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Effect of Salinity Stress on Oxidative Stress Parameters and the Activity of Antioxidant Enzymes in Eight Durum Wheat Genotypes

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Abstract:

This study was carried out at the Tissue Culture Laboratory, which belongs to the General Commission of Biotechnology, in order to evaluate the influence of NaCl-induced salinity stress on some oxidative parameters (MDA and H_2O_2) and antioxidant enzymes (CAT, APX, and SOD) in eight durum wheat genotypes ($Doma_1$, $Bouhoth_{11}$, $Cham_3$, Bezater, $Cham_5$, Aghamatales, Icaverve, and Icamber) *in vitro*, to determine the most salinity tolerant genotypes. Salinity stress was applied by adding different levels of NaCl to the growing medium (0, 50, 100, and 150 mM). The experiment was laid according to a complete randomized design (CRD) with sixteen replicates. Increasing salinity level caused an increase in both the Hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and the activity of the investigated antioxidant enzymes (SOD ‹APX ‹CAT) compared with the control. The leaf content of both H_2O_2 and MDA was significantly higher in the genotype Icaverve at the salinity level of 150 mM NaCl (45.67 and 130.74 µmol g^{-1} fresh wt. respectively), while the activity of the enzymes SOD, APX and CAT were significantly higher in the two durum wheat genotypes, $Bouhoth_{11}$ and $Doma_1$ at the highest salinity level (150 mM NaCl). Antioxidant enzymes play a pivotal role in the defense mechanisms in the durum wheat under salinity stress conditions, and the *in vitro* screening tool can be effectively used to assess the genetic variability for salinity tolerance in the durum wheat crop.

Keywords: Durum wheat, Salinity stress, Oxidative stress, Antioxidant enzymes.

1. Introduction:

Cereal crops in general, and wheat in particular, are the most important component of the human diet. Cereals contribute about 42.5% of the world's food caloric supply, and their contribution comes through the provision of proteins (37%) [1]. In terms of world production, wheat ranks second in the list of cereal crops after Corn (*Zea mays* L.).

Abiotic stresses (drought, heat, salinity) and biotic stresses (pathogens, insect pests) caused by climate change reduce the quantity and quality of agricultural production globally [2]. Salinity causes a decrease in the productivity of the cultivated crop species, estimated at 20% [3]. Salt stress expresses an increase in the concentration of soluble salts in the rhizosphere, which leads to a decrease in the soil water potential, which reduces the water potential gradient (WPG) between the soil solution and the roots, which negatively affects the rate of water and mineral nutrients absorption [4], leading to a decline in plant growth, development, and productivity [5]. The severity of the effect of salt stress on plants varies according to the salt type, concentration, plant species, cultivar and the developmental stage of the plant life cycle

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[6]. The salt stress is due to the osmotic effect and the ionic toxic effect, mainly ions of chlorine (Cl^-) and sodium (Na^+), which can move into and out of cells [7].

Salt stress causes a decrease in the stomatal conductance, which affects the diffusion rate of CO_2, thereby reducing the photosynthetic capacity. It also causes a decline in the turgor pressure in plant cells, due to a reduction in the rate of water absorption, which negatively affects the elongation rate of the plant cells during the early stages (the osmotic effect), and inhibits cell division during the advanced stages (specific ion toxic effect) [8], which leads to a remarkable decrease in the source size (leaf area index), photosynthesis, and as a consequence the total amount of dry matter available for growth and development of different plant parts [9].

