The Effect of Salt Stress on Seed Germination and Some Growth Characteristics in Two Varieties of Barley (Hordeum vulgare L(.

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Abstract :

The research involved conducting a laboratory experiment in plastic pots in the wire house of the Department of Life Sciences / Faculty of Education for Pure Sciences / University of Mosul, for the agricultural season 2024, with the aim of studying the effect of salt stress (sodium chloride) on seed germination and growth and estimating the proline concentration and peroxidase activity of two varieties of barley plants (white barley and black barley) using three levels of salinity (0,25,50 .% (

The results showed a significant variation in germination as the increase in the concentration of added NaCl reduced the percentage of seed germination and the average lengths of both the vegetative and root system and their dry weights, where the lowest percentage of seed germination was recorded in the (black barley) variety (75%) at (50)% NaCl concentration, while the lowest length of the vegetative system was recorded in the (white barley) variety (22. 17) cm at (50) % NaCl concentration. and the lowest root length in the (black barley) variety (19.23) cm at (50) % NaCl concentration, while the lowest dry weight of the vegetative and root group was recorded respectively in (black barley) variety (0.13 and 0.06) g at (50) % NaCl concentration .

While increased concentrations of proline and peroxidase enzyme activity were observed between the two barley varieties under salt stress conditions, as (white barley) variety recorded the highest concentration of proline (8.21) (μmol g-1 FW) at (50) % salinity concentration, While the highest concentration of proline in the black barley variety was (6.82) (μmol g-1 FW) at a salinity concentration of 50 %. The highest concentration of peroxidase enzyme was recorded in (black barley) variety (20.66) (units. min-1. g FW-1) at (50) % salinity, and the highest concentration of peroxidase enzyme was recorded in (white barley) variety (7.78) (units. min-1. g FW-1) at (50) % NaCl concentration .

Keywords: Salt stress, NaCl, barley, reactive oxygen species, antioxidants.

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Introduction

Barley (Hordeum vulgare L.) is one of the main cereals and is known as the salt plant. It contains the largest amount of dietary fiber compared to any type of grain, used for food and industrial purposes, and geographical factors affect the determination of barley crop cultivation areas, as barley is cultivated. The favorable temperature for barley germination ranges between (20-22oC) [1]. Salinity is an important environmental determinant of crop growth and productivity, as plant production and growth are reduced when salinity exceeds the permissible limit for each crop, and barley is one of the plants tolerant to high levels of salinity [2]. Salt stress is emerging as one of the major challenges facing food production worldwide, with its severity increasing as a result of various natural phenomena and human activities. Salt stress results from oxidative stress and ion imbalance, which severely inhibits seed germination, photosynthesis, respiration, nutrient uptake, and other physiological processes, ultimately leading to reduced yield or even plant death [3]. Plants can resist salinity by using a sodium-potassium pump, often in the roots, which works to return sodium to the external environment while allowing potassium to enter, relying on ATPases to provide the energy needed for this process [4], Some plants can also resist salinity through several mechanisms, including osmotic adjustment. Osmotic adjustment has been observed in many plants, including barley, by raising the osmotic pressure of the cellular content due to the accumulation of salts inside the cell as a means of resisting salt stress in the soil [5]. In a study [6], selected varieties of barley were exposed to salinity stress and four different levels (200,100,50,0) mM NaCl, it was found that salt stress negatively affects germination and growth, and early plant growth was found to decrease with increasing salinity stress. Plants are exposed to salt stress, to face this stress conditions, they accumulate proline as a protective mechanism in plants. Proline acts as an osmotic solution, helping to regulate the water balance within plant cells [7]. Another detrimental effect of salinity on intracellular physiological and metabolic activities is the increased activity of reactive oxygen species (ROS). Reactive oxygen species include hydroxyl radicals and their derivatives such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2) and hydrogen radicals (OH) and are considered highly oxidizing compounds and are usually toxic, they can destroy cell components such as proteins, lipids and nucleic acids and can cause cell death [8]. Plants use resistance mechanisms under saline conditions such as antioxidants to minimize the harmful effects on plant cells, such as peroxidase (POD) and catalase (CAT) enzymes, located in cells, especially in chloroplasts and mitochondria, to overcome reactive oxygen species (ROS) [9]. Oxidative stress is a biomarker of salt stress, which is produced by an imbalance between enzymatic antioxidants and reactive oxygen species (ROS) such as peroxidase content, which is the first line of defense to remove ROS under stress conditions [10] [11]. Proline also acts as a hydroxyl radical inhibitor and stabilizes proteins, cytoskeletons and membranes and has the ability to quench reactive oxygen species (ROS), thereby protecting cellular functions by eliminating ROS [12.[

