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RESPONSE TO SOME TOLERANCE STANDARDS OF SALINITY STRESS TO ADAPT THE SEEDS OF WHEAT VARIETIES WITH SALTY WATER AND GROWTH REGULATORS

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Abstract

Salinity is a significant environmental factor that prevents plant germination and growth, thus affecting agricultural productivity globally. Plant growth regulators, such as 6-benzylaminopurine, gibberellin, and melatonin, have been studied for their ability to mitigate the adverse effects of salt stress on wheat, a salt-tolerant staple crop cultivated worldwide. A field experiment was conducted before planting, involving three wheat varieties (Al-Ezz, Dijlah, and Al-Rasheed) subjected to different seed treatment combinations (salt tempering as control, salt tempering + benzyl stimulation, salt tempering + gibberellin stimulation, and salt tempering + melatonin stimulation), all irrigated with saline well water (6 ds m^{-2}) except for the control group irrigated with river water. The findings highlighted the superior performance of the Al-Rasheed variety, exhibiting the highest levels of chlorophyll, relative water content, peroxidase enzyme, and catalase activity (1.993 mg g^{-1}, 83.51%, 40.09 units ml^{-1}, 34.54 units ml^{-1}, respectively). The combination of salt-adaptation treatment and benzyl stimulation resulted in the highest chlorophyll content (2.117 mg g^{-1}), while salt-adaptation treatment and melatonin stimulation showed the highest peroxidase and catalase activity (47.46 units ml^{-1}, 42.71 units ml^{-1}), along with increased grain yield (58.34 g) and harvest index (48.43%). Melatonin and benzylaminopurine have demonstrated potential in alleviating salt stress in plants by influencing various physiological traits.

Keywords: Wheat, Seed priming, Salinity stress, Growth regulators, Antioxidant enzyme.

استجابة بعض معايير تحمل الإجهاد الملحي لتطويع بذور اصناف من الحنطة بالماء المالح ومنظمات النمو

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الخلاصة

الملوحة من الإجهادات اللاحيوية، التي تحد من نمو النباتات والإنتاج الزراعي في جميع أنحاء العالم. ان منظمات النمو النباتية معروف عنها تخفف الاثار السلبية للملوحة. تهدف الدراسة الحالية بيان تأثير البنزيل بيورين ادنين والجبرلين والميلاتونين في أصناف من محصول الحنطة، وهو محصول متوسط 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$^{-2}$) إضافة الى معاملة المقارنة 1 التي لم تعرض للتقسية والتحفيز ورويت بماء النهر. بينت النتائج تفوق صنف الرشيد بأعلى محتوى من الكلوروفيل ومحتوى الماء النسبي وفعالية انزيم البيروكسيديز والكاتاليز (1.993 ملغم غم$^{-1}$، 83.51%، 40.09 وحدة مل$^{-1}$، 34.54 وحدة مل$^{-1}$) على التوالي. أدت توليفات معالجة الملوحة بالتقسية + التحفيز بالبنزيل الى إعطاء اعلى محتوى من الكلوروفيل (2.117 ملغم غم$^{-1}$)، في حين حققت التوليفة تقسية + التحفيز بالميلاتونين اعلى فعالية لأنزيم البيروكسيديز والكاتاليز (47.46 وحدة مل$^{-1}$، 42.71 وحدة مل$^{-1}$) على التوالي، حاصل الحبوب 58.34 غم ودليل الحصاد 48.43%. يتضح انه يمكن ان يخفف الميلاتونين والبنزيل بيورين ادنين من إجهاد الملح في النباتات من خلال التأثير على بعض الصفات الفسيولوجية.

كلمات مفتاحية: الحنطة، معايير الاجهاد الملحي، تحفيز البذور، منظمات النمو، مضادات الاكسدة الانزيمية.

Introduction

Water scarcity is a major issue in arid and semi-arid regions. Iraq is experiencing a lack of freshwater resources for agriculture due to recent droughts and water being held in neighboring countries. The rise in agricultural land, population, and food demand has increased the need for water, leading to the reuse of lower-quality alternative sources like groundwater to make up for the shortage of fresh water.

