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EFFECT OF OSMO-HARDENING SEED ON K AND NA CONCENTRATION AND SOME GROWTH PROPERTIES OF WHEAT UNDER SALT STRESS

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Abstract

Salinity is a major abiotic stress, limiting plant growth and agricultural productivity worldwide. It is known that plant growth regulators mitigate the negative effects of salinity. The current study showed the effect of 6-benzyl aminopurine, gibberellin and melatonin on wheat, which is a moderately tolerant crop to salinity, and it is grown for grain as it is a major crop for most countries of the world. A field experiment was conducted using three cultivars of wheat (Al-Ezz, Dijlah, and Al-Rasheed) under the influence of five combinations of tempering seeds before planting (Seed Hardening with salts (comparison 2), Seed Hardening + Benzyl stimulation, Seed hardening + Gibberellin stimulation, Seed hardening + Melatonin stimulation), all of them. It was irrigated with salty well water (6 dS Siemens m 2 m) in addition to the treatment (comparison 1) that was not subjected to hardening and stimulation and was irrigated with river water. The potassium and sodium content of the leaves were evaluated, The Dijlah and Al-Rasheed cultivars had the highest potassium content 3.40 and 3.10, while the Ezz cultivar had the highest sodium content of 0.238. The area of the flag leaf, the number of branches and the biological yield of Al-Rashid cultivar increased (19.6 cm2 and 43.6 branch cm2 and 126.06g cm-2), while the Dijlah cultivar achieved the highest root weight of 0.568 gm. The combinations of salinity treatment (hardening + melatonin) increased the area of the flag leaf and the concentration of potassium in the leaves and decreased the sodium element (14.03 cm-2, 3.866 and 0.167), Thus, it was superior to the comparison treatment 1 that was not exposed to saline stress, and this was positively reflected on the rest of the studied traits. It turns out that melatonin can relieve salt stress in plants by affecting metabolic or physiological processes. The application of melatonin is an effective management strategy to mitigate salt stress in order to increase agricultural production and sustainability.

Keywords: Wheat, Growth regulators, Seed adaptability, Salt stress.

تأثير تقسية البذور في تراكيز عنصري K وNa وبعض صفات النمو لنبات الحنطة تحت الشد الملحي

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الخلاصة

الملوحة هي إجهاد غير حيوي رئيسي، مما يحد من نمو النباتات والإنتاجية الزراعية في جميع أنحاء العالم. من المعروف ان منظمات النمو النباتية تخفف الاثار السلبية للملوحة. أظهرت الدراسة الحالية تأثير 6-بنزيل امينو بيورين والجبرلين والميلاتونين على الحنطة، وهو محصول معتدل التحمل للملوحة، ويزرع من اجل الحبوب فهو محصول رئيسي لمعظم دول العالم. تم اجراء تجربة حقلية باستخدام ثلاثة أصناف من الحنطة (العز ودجلة والرشيد) تحت تأثير خمس توليفات من تطويع البذور قبل الزراعة (تقسية البذور بالأملاح (مقارنة 2)، وتقسية البذور+ تحفيز بالبنزيل، تقسية البذور+ تحفيز بالجبرلين، تقسية البذور+ تحفيز بالميلاتونين) جميعها رويت بماء بئر مالح (6 ديسي سيمنز م$^{-1}$) إضافة الى معاملة المقارنة 1 التي لم تعرض للتقسية والتحفيز ورويت بماء النهر. تم تقييم محتوى الأوراق من عنصري البوتاسيوم والصوديوم، تفوق صنفي دجلة والرشيد بأعلى محتوى للبوتاسيوم 3.40 و3.10 بينما تفوق صنف العز بأعلى محتوى لعنصر الصوديوم بلغ 0.238. زادت مساحة ورقة العلم وعدد التفرعات والحاصل البايولوجي لصنف الرشيد (19.6 سم^{2}و43.6 فرع سم ^{2} و126.06 غم اصيص)، في حين حقق صنف دجلة اعلى وزن جذر بلغ 0.568 غم. أدت توليفات معالجة الملوحة بالتقسية + الميلاتونين الى زيادة مساحة ورقة العلم وتركيز البوتاسيوم في الأوراق وانخفاض عنصر الصوديوم (14.03 سم $^{-2}$ و3.866 و0.167)، وبذلك تفوقت على معاملة المقارنة 1 التي لم تعرض للإجهاد الملحي، وانعكس ذلك إيجابيا على بقية الصفات المدروسة. يتضح يمكن ان يخفف الميلاتونين من إجهاد الملح في

النباتات من خلال التأثير على الأطر الأيضية أو الفسيولوجية. استخدام الميلاتونين من استراتيجياته إدارة فعالة للتخفيف من الإجهاد الملحي من أجل زيادة الإنتاج الزراعي والاستدامة.