Therefore, this research

was carried out with the aim of studying the effect of salinity (sodium chloride) at three levels (50,25,0) % on the percentage of germination and some growth characteristics represented by the length of the vegetative and root system and their dry weights, and the area of the flag leaf for two varieties of barley (white barley and black barley) in addition to knowing the effect of salinity on the activity of antioxidants such as peroxidase and proline amino acid under salt stress conditions .

Materials and Methods

The experiment was conducted in the wire house of the Department of Life Sciences / Faculty of Education for Pure Sciences / University of Mosul in the winter season 2024-2025, two varieties of barley (white barley and black barley) were selected, prepared from the Seed Inspection and Certification Center / in Ninewa Governorate / Iraq, and their vitality was recorded (white barley 95% and black barley 93%).The soil used in the experiment was collected from an agricultural field in Nineveh Governorate at a depth of 0–30 cm. It was air-dried and passed through a 2 mm sieve to prepare it for the planting process. The experiment was carried out using plastic pots with a diameter (15) cm and a height (20) cm. (5) kg of dry soil was placed in each pot and treated with sodium chloride in concentrations (50, 25, 0) %, barley seeds were planted on 26/11/2024, with 10 seeds / pot and placed under wire house conditions and after 20 days after planting, the number was reduced to (5) coolers / pot and the moisture content of the soil was maintained to field capacity (75) % throughout the duration of the experiment. After (90) days of planting, the plants were removed from the soil and care was taken to use a water sprayer for cleaning the roots, in addition to using a sieve to prevent the loss of any root parts. After cleaning, the plants were separated into vegetative group and root to prepare them for the required measurements.

Traits studied

.1The percentage of germination eight days after planting was calculated according to the following [13] formula:

Germination percentage% = No. of normal

Seedlings/ No. of cultivated seeds ×100

.2Vegetative and root lengths by Graduated scale (cm) of the plants were measured, then air-dried in an electric oven (70°C) for 72 h and weights by electronic scale (g) were recorded, In addition to The total vegetative lengths of the plants were measured during the duration of the by Graduated scale (cm) [14 .[

.3Flag leaf area (cm2): Flag leaf area was calculated according to the following equation [15 :[

Leaf area (cm^2) = Maximum leaf length × Maximum leaf width × 0.95

.4Determination of the amino acid proline in plant tissues (μmol g-1 FW) according to the method of [16:[

Proline content = Absorbance × 20/Weight of plant sample (g) × 1.47

.5Estimation of peroxidase activity (units. min-1. g FW-1) according to the method of

:[17]

Peroxidase enzyme activity =

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Δ × 3Absorbance /Δtime × 0.001

Statistical Analysis

The experiment was designed according to the Randomized Completely Black Design, the data were statistically analyzed using SAS software, and the mean coefficients were distinguished using Duncans Multiple Range Test at the 5% probability level [18.[

Results

Root Group Dry Weight (g)	Root Group Length (cm)	Vegetative Group Dry weight (g)	Vegetative Group length (cm)	Percentage of germination%	Concentrate Salt%	Varieties
0.73a	29.63a	0.52a	27.37b	93.33 a	%0	White barley
0.09c	24.4b	0.26d	24.07c	86.66 b	%25	
0.08c	24.03b	0.21d	22.17c	81.67 c	%50	
0.3a	26.02b	0.33c	24.53c	87.22a	Average white barley variety	
0.17b	38.4a	0.65a	37.94a	91.67 a	%0	Black barley
0.14b	28.3a	0.45b	36.4a	78.33 cd	%25	
0.06c	19.23c	0.13e	27.24b	75d	%50	
0.123b	28.64a	0.41b	33.85a	81.67b	Average barley variety	
0.45a	34.02a	0.58a	32.65a	92.5a	%0	Average salt concentration
0.11b	26.35b	0.35c	30.23b	82.5b	%25	
0.07c	21.63c	0.17e	24.71c	78.33c	%50	

