Study of salt tolerance of germination and growth of barley seedlings *Hordeum vulgare* L. hydroponically grown

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Abstract

A laboratory experiment was conducted during the academic year (2024-2025) in the laboratories of the Department of biology - College of Education for women / University of Kufa, in which a hydroponic culture consisting of Hoagland's solution was used with the aim of studying the salt tolerance of barley seedlings *Hordeum vulgare* L. The experiment was carried out using a completely randomized design (CRD). The seeds were treated before germination with $CaCl_2$ solution at concentrations of (0.025 and 0.25)%, and another quantity of seeds was left without salt treatment as a control treatment. After the seedlings formed, they were treated with the following concentrations (0.1 and 0.5)% of NaCl after Adding it to the Hoagland nutrient solution, and the control treatment was with Hoagland nutrient solution only. The experiment lasted for 21 days, after which the following laboratory measurements and analyses were performed: germination percentage, plumule length, radical length, fresh and dry weights of the seedling, and estimation of the seedling content of the amino acid proline Means were compared using Duncan's multiple range test at the 0.05 probability level. The results showed that increasing the concentration of NaCl in the nutrient solution led to a gradual decrease in height and weight and an increase in the amino acid proline. The results also indicated that seeds previously treated with salt were more tolerant to salinity than those not previously treated, and this was reflected in the traits studied above.

Keywords: *Hordeum vulgare*, Salt tolerance and stress, Hydroponics

Introduction

Barley plant (*Hordeum vulgare* L.) is an important cereal crop in Iraq. It is primarily used in most countries of the world as a fodder crop, either as green fodder or grain. It is also used in various industrial and medical fields, as a laxative, a soothing agent, and as a food for diabetics, as well as in the production of vinegar and yeast [1]. Barley is one of the oldest and most important agricultural crops in the world after wheat, due to its close connection with the livestock sector, as it constitutes the main source of fodder grains, as we mentioned, in addition to the use of the straw resulting from it as animal feed [2,3]. Environmental stresses affect the various stages of plant growth and development and its various physiological functions. Salt stress is one of these stresses that is still the focus of programs and research and that needs great attention, especially in our country, Iraq, which is classified among the countries most affected by salinity in the continent of

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Asia. Studies on salt tolerance cover from During the effect of salinity in causing many phenotypic and physiological changes at the molecular level, there are many studies focusing on developing salt tolerance mechanisms in the germination and early growth stages of seedlings, which has an impact on obtaining plants with good tolerance later [4]. Increasing the concentration of salts in the growth medium (soil or nutrient solution) not only affects the germination rates but also the characteristics of the resulting seedlings. Many strategies have been tested to overcome the harmful effects of salt stress and other types of environmental stresses, such as treating seeds before germination with different materials such as hormones or salts, in order to study the effect of these additives in increasing salt tolerance by modifying the metabolic activities before field emergence of seedlings [5]. Given the economic importance of barley crops in the world and Iraq, and the conditions that agriculture suffers from as a result of environmental and climatic fluctuations, desertification and drought in our country, and the increasing salinization of lands and the scarcity of water allocated for agriculture due to low water levels or the failure to store water during the rainy season properly for later investment, **the aim of this research is:**

- To study and know the effect of treating barley seeds before planting with $CaCl_2$ and its effect on salt tolerance using hydroponic culture on germination and some growth characteristics of barley seedlings as well as some physiological characteristics.

Material and Methods

A laboratory experiment was conducted during the academic year (2024-2025) from 11/1/2024 to 21/11/2024 in the laboratories of the Department of biology / College of Education for women - University of Kufa, and measurements were taken after the completion of the experiment. The present study was carried out on barley (*Hordeum vulgare* L.), a local variety registered by the Ministry of Agriculture, according to a completely randomized design (CRD) as a simple experiment with three replicates and using a split plot design for three levels of $CaCl_2$. The means were compared according to Duncan's multiple range test at a probability level of 5% [6]. Barley seeds were brought and cleaned from impurities and foreign materials accompanying them. After that, healthy seeds were selected and a quantity of them was soaked in a $CaCl_2$ solution with a concentration of (0.025)%. Another quantity of those seeds was soaked in the same solution with a concentration of (0.25)%, in addition to another quantity that was soaked in water as a control treatment for 24 hours. Then the seeds were dried and a group of seeds soaked in salt at the two concentrations above and those soaked in normal water were taken and planted in Petri dishes provided with filter paper moistened with (5) ml of Plain water at a rate of 6 seeds in each dish and one dish was considered an experimental unit and repeated three times and left to germinate for the purpose of obtaining seedlings. Then the well-germinated and consistent seedlings were transferred to cups with a capacity of (100) ml containing Hoagland's nutrient solution [7]. At a rate of four seedlings per cup, the seedlings were fixed well in the holes in the cup cover, with three replicates for each treatment so that the root system was immersed in the solution. The number of treatments reached 27 treatments, Leaving a hole for ventilation and renewal of the nutrient solution, they were distributed on the same cups and the seedlings that had been previously treated with salt concentrations (0.025 and 0.25)% and those that had not been treated were fixed. The salt treatments were represented by adding the following concentrations: (0, 0.1 and 0.5)% of NaCl to the water used to irrigate the dishes later and to the nutrient solution successively. The experiment continued for (21) days, Figure No (1). The germination percentage was calculated according to the total number of germinated seeds, as the seeds were considered germinated when their root length reached 2-5 mm, Figure No. (2), then the results were converted into a percentage [8] according to the following equation: Germination percentage = number of germinated seeds / total number of seeds × 100. The average length of the plumule was measured from the point of its connection to the Radical to the tip, in addition to measuring the average length of the Radical using a graduated ruler for three repetitions [9]. The fresh weight was measured using a sensitive balance as an average of three repetitions. Then the samples used to measure the fresh weight were dried in an electric oven at a temperature of 65°C until the weight was constant. The dry weight was calculated using a sensitive balance [10]. The method of [11] was followed in estimating the proline content of the seedling.