كلمات مفتاحية: Wheat، منظمات النمو، تقسية البذور، الاجهاد الملحي.

Introduction

Water scarcity is one of the major challenges facing arid and semi-arid regions, as Iraq suffers from a shortage of fresh water resources that are used for agricultural purposes due to the drought it has been exposed to in recent years and the continuous storage of water in the neighboring countries where the headwaters of these rivers are located. In view of the great expansion of the agricultural area, the increase in the population and the increasing need for food, the reason for the increase in the demand for water led to the use of alternative water resources of poor quality, including the use of well water to compensate for the shortage of fresh water. High, rapid and homogeneous germination leads to a good field establishment, but salt stress hinders this as it is one of the most important physiological stresses that affect seed germination and seedling growth, which in turn affects the subsequent growth stages as a result of the accumulation or accumulation of dissolved salts to a degree that exceeds their natural rates in the soil, and this leads It leads to the inhibition of germination as a result of a decrease in the amount of water absorbed by the roots and the entry of some ions in quantities that are not commensurate with the cell’s need, thus negatively affecting the vital processes (24). This problem can be overcome through breeding programs or genetic engineering, but it is not so easy because of the complex nature of the characteristic of salt tolerance, as this trait is governed by a large number of genes. Therefore, the use of other methods that shorten time, effort and cost, such as stimulating seeds, can be one of the alternative solutions to reduce the harmful effects of salt stress. One of the alternative methods, simple, inexpensive and easy to apply, is the process of salt hardening of the seeds of selected varieties of wheat to raise their resistance to salt stress under irrigation conditions saline water, as well as stimulation by plant hormones that have an important role in seed germination, including gibberellin acid, 6-benzyl-aminopurine and melatonin Which is one of the hormones that increase the speed of germination by stimulating the hydrolytic enzymes necessary for the breakdown of nutrients and cell division, It regulates physiological processes including regulation of ion uptake, hormonal balance, stomata movement and photosynthesis (1 and 30). In addition, these organizations have an important role in regulating the plant response to environmental stress conditions, as it was found that these compounds provide protection against types of environmental stress such as salt stress, drought stress and thermal stress (9, 4 and 15). Thus, it is possible to increase the ability of the wheat crop to tolerate salinity using plant hormones by soaking the seeds with these compounds before planting, and thus leads to the seeds being impregnated with soaking materials, which provides an additional store for the embryo (22). Therefore, this study aims to reduce the negative effect of salinity of irrigation water by using salt osmo-hardening of seeds with stimulation of 6-benzylaminopurine, gibberellin or melatonin through growth indicators and concentrations of elements.

Materials and Methods

A factorial field experiment was carried out according to a complete random sector design with three replications in the fields of the College of Agriculture / University of Anbar / Field Crops Department, to know the effect of adapting seeds before planting to wheat to bear salt stress. The first factor included three varieties of wheat, Al-Ezz, Dijlah and Al-Rasheed, and the second factor included four combinations of seed tempering (1- Hardening of seeds, 2- Hardening of seeds + stimulation with 6-benzyl aminopurine, 3- Hardening of seeds + stimulation with gibberellin, 4- Hardening of seeds + Stimulating it with melatonin) in addition to 5- The comparison treatment that was irrigated with river water and and its seeds were not hardened and stimulated. The process of hardening the seeds of wheat cultivars was done by placing them in a solution of well water whose salinity was diluted from 10 ds m^{-2} to 6 ds m^{-2} for 12 hours, then they were placed in solutions of growth regulators according to the above treatments for another 12 hours. The seeds treated with the mentioned treatments were planted in the pots with the surface area of the pot 962.5 cm2, height 40 cm and capacity 35 kg, placing a small amount of gravel, then a light layer of glass wool. A silty clay mixture from a depth of (0-30 cm) mixed with phosphate fertilizer (160 kg P ha) in one batch in the form of triple superphosphate (45% P2O5) and nitrogen fertilizer (200 kg N ha) in three batches in the form of urea. The first payment when planting, the second batch in the branching stage and the third in the al-booting stage of wheat crop (16).

