنفي وادي عتبة والريحان - ليبيا كأصناف شعير لموضوع الدراسة. حددت التركيزات (0.0 ,100 , 300 mM) من ملح كلوريد الصوديوم NaCl كمستويات ملوحة مختلفة . حدد تركيزان 0.0، 100 ميكرومول (µM) من حامض الجبريليك (GA_3) لمعاملة النباتات المختبرة.
استخدمت طريقة الورقة الملفوفة التي وصفها Hampton, and Tekrony سنة ١٩٩٥ للاستنبات حبوب الشعير لما لهذه الطريقة من مميزات في هذا الجانب. استخدمت مقاييس النمو كمؤشر لاستجابة النباتات لمعاملات الملوحة والهرمون المختلفة وهي نسبة الإنبات، طول المجموع الخضري، طول المجموع الجذري، الوزن الخضري الطازج، والوزن الخضري الجاف، الوزن الجذري الطازج، الوزن الجذري الجاف، المحتوى المائي النسبي للمجموع الخضري، المحتوى المائي النسبي للمجموع الجذري . وقد أظهرت النتائج أن للملوحة تأثيراً سلبياً على معظم المقاييس المستخدمة خاصة في نباتات صنف وادي عتبة والذي أظهر حساسية أكثر للملوحة عنه في صنف الريحان الذي أبدى بعض المقاومة وكانت واضحة في أنه أقل انخفاضاً في نسبة الإنبات تحت تأثير الملوحة كذلك الزيادة في الوزن الخضري والمحتوى المائي النسبي للمجموع الخضري وعدم تأثر معظم المقاييس الأخرى تحت ظروف الملوحة، والذي يقابله نقص معنوي في معظم المقاييس المستخدمة بالنسبة لصنف وادي عتبة. أظهر صنف وادي عتبة استجابة كبيرة للتداخل بين هرمون GA_3 والملوحة في مستوى الملوحة المتوسطة (100 mM) حيث عكس تأثير الملوحة في جميع المقاييس المستخدمة تقريبا مع إظهاره إلى حساسية أكبر للمعاملة بالملوحة فقط. ومن ذلك يمكن أن نستنتج أن معاملة نباتات الشعير بهرمون حامض الجبريليك أدت إلى زيادة مقاومة أصناف الشعير للملوحة خاصة صنف وادي عتبة والنامي تحت ظروف الملوحة المتوسطة (100 mM) كذلك نستنتج من هذه الدراسة أن صنف الريحان أكثر مقاومة لظروف الملوحة عن الصنف المختبر الأخر.

قام بتحكيم البحث

أ.د / محمود محمد درويش	**كلية الزراعة – جامعة المنصورة**
أ.د / محب طه محمد صقر	**كلية الزراعة – جامعة المنصورة**