Fast and efficient seed germination is crucial for successful field establishment (3), however, saline stress poses a challenge as it significantly impacts the physiological processes involved in seed germination and seedling growth. Excess soluble salts in the soil beyond natural levels can prevent water absorption by roots and disrupt biological processes (18 and 20). This problem can be overcome through breeding programs or genetic engineering, but it is not that easy due to the complex nature of the trait of salt tolerance, as this trait is governed by a large number of genes (26). Therefore, using other methods that reduce time, effort, and cost, such as seed stimulation, can be one of the alternative solutions to reduce salt tolerance. One of the harmful effects of salt stress. The process of salt hardening of seeds of some selected local varieties of wheat to increase their resistance to salt stress under irrigation conditions with water with different levels of salinity. Plant hormones also play an important role in seed germination, including gibberellic acid, 6-benzylaminopurine, and melatonin, which are among the hormones that increase the speed of germination by stimulating the hydrolysis enzymes necessary to analyze nutrients and cell division, and work to regulate physiological processes, including regulating the absorption of ions. Hormonal balance, stomatal movement, and photosynthesis (2, 12 and 24). These hormones are important and necessary for regulating a plant's response to environmental stresses such as salt, drought, and heat (7, 10 and 15). Improving the salt tolerance of wheat crops can be accomplished by treating seeds with plant hormones, enabling them to take in the solution to promote embryo growth. This study aims to reduce the negative impacts of salinity in irrigation water by enhancing seed resilience through seed priming by 6-benzyl amino purine, gibberellic acid, and melatonin, via analysing various physiological indicators.

Materials and Methods

A field experiment factorial was conducted according to the randomized completely block design with two factors and three replications in the fields of the College of Agriculture/ University of Anbar/ Department of Field Crops to determine the effect of seed priming before planting on wheat tolerance to salinity stress. The first factor included three wheat varieties (Alaz, Dajla, and Rasheed), while the second factor comprised four seed priming treatments (1- hardening, 2-hardening + priming with 6-benzyl amino purine, 3- hardening + priming with gibberellin, 4- hardening + priming with melatonin) in addition to 5- the control treatment where seeds were soaked in river water without priming. The seeds of the wheat varieties were primed by immersing them in a solution of well water with reduced salinity from 10 Deci Siemens m^{-2} to 7.5 Deci Siemens m^{-2} for 12 hours, followed by soaking in growth regulators according to the mentioned treatments for another 12 hours. The seeds that had been treated were planted in pots measuring 40 cm in height and with a capacity of 35 kg. These pots were filled with a soil mixture obtained from a field within the Department of Field Crops (the experimental site) at a depth ranging from 0 to 30 cm. A phosphatic fertilizer in the form of triple superphosphate (containing 45% P2O5) at a rate of 160 kg P2O5 ha^{-1}, and nitrogen fertilizer (200 kg N ha^{-1}) applied in three stages using urea (with 46% N content) were incorporated.

The seeds were sown at 1.20 g per pot (130 kg ha^{-1}). Plants were irrigated as needed with saline water of 6 Deci Siemens m^{-2} (wheat threshold). The control treatment meets the 20% leaching requirement to ensure ion balance equilibrium as much as possible. The pots were placed in uncovered plastic houses from the sides and covered with polyethylene from the top to protect against rainfall. Irrigation water was prepared from one of the wells at the study site (salinity 10 Deci Siemens m^{-1}) using the following equation (11).

EC1= ECa x a + ECb (1-a).

The chlorophylls measured using (17), The relative water content was measured using the equation described earlier (21).

$$\text{R.W.C \%} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

The peroxidase enzyme was quantified using method (19), and the catalase enzyme was quantified using method (1).

Results and Discussion

Chlorophyll content of leaves: The findings in Table 1 indicate that the osmo hardening + benzyl adenine treatment outperformed others, resulting in the highest average chlorophyll content of 2.117 mg g^{-1} fresh weight. This was followed by the osmo hardening + melatonin treatment with an average of 1.992 mg g^{-1} fresh weight. In contrast, the osmo hardening + gibberellin treatment showed the lowest average of 1.692 mg g^{-1} fresh weight. Stimulation treatments have a positive impact on boosting chlorophyll levels due to various factors. These include shielding chloroplasts and other cell structures from the harmful effects of free radicals induced by salt stress. Moreover, benzyl adenine can help sustain elevated chlorophyll levels by managing leaf aging, promoting nutrient accumulation, and hindering the activity of the chlorophyllase enzyme responsible for chlorophyll breakdown. Moreover, melatonin also helps safeguard chloroplasts from oxidative harm. These results align with earlier research (16) on benzyl adenine treatment in wheat plants exposed to heat and drought stress, highlighting melatonin's significant role in enhancing chlorophyll levels during salt stress.