The seeds were sown at a rate of 1.20 g per pot (130 kg ha-1). The plants were irrigated when needed with water with a salinity of 6 decimens m (wheat threshold) for the saline stress treatments, and with river water for the comparison treatment, and in sufficient quantity to achieve a 20%, washing requirement to ensure that the ionic equilibrium was maintained as much as possible. The pots were placed in a plastic house, exposed from the sides, and covered with polyethylene only from the top, in anticipation of the amount of rain falling. Irrigation water was prepared from the water of one of the wells in the study site (salinity of 10 ds m^{-2}) using the following equation (5):

$$EC_1 = ECa \times a + ECb\ (1-a)$$

Varieties differed in the time of harvest. The area of the flag leaf was measured by measuring the length of the leaf x maximum width x 0.95, calculating the number of tillers in the pot (cm-2), calculating the root weight after the end of the experiment, estimating the biological yield (gm-pot^{-2}) from the average quotient of the pot area. Samples were taken from the leaves of the plant and for each experimental unit, and then dried in the oven at a temperature of 65 0 C, until the weight was stable, and then the samples were ground to estimate their content of some mineral elements K and Na.

Results and Discussion

Flag Leaf Area: Table 1 results showed that Adaptability and stimulation treatments have a significant effect in the trait of flag leaf area; so, the mix (Adaptability + benzyl stimulation) has dominated to achieve the highest average flag leaf area as 19.67cm^2 to differ significantly from all the treatments included in the study like the control treatment irrigated with river water that made 13.51cm^2, while the mix (Adaptability + gibberellin stimulation) has recorded the least trait average value as 12.47cm^2. the reason of dominance for mix (Adaptability + benzyl stimulation) is attributed to benzyl purine adenine positive effect to reduce the harmful effects of salt stress, keep leaves green and delaying leaves aging due to the mobility of some cytokine types from their xylem vessels extract to their blade cells that keep their being green and prevent their food content for long time (23) and this agrees with the conclusions of (3 and 6) that spraying and embedding in benzyl aminopurine has led to increase flag leaf area.

It is noticed from table 1 the presence of cultivars existence on flag leaf area; so, Al-Rasheed Cultivar has dominated significantly giving the highest average value of flag leaf area as 19.06%cm^2 with increase percentage that exceeded 100% compared to Dijlah Cultivar that made the least average value as 9.13cm^2. the reason of this contrast among cultivars is the resonance of flag leaf area with cultivars genetic nature, due to their difference in duration from culture to 50% flowering which include the duration for flag leaf to grow and elongate (12) and it agrees with what's obtained by (17 and 27), who referred to wheat cultivars contrast among themselves in flag leaf area.

Table 1 Effect of Adaptability and Stimulation Treatments for Three Wheat Cultivars in Some Growth Traits and Both Potassium and Sodium Elements under Salt stress.

Traits Treatment	Flag Leaf area (cm^2)	N.Tillrin tiller pot	Weight of root (gm)	BIO-Yield g pot	Conc. of K%	Conc. of Na%
A – cultivars						
Al- Izz	15.66	39.13	0.226	108.01	2.457	0.238
Dijlah	9.13	42.6	0.568	104.07	3.405	0.145
Al- Rasheed	19.06	43.6	0.358	126.06	3.109	0.183
LSD (5%)	0.974	1.611	0.006	3.945	0.064	0.0039
B -Treatment						
No Adaptability + No Stimulation (Control 1)	13.4	44.56	0.530	124.83	3.428	0.204
Adaptability without any Stimulation (Control 2)	13.51	42.56	0.395	108.44	2.864	C0.156
Adaptability + Benzyl Stimulation	19.67	36.78	0.183	102.69	2.623	0.215
Adaptability + Gibberellin Stimulation	12.47	41.56	0.333	107.46	2.170	0.200
Adaptability + Melatonin Stimulation	14.03	43.44	0.479	120.15	3.866	0.167
LSD (5%)	1.258	2.079	0.008	5.092	0.083	0.0050