Environmental stresses often lead to the formation of reactive oxygen species (ROS) in plant cells beyond the ability of antioxidant systems to eliminate them, causing oxidative stress [6]. Free oxygen radicals cause lipid peroxidation in the cytoplasmic membranes, inhibit protein synthesis, and damage nucleic acids (DNA, RNA), which ultimately leads to the death of plant cells [10]. The chloroplasts, mitochondria, and the cytoplasm are the main sites of free radical production in the plant cells during exposure to abiotic stresses, in addition to their natural form as by-products of metabolic processes [11]. Free radicals mainly consist of a superoxide radical ($O_2^{\cdot-}$), Hydrogen peroxide (H_2O_2), Hydroxyl radical ($OH^{\bullet-}$), and singlet oxygen ($^1O_2$) [12]. Oxidative stress is defined as the formation of reactive oxygen species (ROS), enzymatic and non-enzymatic antioxidants, as a result of plant exposure to abiotic stresses, such as drought, heat, and salinity, due to a decrease in the rate of photosynthesis, which leads to a decrease in the consumption of the energy rich compounds (ATP, NADPH), which prevents the formation of the final acceptor of electrons ($NADP^+$), then the molecular oxygen (O_2) will capture those electrons, forming a free superoxide radical ($O_2^{\cdot-}$), which, once it reacts with the Hydrogen peroxide (H_2O_2) will form the highly effective $OH^{\cdot-}$, which is characterized by a great reactive ability, which attacks the proteins and phosphorylated fatty substances that are part of the composition of the cytoplasmic membranes and works to destroy them, so that these membranes will lose their selective property, plant cells die, as a result of the exit of many organic and mineral solutes useful for the life of plant cells, and the entry of toxic substances [13].

Lipid oxidation is often the main criterion that researchers resort to when they need to determine the rate of formation of oxygen free radicals in damaged plant cells [14], by measuring the concentration of malonyldialdehyde (MDA). Increased MDA content has been observed in various plant species under saline stress conditions [15].

Hydrogen peroxide (H_2O_2) in plants is one of the most stable active oxygen free radicals, regulating basic processes such as acclimation, defense, and development [16]. Hydrogen peroxide (H_2O_2) plays a dual role in plants. At low concentrations, it plays a role as an acclamatory signal, enabling plants to withstand stress by activating the enzymatic antioxidant defense mechanism [17], and at high concentrations, it regulates the programmed plant cell death [18]. The addition of Hydrogen peroxide (H_2O_2) from external sources at very low concentrations allows the induction of defense responses in plants against oxidative stress, and contributes to the activation of many tolerance mechanisms to different abiotic stresses [19]. The defense mechanisms when hydrogen peroxide is formed include two main types of antioxidants, enzymatic antioxidants, which include several enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), monodic hydro ascorbate reductase (MDHAR) and dihydro ascorbate reductase (DHAR), and non-enzymatic antioxidants, which include many compounds, the most important of which are phenols (flavonoids and anthocyanin), carotenoids, ascorbic acid (vitamin C), α-tocopherol and glutathione [20]. Several studies have shown genetic variation in the level of gene expression

and the activity of these enzymes, which is usually associated with an increase in the adaptive capacity and the level of tolerance to various abiotic stresses [21], and the enzymes play an effective and vital role in reducing salinity toxicity [22], where the enzyme superoxide dismutase (SOD) disassembly or breaks down the superoxide free radicle into molecular oxygen (O_2) and Hydrogen peroxide (H_2O_2), and the Hydrogen peroxide is removed by catalase (CAT) and peroxidase (APX) enzymes [10].

Objectives

1. Evaluating the influence of NaCl-induced salinity stress on some oxidative parameters (MDA and H_2O_2) and antioxidant enzymes (CAT, APX, and SOD) in eight durum wheat genotypes.
2. Determining the most salinity tolerant genotypes in response to NaCl-induced salinity stress using tissue culture technique.

2. Materials and methods:

2-1-Time and site of experimentation:

The research was carried out in the Plant Biotechnology Laboratory at the General Commission for Biotechnology in Damascus, during the year 2021.

2-2-Plant material:

The study was carried out on eight genotypes of durum wheat, four of which are locally certified cultivars ($Doma_1$, $Bouhoth_{11}$, $Cham_3$, and $Cham_5$), and four are promising lines (Aghamatlas, Bezater, Icaverve, Icambel).

2-3- Methodology:

The seeds were washed with running water and then soaked in ethyl alcohol (70%) for one minute while stirring, then they have been treated with sodium hypochlorite (NaOCl) solution (4%) for 20 minutes with the addition of 20 TWEEN (Polysorbate 20) solution to increase the effectiveness of the sterilization process and reduce surface tension, then the seeds were washed with sterile distilled water three times in succession, at an average of 5 minutes for each time. Wheat grains were then grown in MS medium [23], which has been supplemented with 30 $g.l^{-1}$ sucrose and 7 g l^{-1} agar (pH = 5.8), in 2.5 x 20 cm test tubes, containing 12.5 ml of the nutrient medium, then the tubes were sterilized in a wet autoclave at 121 °C at a pressure of 1.04 $kg.cm^{-2}$ for 20 minutes. Tubes grown in the growth chamber were incubated at 22 ± 2 °C, 16 h/8 darkness and 3000 lux [24]. Salt stress was applied, by adding different concentrations of sodium chloride (NaCl) (0, 50, 100, and 150 mM) to the growth medium at the time of sowing directly, and the experiment was repeated twice despite the similarity of the cultivation conditions within the laboratory to a large extent, to ensure the accuracy of the results obtained, with an average of 16 replicates (tube) for each treatment, and for each experiment.