The Rasheed variety had the highest average chlorophyll content of 1.993 mg g^{-1} fresh weight, which was not significantly different from the Dijlah variety (1.985 mg g^{-1}). On the other hand, the Al-Ezz variety had the lowest average of 1.652 mg g^{-1} fresh weight, due to differences in growth nature and genetic material among the varieties. These results are consistent with a study involving thirty-four wheat varieties (9).

Table 1: The impact of seed priming and Osmo Hardening on three wheat varieties in some physiological traits under salt stress.

Traits Treatment	Chlorophyll in Leaf	Relative water content	Peroxidase enzyme	Catalase enzyme
z	1.652	70.25	36.77	33.61
Dijlah	1.985	81.81	34.51	32.57
Al- Rasheed	1.993	83.51	40.09	34.54
LSD (5%)	0.0951	2.923	1.303	1.220
No Adaptability No Stimulation (Control 1)	1.754	78.64	23.87	21.01
Adaptability without any Stimulation (Control 2)	1.827	76.52	33.09	31.24
Adaptability + Benzyl Stimulation	2.117	78.94	40.34	36.5
Adaptability + Gibberellin Stimulation	1.692	77.07	40.87	36.4
Adaptability + Melatonin Stimulation	1.992	81.44	47.46	42.71
LSD (5%)	0.1228	N.S	1.682	1.575

Table 2 demonstrates a significant interaction between the hardening and seed priming treatments and wheat varieties, indicating varied responses among the varieties to the stimulation treatment combinations. For example, the "Al-Ezz" variety exhibited a difference in response to the hardening and seed priming treatments, yielding 1.997 mg g^{-1} fresh weight with the hardening + priming by benzyl treatment, and 1.283 mg g^{-1} fresh weight with the hardening + priming by gibberellin treatment. The most notable interaction occurred with the "Rasheed" variety and the hardening + melatonin treatment, resulting in 2.223 mg g^{-1} fresh weight.

Relative water content %: Table 1 displays a notable distinction among the varieties, whereas no significant variations were observed in the osmotic hardening concerning the relative water content of the leaves. The data indicates that the Rasheed variety performed exceptionally well, having the highest average relative water content at 83.51%, while the Dijlah variety showed a comparable percentage of 81.81%. The Azz variety had the lowest average water content at 70.25%. Differences in water content among the varieties may be due to variations in their ability to regulate osmotic pressure in cells and retain high water content in tissues (4). This corresponds with (7) a study involving four wheat varieties exposed to salt stress.

Table 2: The impact of introduction in seed priming and Osmo Hardening on three wheat varieties in some physiological traits under salt stress.

	Traits Treatment	Chlorophyll in Leaf	Relative water content	Peroxidase enzyme	Catalase enzyme
Al- Ezz	**No Adaptability + No Stimulation (Control 1)**	**1.36**	**75.77**	**25.07**	**21.17**
	Adaptability without any Stimulation (Control 2)	**1.737**	**63.2**	**32.73**	**32.07**
	Adaptability + Benzyl Stimulation	**1.997**	**66.32**	**39.83**	**35.6**
	Adaptability + Gibberellin Stimulation	**1.283**	**72.9**	**38.73**	**36.07**
	Adaptability + Melatonin Stimulation	**1.883**	**73.07**	**47.47**	**43.17**
Dijlah	**No Adaptability + No Stimulation (Control 1)**	**2.05**	**75.04**	**20.27**	**19.6**
	Adaptability without Stimulation (Control 2)	**1.86**	**78.6**	**30.93**	**28.07**
	Adaptability + Benzyl Stimulation	**2.22**	**92.47**	**38.27**	**36.83**
	Adaptability + Gibberellin Stimulation	**1.923**	**72.95**	**38.6**	**36.4**
	Adaptability + Melatonin Stimulation	**1.87**	**90**	**44.5**	**41.93**
Al- Rasheed	**No Adaptability + No Stimulation (Control 1)**	**1.853**	**85.12**	**26.27**	**22.27**
	Adaptability without Stimulation (Control 2)	**1.883**	**87.77**	**35.6**	**33.6**
	Adaptability + Benzyl Stimulation	**2.133**	**78.02**	**42.93**	**37.07**
	Adaptability + Gibberellin Stimulation	**1.87**	**85.36**	**45.27**	**36.73**
	Adaptability + Melatonin Stimulation	**2.223**	**81.27**	**50.4**	**43.03**
	F- test				
	LSD (5%)	**0.2127**	**6.535**	**N.S**	**N.S**