The interaction results in table 2 showed that there's a significant effect in response size among cultivars and mixes to indicate that cultivars have made a different behavior to Adaptability and stimulation treatments; for example, it's found that cultivar Al- Izz has differed in its response to mixes to give 21.41cm^2 with mix (Adaptability + benzyl stimulation), while it gave 13.49cm^2 with mix (Adaptability + gibberellin stimulation) and the highest interaction between classes and mixes was between Al- Rasheed Cultivar and mix (Adaptability + benzyl stimulation) who gave the highest average value as 26.5cm^2. it returns to cytokines role in plant cells enhancement and their positive effect in metabolic and physiologic processes that happen in plant cells like carbohydrate, proteins and saccharide catabolism as cytokines prolong the time and number of divisions, widening and elongation (13).

Table 2 Effect of Adaptability and Stimulation Treatments for Three Wheat Cultivars in Some Growth Traits and Both Potassium and Sodium Elements under Salt stress.

	Traits / Treatment	Flag Leaf area(cm^2)	N.Tillring tiller pot	Weight of root (g)	BIO-Yield g pot^{-1}	Conc. of K%	Conc. of Na%
Al- Izz	No Adaptability + No Stimulation (Control 1)	11.8	45.33	0.321	119.53	2.847	0.216
	Adaptability without any Stimulation (Control 2)	15.22	38	0.233	100.71	2.257	0.224
	Adaptability + Benzyl Stimulation	21.47	34	0.119	99.40	2.847	0.306
	Adaptability + Gibberellin Stimulation	13.49	37	0.164	102.68	1.11	0.237
	Adaptability + Melatonin Stimulation	16.32	41.33	0.292	117.75	3.223	0.210
Dijlah	No Adaptability + No Stimulation (Control 1)	8.91	43.33	0.658	109.03	2.947	0.212
	Adaptability without Stimulation (Control 2)	8.46	47	0.560	106.96	3.087	0.096
	Adaptability + Benzyl Stimulation	11.04	35	0.304	91.31	4.073	0.149
	Adaptability + Gibberellin Stimulation	8.12	43	0.652	102.45	2.553	0.148
	Adaptability + Melatonin Stimulation	9.13	44.67	0.668	110.59	4.363	0.118
Al- Rasheed	No Adaptability + No Stimulation (Control 1)	19.48	45	0.611	145.94	2.8	0.184
	Adaptability without Stimulation (Control 2)	16.85	42.67	0.391	117.65	2.527	0.149
	Adaptability + Benzyl Stimulation	26.5	41.33	0.126	117.36	3.363	0.191
	Adaptability + Gibberellin Stimulation	15.81	44.67	0.182	117.25	2.847	0.215
	Adaptability + Melatonin Stimulation	16.64	44.33	0.477	132.12	4.01	0.174
	F- test						
	LSD (5%)	2.179	3.601	0.0136	NS	0.143	0.0087

Tillers Number (tiller. pot^{-1}): Results of table 1 showed that adaptability and stimulation treatments have a significant effect in tillers number; so, the treatment (Adaptability + melatonin stimulation) has dominated making 43.44 tiller pot^{-1} and didn't differ significantly from the control treatment (river water irrigated) that made the highest average value as 44.56 tiller pot^{-1}, while the treatment (Adaptability + benzyl stimulation) has made the least average value as 36.78 tiller pot^{-1}. The control treatment 1 being not stressed with salt stress has increased the number of anvils. But melatonin had a positive role to reduce harmful salt stress effects through regulating the cytokine activities that support growth and free them of apical dominance (8 and 32). For information plant expose to salt stress would cause tillers number reduction due to the lower photosynthesis efficiency that reduce the nutritional assimilations and thus it increases competition among tillers (21). These results agree with (20) who found that increasing river water salinity has led to reduce the tillers number for wheat plant and with (30) who found that embedding wheat seeds in melatonin has increased the plant tillers number.