Table (1) Effect of adding sodium chloride salt at three levels (0,25,50 %) on seed

germination and growth in two varieties of barley (white barley and black barley(

The results of table (1) showed that the treatment of the soil with concentrations of sodium chloride (50,25,0%) led to a significant decrease in the percentage of germination of seeds of the two varieties of barley (white and black) and recorded the lowest percentage of germination (78.33%) at concentration (50)% and from the comparison of varieties it was observed that the variety (black barley) gave the lowest percentage of germination (81.67%). The interaction between varieties and salt concentrations shows that the treatment of the soil with sodium chloride caused a significant decrease in the percentage of seed germination and the lowest germination percentage (75%) was recorded in the seeds of black barley grown under the level of salinity (50.% (

As for the plant growth indicators represented by the length of the vegetative and root group and their dry weights, the length of the vegetative group and its dry weight decreased significantly from the control, and the lowest length of the vegetative group (24.71) cm and the lowest dry weight of the root group (0.07) g at the same concentration (50) %. As for the barley varieties, (white barley) showed the lowest length of the vegetative and root group and recorded (24.53 and 26.02) cm, respectively, except for the dry weight of the roots, as the lowest dry weight of the roots

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(0.123) g was recorded in the roots of black barley. From the interaction between varieties and salt concentrations, significant differences were observed in the growth indices of the two barley varieties, and the lowest vegetative length was recorded (22.17) cm in the variety (white barley) and the lowest root length was recorded (19.23) cm in the variety (black barley), under the level of salinity (50%). While the lowest dry weight of the root and vegetative (0.06 and 0.13) g, respectively, was recorded in the (black barley) variety grown under 50 % salinity level. The results in Figure (1) shows a noticeable decrease in the leaf area of the white barley variety with increasing sodium chloride concentration, indicating a high sensitivity of the white barley variety to elevated sodium chloride levels. This may negatively affect growth efficiency and photosynthesis. In contrast ,