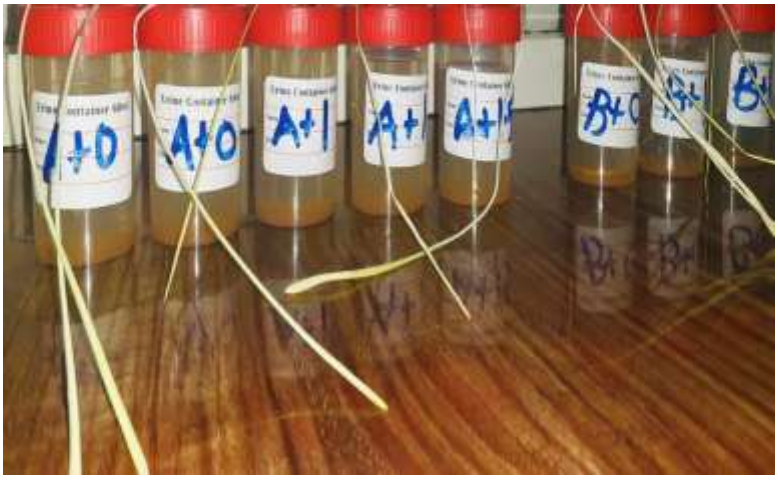

Figure 1: hydroponically grown barley seedlings

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Figure 2: Seed germination

Results and Discussion

Tables (1, 2, 3, 4, 5, and 6) The effect of pre-salt treatment with $CaCl_2$ on salt tolerance to different concentrations of NaCl in the germination medium and the interaction between them was shown on the following properties: Germination percentage % , plumule length, radical length, fresh weight, dry weight, and Proline content of seedling In light of the results obtained, it was found that the highest rates were in the pre-seed treatment, especially at a concentration of (0.25%), which reached (88.29%, 11.03cm, 5.38cm, 0.64 mg, 0.45mg, 0.503 (µmol.mg-1) respectively compared to the rest of the treatments. While the above-mentioned traits were negatively affected by the increase in salinity concentrations in the irrigation of the germination medium, this was evident in a significant difference at the two concentrations (0.1 and 0.5%), and the lowest rates were recorded at the concentration(0.5%),reaching (67.22%,7.63cm,2.99cm,0.35mg,0.24mg).
However, increasing the salt concentrations of NaCl in the seedling growth medium had a significant effect on increasing the accumulation of the amino acid proline, as the rate reached (0.528 (µmol.mg-1) at the salt concentration (0.5%) compared to the control treatment, which reached (0.251 $Mm.mg^{-1}$). As for the interaction, the results showed that the seeds that were previously treated with salt without adding salt during irrigation achieved the highest rates with a clear significant difference from those that were not previously treated. While the accumulation of proline in the interaction treatments of seedlings pre-treated with CaCl2 salt achieved a higher rate than those that were not treated, the interaction treatment between seedlings pre-treated with calcium chloride and the salt concentration of NaCl (0.5%) recorded the highest values compared to the rest of the treatments, while the lowest rate was in the control treatment.

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Table 1. Percentage of germination of barley seeds

$CaCl_2$ %	NaCl %			Mean
	0	0.1	0.5	
0	73.15 f	65.33 h	56.00 i	64.82 c
0.025	88.31 c	80.00 d	68.22 g	78.84 b
0.25	96.82 a	90.65 b	77.42 e	88.29 a
Mean	86.09 a	78.66 b	67.22 c	

Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 2. Length of the plumule (cm) of a barley plant seedling

$CaCl_2$ %	NaCl %			Mean
	0	0.1	0.5	
0	12.00 c	9.32 f	5.99 i	9.10 c
0.025	12.41 b	10.56 e	7.88 h	10.28 b
0.25	13.42 a	11.45 d	8.22 g	11.03 a
Mean	12.61 a	10.44 b	7.63 c	

Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

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Table 3. Radical length (cm) of barley seedling

$CaCl_2$ %	NaCl %			Mean
	0	0.1	0.5	
0	5.55 c	4.21 f	2.52 i	4.09 c
0.025	6.24 b	4.87 e	2.78 h	4.63 b
0.25	7.35 a	5.12 d	3.67 g	5.38 a
Mean	6.38 a	4.73 b	2.99 c	

Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 4. Fresh weight (mg seedling^{-1}) of barley seedling

$CaCl_2$ %	NaCl %			Mean
	0	0.1	0.5	
0	0.69 c	0.45 e	0.29 g	0.47 c
0.025	0.75 b	0.52 d	0.35 f	0.54 b
0.25	0.85 a	0.66 c	0.43 e	0.64 a
Mean	0.76 a	0.54 b	0.35 c	

Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 5. Dry weight(mg seedling-^{1}) of barley seedling

$CaCl_2$ %	NaCl %			Mean
	0	0.1	0.5	
0	0.46 c	0.33 e	0.18 g	0.32 c
0.025	0.51 b	0.39 d	0.24 f	0.38 b
0.25	0.60 a	0.45 c	0.31 e	0.45 a
Mean	0.52 a	0.39 b	0.24 c	

Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

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Table 6. Barley seed content of the amino acid proline (μmol.mg^{-1})

$CaCl_2$ %	NaCl %			Mean
	0	0.1	0.5	
0	0.105 h	0.220 g	0.325 e	0.216 c
0.025	0.265 f	0.393 d	0.575 b	0.411 b
0.25	0.383 d	0.442 c	0.685 a	0.503 a
Mean	0.251 c	0.351 b	0.528 a	

Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

The results obtained in Tables (5, 4, 3, 2, 1 and 6) respectively can be interpreted as exposing the seeds to pre-treatment with calcium chloride salt, which led to improved germination as a result of activating the seeds before the germination stage and thus improving the potential performance of the seed in a wide range of environmental conditions and causing Molecular and physiological changes in the seed, as the activation mechanism works to accelerate the production of ATP and increase the activity of enzymes, especially energy-forming enzymes, with an increase and repair of RNA and DNA. The stimulation and increase in the germination rate of activated seeds is due to the integration of cell membranes, the stimulation of protein and nucleic acid production, and the increase in the effectiveness of antioxidants. During the soaking stage, the level of some Enzymes trigger some metabolic processes that result in the formation of some simple sugars that the embryo can absorb immediately upon the onset of germination [12,13] As for the decrease in the germination rate when salt concentrations in the medium increase, it is attributed to the gradual decrease in the water potential between the seeds and the germination medium and the slow entry of water, which leads to slowing down the vital metabolic activities in the embryos, leading to uneven germination [14]. The results, as we have mentioned, showed an increase in the average length of the shoot and root, as well as an increase in the fresh and dry weights of the seedlings whose seeds were previously treated with CaCl2, as a result of a decrease in the osmotic potential in the seedling cells (it became more negative) compared to the growth medium (Hoagland solution) at the beginning of the first stages of growth, which helped in the entry of water, swelling of the cells, and an increase in size and division, which was reflected in the improvement of the above growth characteristics. These results are consistent with what was reached by [15]. However, with the passage of time and days, with the increase in the effect of salt concentrations in the nutrient solution during

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the stages of seedling growth, the rate of water absorption by seedlings began to decline as a result of the decrease in water potential in them and the increase in osmotic potential in the growth medium, so the filling potential inside the cells decreases, and this is negatively reflected on all the vital processes inside the seedling and the growth characteristics studied, and this is consistent with what was reached by [16,17]. As for the amino acid proline, the relationship between the increase in salts and its content inside the plant is a direct relationship. The increasing salinity causes an increase in the accumulation of the amino acid proline inside the seedling, as it has an important role in the process of osmotic regulation during salt stress and reducing it [18].

Conclusion

From the results obtained, we conclude the following: Soaking barley seeds with different levels of calcium chloride before planting increased seedling tolerance and adaptation to salinity conditions. Pre-treatment of seeds at a concentration of 0.25% was the best in accelerating germination and increasing germination percentage as well as growth characteristics. Increased accumulation of sodium chloride salts in the nutrient solution also reduced growth indicators. Seedling growth characteristics, especially at a concentration of 0.5%, showed a significant difference compared to other treatments. Furthermore, increased salinity led to an increase in the seedlings' content of the amino acid proline, which plays a significant role in reducing salt stress and its impact on plants.

Recommendations

Based on the above, we recommend the following: Soaking seeds before planting in different levels of hormones to cause physiological changes in seed embryos and to study the extent of adaptation and improvement of the growth characteristics of seedlings and resulting plants to stressful environmental conditions. Study and focus on hydroponics using nutrient solutions as an important means of growing crops as one of the points of finding a solution to the problem of water scarcity, in addition to improving plant growth and increasing its quality in less time and at a lower cost under stressful conditions.

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