It's also obvious in table 1 results the presence of cultivars effect; so, Cultivar Al-Rasheed has significantly dominated with the highest average tillers number value as 43.60 tillers, followed by Cultivar Dijlah that didn't differ significantly from it (42.6 tillers.pot^{-1}), while cultivar Al- Izz has made the least average value as 39.13 tillers.pot^{-1}. The reason of cultivars difference is attributed by their genetic differences. Cultivars branching capacity is considered a characteristic that has a combination to genetic structure which is variously affected with environmental factors (12). This result agrees with (7) in wheat cultivars difference in tillers number.

Table 2 shows the presence of significant interaction in response size and direction between cultivars and Adaptability with stimulation mixes in tillers number. It showed that Al- Rasheed cultivar is more responsible to adaptability and stimulation treatments, for example, it's found that mix (Adaptability + gibberellin) has given the highest value with Al- Rasheed cultivar as 44.67 tiller pot^{-1}, and inversely, the same mix has given the least value with Cultivar Al- Izz as 37 tiller pot^{-1}. It's explained by the growth supporting and salinity resisting effects of these plant hormones, as treating with it has reduced the salinity inhibiting effect.

Root System Dry Weight (g): Results of table 1 showed that Adaptability and stimulation treatments have a significant effect in root system dry weight trait. The dominance of control 1 treatment (no Adaptability and no stimulation) with the highest average value as 0.5301g is a result of not exposing to salt stress, but comparison between Adaptability and stimulation treatments shows that all Adaptability + stimulation treatments have significantly dominated on control 2 treatments (Adaptability only) that made the least average root weight as 0.1830g; so, the mix (Adaptability + melatonin) has given the highest root weight as 0.4788g, followed by treatment (Adaptability + benzyl) (0.3948g) and the treatment (Adaptability + gibberellin stimulation) (0.3330g) and the reason returns to the positive role of growth regulators to reduce salt stress harm and increase the root system. Melatonin stimulation leads to develop a strong root system (26) and it agrees

with (31) for wheat crop treated with melatonin under salt stress. For information, the salt stress effects negatively the root growth and reduces soil fresh water content and it agrees with (28) who noticed that salts level increase has cause to reduce root system dry weight.
It's noticed from table 1 the cultivars effect on root system weight trait; so, Cultivar Dijlah has significantly dominated with the highest average root system dry weight as 0.5683g on the other cultivars, while Cultivar Al- Izz has made the least average value as 0.2259g. the difference among cultivars is attributed to their genetic differences, and it agrees with the conclusions of (14). In cultivars root weight differences in their study.

Table 2 shows a significant interaction between cultivars and mixes to indicate that cultivars have acted a different behavior toward Adaptability and stimulation treatments. For example, it's found that Cultivar Dijlah with the treatment (Adaptability + gibberellin) and treatment (Adaptability + melatonin) have made the highest interaction value as 0.652 and 0.668g without significant difference from the control 1 treatment for the same cultivar which made 0.560g. the inverse were Cultivars Al- Rasheed and Al- Izz toward Adaptability and gibberellin stimulation treatment.

Biologic Yield: Results stated in table 1 showed Adaptability and stimulation treatments have a positive effect in increasing biologic yield for reducing the harmful effect of salt stress; so, treatment (Adaptability + melatonin) has made 120.15 $g.pot^{-1}$ of dry matter yield; so, it didn’t differ significantly from control 1 treatment that made the highest average value as 124.83 $g.pot^{-1}$. The reason, perhaps, is the same treatment dominance in tillers number (table 1); so, melatonin functions enhancing growth properties for plants exposed to salt stress by its role to activate photosynthesis through maintaining enzymes involved in this process, plasma membrane permeability and increasing photosynthetic stains. This result has agreed with (31) for melatonin treated wheat plant under salt stress conditions.
It's clear from table 1 the cultivars effect in biologic yield trait. Cultivar Al- Rasheed has significantly dominated giving the highest bio-yield average value as 126.06g with increase percentage 21.1%, compared to Cultivar Dijlah that made the least average value as 104.07g. the reason is that Cultivar Al- Rasheed has dominated in traits of flag leaf area and tillers number (table 1). In addition to cultivars difference in their response to environmental conditions. This result agrees with (2) about cultivars difference in biologic yield trait.