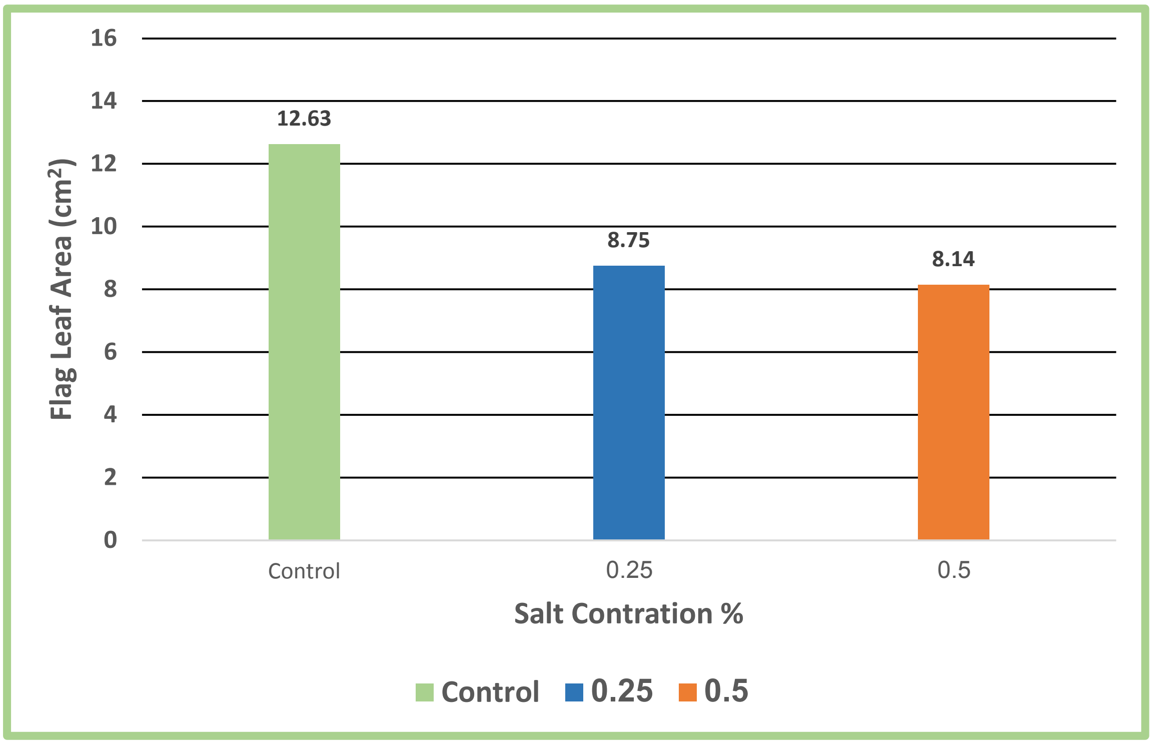

Figure (1) Effect of adding sodium chloride salt at three levels (50,25,0%) on the leaf area trait of barley (white barley) plants

Figure (2) reveals that the black barley variety maintained stable leaf area, with only a slight decrease, demonstrating its resistance to increased sodium chloride concentration compared to the white barley variety .

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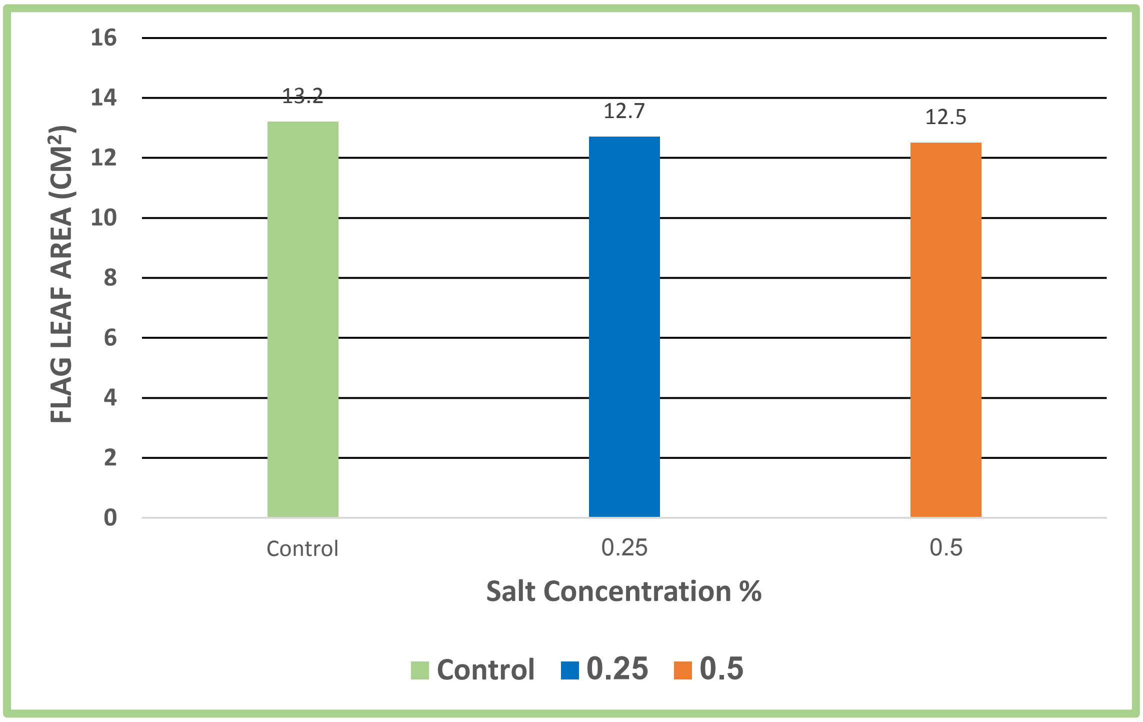

Figure (2) Effect of adding sodium chloride salt at three levels (50,25,0%) on the leaf area trait of barley (black barley) plants

Plant Height Over Weeks Figure (3) illustrates a decline in the growth of the white barley variety, highlighting the cumulative negative impact of salinity across different growth stages during the experiment, particularly at the highest conce ntration .