Table 2 demonstrates a notable interaction between seed priming, osmo hardening, and wheat varieties, revealing distinct responses in hardening and stimulation treatments among the varieties. For instance, the Dijlah variety exhibited variations in its reaction to the treatments, hardening+ priming by benzyl 92.47%, and hardening+ priming by gibberellin 72.95%. The Azz variety interaction with control treatment 1 (without Hardening and priming) reached the highest average of 75.77%. This could be due to the significant role of benzyl aminopurine in preserving leaf hydration levels during saline stress. It also plays a vital role in safeguarding the cell membrane during water stress and significantly improves nutrient absorption, thus facilitating water uptake (13). This aligns with the pointed-out study (16) where wheat plants treated with benzyl aminopurine showed enhanced resilience under heat and drought stress.

Peroxidase enzyme: The findings in Table 1 demonstrate that pre-planting seed priming and hardening have mitigated the negative impacts of salt stress. This was evidenced by a positive effect on the average peroxidase enzyme activity, with the hardening + priming with melatonin treatment surpassing others and reaching the highest average of 47.46 units mL^{-1}.

The results shown in Table 1 indicate that seed priming and hardening before planting have reduced the harmful effects caused by salt stress. This was reflected positively in the average activity of the peroxidase enzyme, with the hardening+ priming with melatonin treatment outperforming and achieving the highest average of 47.46 units mL^{-1}. In contrast, the treatment without hardening and seed priming (control treatment 1) exhibited a lower average of 23.87 units mL^{-1}, due to no exposure to salt stress, while control treatment 2 (hardening + without priming) exhibited an average of 33.09 units mL^{-1}. The effectiveness of hardening and priming with melatonin treatment may be due to melatonin's significant role in stimulating antioxidant enzyme systems to combat stress and minimize oxidative harm (21). This result agreed with (15 and 25) on corn plants under salt stress and wheat plants exposed to salt stress.

Table 1 shows a significant impact among the varieties. The Rasheed variety had the highest average enzyme activity at 40.09 units mL^{-1}, followed by the Al-Azz variety with 36.77 units mL^{-1}, and the Dajla variety with the lowest average at 34.51 units mL^{-1}. Wheat varieties differ in their genetic composition and capacity to improve the activity of antioxidant enzymes. Previous studies (16) have also indicated variations in antioxidant enzyme levels among different wheat varieties.

Catalase enzyme: The results in Table 1 showed the osmo-hardening and seed priming treatment reduced the harmful effects of salt stress, which was positively reflected in the average catalase enzyme activity. The hardening+ priming by melatonin treatment achieved the highest average of 42.71 units mL^{-1}, meanwhile, the control treatment 1 (without hardening and priming treatment) had the lowest average of 21.01 units mL^{-1} due to not being exposed to salt stress compared to the other treatments. Control treatment 2 (hardening+ without treatment) had an average of 31.24 units mL^{-1}. The superiority of seed priming + melatonin treatment might be attributed to the significant role of melatonin in inducing antioxidant enzyme systems to resist stress and reduce oxidative damage. These findings are consistent with (11 and 25) previous studies on corn and wheat crops under salt stress.

The same table shows a significant impact among the varieties, with the Rasheed variety achieving the highest average enzyme activity of 34.54 units mL^{-1}, which is not significantly different from the Al-Azz variety (33.61 units mL^{-1}). On the other hand, the Dajla variety achieved the lowest average of 32.57 units mL^{-1}. The variation among the varieties may be attributed to the genetic nature of the varieties in their ability to increase the activity of antioxidant enzymes. This is consistent with previous findings (3) that highlighted differences among wheat varieties in their content of antioxidant enzymes.