2-4-Investigated traits:

After 45 days of applying salt stress (the seed length ranges between 15-18 cm), the entire vegetative growth of the saline-stressed plants and the control was taken, and they were directly frozen and ground in liquid nitrogen, then the crushed samples were placed in plastic containers and stored at -80°C until the analysis was carried out. The following traits were recorded:

2-4-1-Malondialdehyde content in terms of the destruction of fatty substances (MDA $nmol.g^{-1}$ wet weight):

At the end of the stress period, the level of destruction of fatty substances was estimated in the leaves by adopting the method of estimating Malondialdehyde (MDA) by the reaction of 2-thiobarbituric acid (TBA). Full-elongated leaves of each genotype for each treatment and

replication were separately ground with 0.3% TBA in 10% Trichloracetic acid (TCA) solution at 4°C, and the Malondialdehyde concentration was calculated from the difference between absorption at 532 nm and 600 nm wavelength using an extinction coefficient of 155 mmol cm^{-1}, expressed as one nmol $MDA.g^{-1}$ fresh weight [25].

2-4-2 Hydrogen Peroxide Concentration (µmol. g^{-1} wet weight):

The content of leaves of Hydrogen peroxide (H_2O_2) was measured by taking 0.25 g of frozen and ground powder samples in liquid nitrogen at the rate of 4 replicates from each genotype, treatment and replication, then homogenized in 1 ml of 0.1% (w/v) trichloracetic acid (TCA) in an ice bath. Then the mixture was centrifuged at 12000g, for 15 minutes at 4°C. Then 500 µL of the resulting filtrate was taken and 250 µL of potassium phosphate solution at a concentration of 10 mM at pH 7, and 500 µL of potassium iodine (KI) with a concentration of 1 M were added to it. After good stirring, the samples were left for 30 minutes at normal temperature, then the absorption reading was recorded. The sample absorbance was measured at a wavelength of 390 nm by a spectrophotometer. A standard curve was used to calculate the H_2O_2 concentration in the sample.

2-4-3 Estimation of the activity of antioxidant enzymes:

All enzymatic tests were carried out at a temperature of 25°C using a spectrophotometer according to the method [26]. To extract the studied enzymes, powdered and frozen samples were taken and ground in liquid nitrogen (0.2-0.4 g), homogenized in 1 ml MES/KOH solution at a concentration of 50 mM, pH= 6, containing 40 mM KCl, 2 mM $CaCl_2$, and 1 mM ascorbate, then placed in a centrifuge for 15 min at 16000xg, at a temperature of 4° C. Then the resulting filtrate was used to measure the activity of antioxidant enzymes directly, as well as to measure the concentration of proteins, which was done by Bradford method [27].

2-4-4 Estimation of the activity of the enzyme superoxide dismutase (SOD):

The activity of the enzyme was estimated by adding 10 µL of the enzyme extract to 1 ml of the reaction solution containing potassium phosphate at a concentration of 50 mmol, pH= 7.8, methionine at a concentration of 13 mm, NBT at a concentration of 75 mm, EDTA at a concentration of 0.1 mm, and riboflavin at a concentration 2 micromole. After good stirring, the mixture was exposed to artificial lighting for 5 minutes, and then the sample's absorption at a wavelength of 560 nm was read by a spectrophotometer. A standard curve, plotted using known concentrations of commercial SOD, was used to calculate the SOD activity in the sample.

2-4-5 Determination of the enzyme activity of catalase (CAT):

The activity of CAT enzyme was estimated by estimating the decrease in H_2O_2 concentration by measuring the decrease in absorbance for 3 minutes at a wavelength of 240 nm for the reaction solution (1 ml) containing a phosphate buffer solution at a concentration of 50 mmol, pH= 7 and 15 mmol H_2O_2 and 100 µL of the enzymatic extract. CAT activity was calculated using a degradation factor of 43.6 mol/cm.