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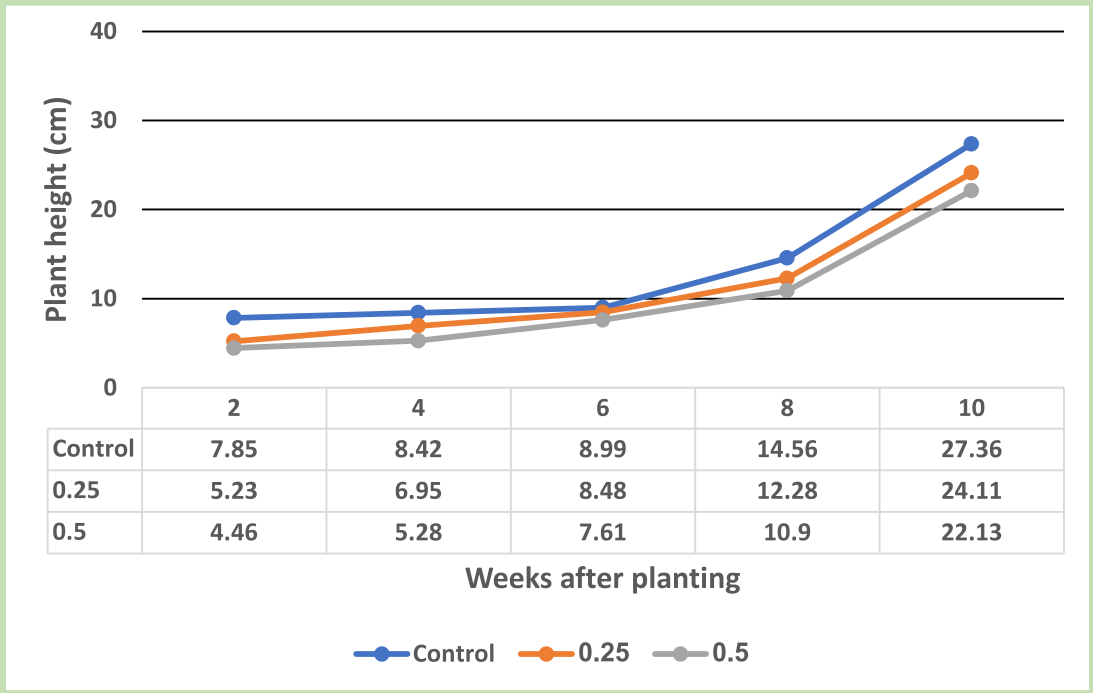
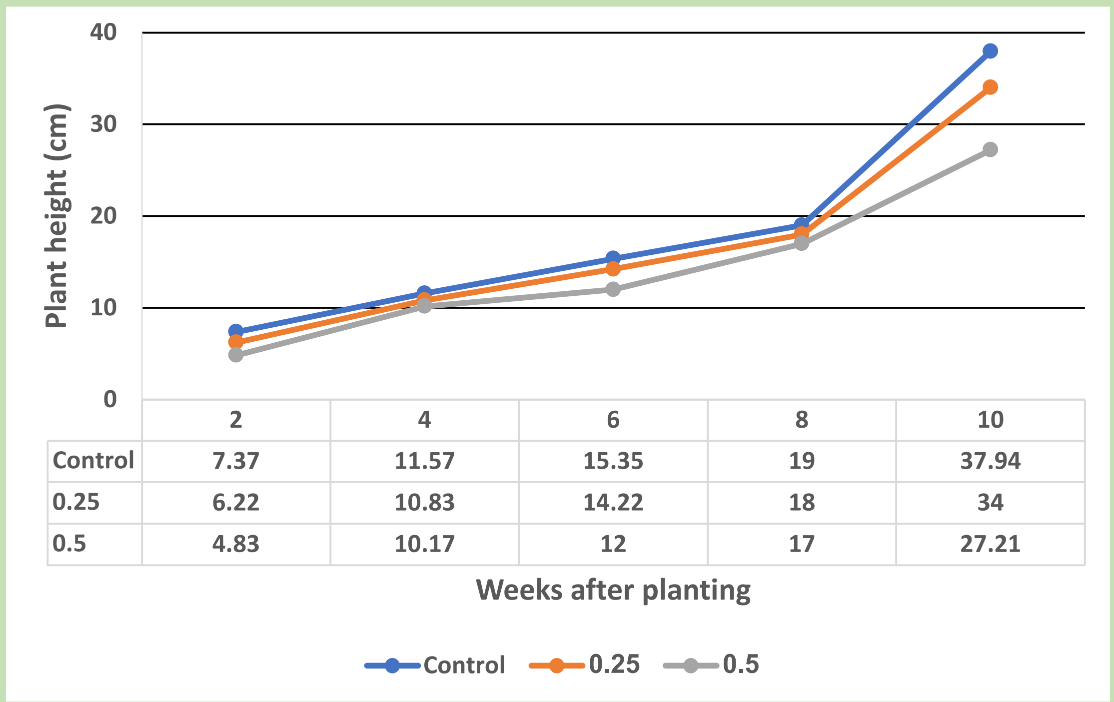