Total grain yield (gm): The results of Table 3 showed significant differences between the effects of hardening and priming on grain yield. priming treatments with growth regulators all increased the grain yield, with the highest increase observed in the treatment (hardening + melatonin stimulation) which yielded the highest average of 58.34 gm, a 32.32% increase compared to the control treatment 2 (hardening without stimulation) which yielded the lowest average of 44.09 gm. The melatonin stimulation treatment did not differ significantly from control treatment 1 (without hardening and priming) which yielded 58.01 gm. The superiority of the (hardening + melatonin) treatment may be attributed to the role of melatonin in mitigating salt stress damage and its contribution to increasing the components of yield such as the number of spikes and number of grains per spike, resulting in an overall increase in yield. These findings align with the results of (22 and 24), who indicated that melatonin-treated wheat plants through soaking or spraying increased yield components under salt stress conditions. Table 3 also revealed that the Rasheed variety achieved the highest average grain yield of 59.29 gm, a 55.94% increase compared to the Dajla variety which yielded the lowest average of 38.02 gm. The superiority of the Rasheed can be attributed to its highest results in terms of the number of spikes and grains per spike leading to an increase in the final yield. These results are consistent with those found by (5).

Table 3: The impact of varieties, hardening treatments, and stimulation in growth regulators and their interaction in the total yield (tan ha^{-1}).

Varieties	Trait					Average
	Adaptability + Melatonin Stimulation	Adaptability + Gibberellin Stimulation	Adaptability + Benzyl Stimulation	Adaptability without Stimulation (Control 2)	No Adaptability + No Stimulation (Control 1)	
Al-Ezz	64.73	54.7	49.86	47.67	65.14	56.42
Dijlah	44.37	36.01	34.92	34.02	40.62	38.02
Al-Rasheed	65.92	55.76	56.09	50.42	68.27	59.29
Average	58.34	48.82	46.96	44.09	58.01	
	Trait	varieties		introduction		L.S.D 0.05
	4.780	3.703		Ns		

Harvest Index: The results in Table 4 show that the treatment (hardening+ melatonin) significantly outperformed and achieved the highest average of 48.43%. On the other hand, the treatment (hardening without priming) yielded a lower average of 40.59%, which decreased significantly compared to the other treatments. It is known that growth regulators play a significant role in influencing growth patterns, thus some are used to increase dry matter production and enhance its distribution within plant parts to boost economic yield (4). The reason for the superiority of the combination (hardening + melatonin) lies in the role of melatonin in enhancing photosynthesis and expediting the transfer of photosynthetic products from sources to sinks, significantly contributing to increasing final yield components such as spike number, grain number per spike, and weight of 500 grains. These findings align with (24) in observing an increased harvest index when wheat seeds were stimulated with melatonin for 24 hours.

Table 4: The impact of varieties, hardening treatments, and stimulation in growth regulators and their interaction in the harvest index (%).

Varieties	Trait					Average
	Adaptability + Melatonin Stimulation	Adaptability + Gibberellin Stimulation	Adaptability + Benzyl Stimulation	Adaptability without Stimulation (Control 2)	No Adaptability + No Stimulation (Control 1)	
Al-Ezz	54.98	53.26	50.18	47.24	54.42	52.02
Dijlah	40.44	35.19	38.26	31.71	36.94	36.51
Al-Rasheed	49.88	47.56	47.77	42.83	46.86	46.98
Average	48.43	45.33	45.40	40.59	46.07	
	Trait	varieties		introduction		L.S.D 0.05
	1.783	1.381		3.089		

The Alaz variety had the highest average harvest index of 52.02%, surpassing the Rasheed variety at 46.98% and the Dajla variety at 36.51%. This difference could be due to the Alaz variety's effectiveness in distributing photosynthetic products to sinks. This outcome aligns with (23) research on twenty wheat genotypes, which also noted significant variations in harvest index values.

Conclusions

The study results show that combining the osmo-hardening technique with seed priming using melatonin, 6-benzyl amino pyrene, and gibberellin improved the yield and physiological traits of a wheat crop in saline conditions. The seed priming treatments helped counteract the negative effects of salinity by positively affecting different traits, with the hardening + melatonin treatment showing enhancements in antioxidant enzymes and yield.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author A. S. F. Al-jughaif; methodology, writing—original draft preparation, Author B. Sh. J. Alobaidy writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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