2-4-6 Determination of ascorbate peroxidase activity (APX):

The activity of APX enzyme was estimated by measuring ascorbate oxidation, by recording the decrease in absorbance for 3 minutes at a wavelength of 290 nm for the reaction solution (1 ml) containing a phosphate buffer solution at a concentration of 50 mmol, pH= 7, 0.25 mmol of ASA, 5 mmol H_2O_2 and 100 µL of enzymatic extract. The enzymatic activity was

measured by tracking the oxidation of ascorbate at a wavelength of 290 nm for 3 minutes. APX activity was calculated using a degradation factor of 2.8 mmol/cm.
2-5-Experimental design and statistical analysis:

The experiment was laid according to a simple random design with an average of 16 replications for each treatment. The results were analyzed (the average of the two experiments) using the MSTAT-C program, and an analysis of variance was conducted, where the means were compared and the value of the least significant difference (LSD) was calculated at the level of significance of 1%.

3. Results and discussion:

3-1- Leaf content of MDA (nano mol.g^{-1} wet weight): The results showed significant differences ($P \leq 0.01$) in the MDA content between the different salt levels (NaCl), genotypes, and the interaction between them. It was noticed that the MDA concentration was significantly higher at the two highest salt levels (150, and 100 mM NaCl) without significant differences between them (32.90, 30.06 nmol.g^{-1} wet weight, respectively). MDA concentration decreased exponentially with the decrease in the concentration of NaCl salt in the growth medium, where the MDA content was significantly the lowest in the control treatment (without NaCl) and the salinity level of 50 mM NaCl, without significant differences between them (24.06, 26.63 nmol.g^{-1} wet weight, respectively) (Table, 1). The mean concentration of MDA was significantly higher in the Icaverve cultivar (37.39 nmol.g^{-1} fresh weight), followed by significant differences in the cultivar Cham3 (34.92 nmol.g^{-1} fresh weight), while it was significantly the lowest in Doma1(17.38 nmol.g^{-1} fresh weight)) (Table 1), indicating the ability of the Doma1 cultivar to maintain the stability of cytoplasmic membranes from damage during exposure to salt stress in comparison with the rest of the studied genotypes. Taking into account the interaction between genotypes and salinity levels, the content of MDA was significantly higher for Icaverve at the highest salt concentration (150 mM NaCl) (45.67 nmol.g^{-1} fresh weight), while it was significantly lower in the control treatment (without NaCl) for the cultivar Doma1 (14.71 nmol.g^{-1} fresh wt.) (Table 1). The variation in the leaf content of MDA is due to the differences in the rate of production of oxygen free radicals such as superoxide radical and Hydrogen peroxide, which react with each other to produce the most detrimental Hydroxyl free radical. This is confirmed by the fact that the average concentration of Hydrogen peroxide (H_2O_2) was significantly higher in the Icaverve cultivar (98.17 µmol.g^{-1} fresh weight), followed by the cultivar Cham3 (89.83 µmol.g^{-1} fresh weight), while the concentration of H_2O_2 was significantly lower in Bouhoth11 and Doma1 cultivars (26.00, 35.42 µmol.g^{-1} fresh weight, respectively) (Table 2). The mean SOD activity was also significantly higher in the Doma1 variety (77.68 units/mg protein), followed by Bouhoth11 (72.95 units/mg protein) (Table 3), which reduces the rate of superoxide radical formation (O_2^{-}). The average activity of CAT enzyme was significantly higher in Doma1 (45.64 mmol/min/mg protein), followed by Bouhoth11 (43.03 mmol/min/mg protein) (Table 5), which reduces the rate of Hydrogen peroxide accumulation. In general, the increase in the concentration of malondialdehyde at the high level of salt stress in the growth medium is attributed to the decrease in the rate of carbon fixation during the dark reactions in the C3-photosynthetic carbon reduction cycle, which leads to a decrease in the rate of consumption of energy-rich compounds, and then a decrease in the reformation of the final electron acceptor ($NADP^{+}$), then molecular oxygen (O_2) grabs electrons to form the free superoxide radical ($O2^{\bullet-}$), which reacts with Hydrogen peroxide (H_2O_2) to form the free Hydroxyl radical ($OH^{\bullet-}$), which is characterized by great reactive ability, as it attacks the phosphorous fatty substances included in the composition of the cytoplasmic membranes destroying them, thus creating a porous membrane, losing its selectivity property, and plant

cells die as a result [28+29]. This is consistent with what [30] found for wheat under saline stress conditions

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Table 1: Effect of salt stress on the concentration of Malondialdehyde in durum wheat genotypes.