	2	4	6	8	10
Control	7.85	8.42	8.99	14.56	27.36
0.25	5.23	6.95	8.48	12.28	24.11
0.5	4.46	5.28	7.61	10.9	22.13

Figure (3) Effect of adding sodium chloride salt at three levels (50, 25, and 0%) on plant height of barley (white barley) plants

Figure (4) shows that the black barley variety was also affected by salinity but maintained a relatively better growth rate compared to the white barley variety, indicating a higher relative resistance to salinity in this variety.



	2	4	6	8	10
Control	7.37	11.57	15.35	19	37.94
0.25	6.22	10.83	14.22	18	34
0.5	4.83	10.17	12	17	27.21

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Figure (4) Effect of adding sodium chloride salt at three levels (50, 25, and 0%) on plant height of barley (black barley) plants

Table (2) Effect of sodium chloride salt addition on proline concentration (µmol g-1 FW) and peroxidase activity (units. min-1. g FW-1) in two varieties of barley plants (white barley and black barley(

Peroxidase	Proline	Salt Concentration%	Varieties
3.07c	5.56b	%0	White barley
4.65bc	7.49a	%25	
7.78b	8.21a	%50	
5.15bc	7.08a	Average white barley variety	
5.08bc	4.32c	%0	Black barley
5.19bc	6.51b	%25	
20.66a	6.82b	%50	
10.31a	5.88b	Average barley variety	
4.07c	4.94c	%0	Average salt concentration
4.92bc	7.02a	%25	
14.22a	7.51a	%50	

*Numbers with similar letters mean that there are no significant differences between the coefficients below the 5% probability level according to Duncan's multiple range test at each factor and at each interaction.

The results of table (2) showed that the treatment of the soil with concentrations of sodium chloride at three levels (50, 25 and 0%) led to a variation in the concentration of proline in barley varieties and showed that the treatment with a concentration of (50%) led to a significant increase in the concentration of proline in the barley varieties used in the experiment, and by comparing the varieties, it is noted that the variety (white barley) gave the highest concentration of proline (7. 08) (µmol g-1 FW), and the interaction between varieties and NaCl concentrations showed that it caused a difference in proline concentrations between the two varieties, and the variety (white barley) recorded the highest concentration of proline (8.21) (µmol g-1 FW) at (50) % NaCl concentration. Treatment of barley varieties with increasing concentrations of NaCl caused significant differences in the concentration of peroxidase enzyme and the enzyme concentration was significantly higher than the control at (50) % salinity and recorded the highest concentration (14.22) (units. min-1. g FW-1). From the comparison of varieties, it was observed that the variety (black barley) gave the highest concentration of peroxidase (10.31) (units. min-1. g FW-1.(

The overlap between varieties and NaCl concentrations shows a variation in peroxidase concentration between the two varieties. The (black barley) variety recorded the peroxidase concentration (7.78) (units. min-1. g FW-1) at (50 % NaCl). (50 % NaCl), while the black hair variety showed high stress tolerance with the highest value of peroxidase (20.66) (units. min-1. g FW-1) at 50 % NaCl.