Table 2 shows interaction between cultivars and Adaptability with stimulation treatments but it isn’t significant yet, for example, interaction of each Dijah, Al- Izz and Al- Rasheed cultivars with treatment (Adaptability + benzyl) has made the least values of biologic yield (99.40, 91.31 and 117.25 g pot^{-1}); thus, cultivars have differed in their response to benzyl stimulation. In the same direction, cultivars made it with other stimulators.

Leaves Potassium Content %: Table 1 shows the effect of Adaptability and stimulation treatments in leaves potassium concentration; so, melatonin has reduced the harmful effects of salt stress and it reflected positively on leaves potassium content; so, the treatment (Adaptability + melatonin) is featured with its significant dominance on all the other treatments with significant increase 12.78% to control 1 treatment which potassium leaves content has reached 3.428, while the mix (Adaptability + gibberellin) has given the least average value as 2.170%. the reason is explained by melatonin role to reduce the salt stress affection due to its positive effect to maintain high selectivity of potassium ion despite the presence of excessive sodium ions (11). This result agrees with (10 and 29) for corn and wheat plants treated with melatonin under salt stress.
It's clear from table 1 the effect of cultivars; so, Dijlah cultivar has significantly dominated on other cultivars with making the highest potassium concentration as 3.405, while Cultivar Al- Izz has made the least average value as 2.457%. the reason is attributed to genetic differences among cultivars. It agrees with (2) about wheat cultivars difference in their potassium content.

Table 2 states the presence of significant interaction between cultivars and adaptability with stimulation treatment in potassium concentration to indicate that cultivars have acted a different behavior toward Adaptability and induction treatments, for example, it's found that treatment (Adaptability + benzyl) has increased potassium concentration for all the cultivars compared with control treatment interaction with all cultivars, but its effect size exceeds Dijlah cultivar. In the same direction, treatment (Adaptability + melatonin), interacted with all cultivars to function increasing potassium percentage to moderate plant adaptation with different stresses by reducing the transpiration process.

Leaves Sodium Content %: Table 1 shows the effect of Adaptability and stimulation processes in leaves sodium concentration. Sodium concentration has decreased in both of (Adaptability only) and (Adaptability + melatonin) to 0.156 and 0.167 with a significant decrease as 23.5 and 18.1% compared to control 1 treatment which isn't exposed to salt stress and had average value 0.204. these results came opposite to plant potassium concentration results and it confirms the conclusions of (18) about opposing status in sodium absorption on a hand and potassium absorption on the other. Melatonin functioned also keeping the plasma membrane activities of plant salts harm and potassium absorption enhancement that acted an important role maintaining low osmotic pressure inside plant cells cytoplasm to activate absorbing water and other nutrients like magnesium and calcium and reduced the poisonous effect of sodium ion.
Table 1 shows that there is an effect of the cultivars on the sodium content of leaves, as Al-Ezz cultivar achieved the highest average sodium content of leaves of 0.2384%, while we notice that the Dijlah cultivar reduced the sodium content of leaves by 0.1447%. It may be attributed to reasons including the existence of a mechanism of exclusion of sodium ion by the roots of the plant, or that the plant may take the sodium ion and collect it in the root area and not allow it to move to the upper part of

the plant. The mechanism of sodium ion exclusion is the likely mechanism of salt tolerance of wheat (25). This makes the al-Ezz cultivar sensitive to salinity in this study due to the lack of a sodium ion exclusion mechanism in its leaf cells. While the leaves of the Dijlah cultivar contained low concentrations of sodium and high concentrations of potassium (Table 1). This result agrees with. (19) in their study of sixteen wheat cultivars.

Table 2 shows that there is a significant interaction in the response volume between the cultivars and the Adaptability and stimulating treatments. For example, it was found that the interaction of Dijlah cultivar with treatment 2 (Adaptability only) recorded a decrease in sodium concentration, on the contrary, the wire of Al-Ezz cultivar when it was overlapped with the control treatment 2. This indicates that the cultivars exhibited different behavior in the direction of tempering and stimulation treatments.

Conclusions: It is clear from the results obtained that the use of soaking technique can be one of the important solutions to maintain high potassium level and reduce sodium level under saline conditions. Especially the soaking with melatonin, which excels in most of the studied traits and could be applied to other crops species under saline conditions.

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