Discussion

The results showed that the seed treatment of two barley varieties (white barley and black barley) in soil containing increasing concentrations of sodium chloride (salinity) (50.25) % caused a significant decrease in seed germination, vegetative and root length and dry weights, 0) % caused a significant decrease in seed germination, total vegetative and root length and dry weights, as can be seen from Table (1) that the lowest seed germination and total vegetative length was influenced by NaCl concentrations as salinity is one of the most important environmental stresses that affect the germination and growth of crops[19.[

The study of [20] showed that one of the most important effects of salt stress in crops is the negative impact on germination, as it causes slow germination or failure as a result of the effect of salinity in raising the osmotic pressure around the seed in addition to its effect on physiological and biological processes, and the results agree with the study of [21] as salinity caused a decrease in the percentage of seed germination of three varieties in barley (Nemesia, Erica and Hesikoa) and the decrease increased with the increase in salinity. The decrease in the growth indicators of barley varieties (white and black) is attributed to the increase in osmotic stress mainly due to sodium and chloride ions in the soil solution, which reduces the availability of water to the roots. When the plant roots absorb these ions, they accumulate to harmful levels and cause ionic toxicity, which reduces plant growth by affecting root length, vegetative total and leaf area and reduces the ability of plants to absorb water and this causes a decrease in growth and significantly reduces the yield [22] .The results of the current study also agree with the study of [6] that the exposure of barley to salinity stress and four levels (200, 100, 50 and 0) Mmol of sodium chloride negatively affected the germination and growth characteristics of the studied barley varieties, as the plant growth characteristics represented by the length of the vegetative and root total and leaf area decreased with increasing salinity stress. The decrease in seed germination, vegetative and root length, dry weights, and leaf area of the two barley varieties and the salinity caused a decrease in leaf area, and the results are consistent with the study of [23] which indicated that the growth rate of barley plant leaves decreased under salt stress conditions, and a decrease in the size of wheat plant leaves was also observed. Salt stress affects the growth, phenotype, and anatomical structure of leaves, as well as its contribution to inhibiting the growth rate of leaves in barley and wheat crops, leading to a reduction in leaf area. It was also found that the decrease in leaf area increases with increasing NaCl concentrations, and this is attributed to a decrease in the amount of photosynthetic products available to plants. The results of the current study showed that salinity stress caused an increase in the accumulation of soluble substances in plant cells such as proline, as shown in Table (2), as the treatment with a concentration of (50) % caused an increase in the concentration of the amino acid proline, as it contributes to the ability of plant cells to withstand salt stress by raising the osmotic potential in the cytoplasm of cells, which helps maintain the relative water content inside the cell, a factor necessary for continued plant growth under stress conditions, and the results agree with the study[24] that proline acid increased and Significant when barley variety Irwan was treated with different levels of salinity. It also agrees with the study [25] that the treatment of wheat varieties with different concentrations of sodium chloride caused a difference in proline concentration among wheat varieties. The increase in proline within the cytoplasm of cells causes an increase in osmotic potential and the continuation of water entry from outside the cells under salt stress conditions

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and the continuation of intracellular activities [26]. As well as the elimination of reactive oxygen species to increase plant tolerance to salt stress[27]. Salt stress also caused an increase in peroxidase activity, as shown in Table 2, as salinity (50) % caused a significant increase in enzyme activity and recorded (14.22) (units. min-1. g wet weight-1). Several studies have shown that proline accumulation is associated with increased activity of antioxidant enzymes, which enhances the plant's ability to resist salinity stress [28] .Where this enzyme performs metabolic and physiological roles under conditions of salinity stress, including contributing to the growth of the cell wall by promoting the process of division and elongation, as well as promoting the binding of cell wall components to each other and is one of the most important redox enzymes (enzymatic antioxidants) that work to oxidize a wide range of compounds, as it oxidizes phenolic compounds, amines and lipids, and the effectiveness of the enzyme is directly related to the induced resistance against stress triggers during exposure to them[29] .Increased antioxidant enzyme activity is an indicator of high levels of reactive oxygen species (ROS) production and activation of protective mechanisms aimed at reducing oxidative damage caused by the response of plants to salt stress, which arises as a result of the imbalance between enzymatic antioxidants and reactive oxygen species (ROS) such as peroxidase concentration[30] and the research results agree with the study[31] on the occurrence of changes in the activity of enzymatic antioxidants such as peroxidase in barley plant parts under salt stress conditions .

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