Genotypes	Salinity levels (mM NaCl)				Average
	0	**50**	**100**	**150**	
Bezater	24.15 MNO	25.40 KLMN	27.56 IJKLM	29.26 GHIJK	**26.59 D**
Cham5	24.62 LMNO	26.63 JKLMN	29.42 FGHIJ	32.67 DEFG	**28.33 D**
Aghamatales	25.39 KLMN	29.73 FGHIJ	33.29 DEF	34.84 CD	**30.81 C**
Bouhoth11	17.18 QR	19.35 PQ	20.90 OPQ	23.07 NOP	**20.13 E**
Icamber	25.86 JKLMN	28.33 HIJKL	34.84 CD	37.94 BC	**31.74 C**
Cham3	29.73 FGHIJ	32.22 DEFGH	37.32 BC	40.41 B	**34.92 B**
Doma1	14.71 R	17.19 QR	18.27 QR	19.35 PQ	**17.38 F**
Icaverve	30.81 EFGHI	34.22 CDE	38.86 B	45.67 A	**37.39 A**
Treatment Average	**24.06 B**	**26.63 B**	**30.06 A**	**32.90 A**	**-**
LSD (0.01)	**Salinity levels**		2.906		
	Genotypes		1.973		
	Interaction		3.946		
CV (%)			6.38		

The same letters at the level of columns and lines indicate no significant differences at the 0.01 significance level.

3-2- Leaf content of hydrogen peroxide (micromol. g^{-1} fresh weight): Results showed significant differences ($P \leq 0.01$) in the Hydrogen peroxide content (H_2O_2) between different salt (NaCl) levels, genotypes, and interaction between them. It was noted that the concentration of H_2O_2 was significantly higher at the salt level of 150 mM NaCl (85.83 µmol.g^{-1} fresh weight), while it was significantly lower in the control (36.42 µmol.g^{-1} fresh weight) (Table 2). It is noticed that the Hydrogen peroxide increases significantly with the increase in the concentration of NaCl in the growing medium. The concentration of Hydrogen peroxide was significantly the highest for Icaverve (98.17 µmol.g^{-1} fresh weight), while it was significantly the lowest in Bouhoth11 (26.00 µmol.g^{-1} fresh weight) (Table 2). The content of H_2O_2 was significantly higher for the Icaverve cultivar at the highest salt level (150 mM NaCl) (130.7 µmol.g^{-1} fresh weight), while it was significantly lower in the control for Bouhoth11 (11.33 µmol.g^{-1} fresh weight) (Table 2). In general, the variation in H_2O_2 between the genotypes is attributed to the variation in the activity of the enzymes responsible for its formation under salt stress, where it was noticed that the average activity of the enzyme (SOD) was significantly higher at the highest salt level (150 mM NaCl) (72.32 units/mg protein), and its concentration increases with the increase in the level of salt stress in the growth medium due to the increase in the accumulation rate of free radical superoxide, as a result of the steady decline in the photosynthetic efficiency of plants. This is consistent with what was obtained by [18] in wheat under salinity stress conditions

Table 2: The effect of salt stress on hydrogen peroxide concentration in the durum wheat genotypes.

Genotypes	Salinity levels (mM NaCl)	

	0	50	100	150	Genotypes average
Bezater	27.33$_{R}$	35.33$_{P}$	53.33$_{L}$	58.67$_{K}$	**43.67$_{F}$**
Cham$_{5}$	30.67$_{Q}$	40.67$_{N0}$	64.67$_{I}$	85.33$_{E}$	**55.33$_{E}$**
Aghamatales	39.33$_{O}$	47.33$_{M}$	78.67$_{FG}$	93.33$_{D}$	**64.67$_{D}$**
Bouhoth$_{11}$	16.67$_{S}$	29.33$_{QR}$	43.00$_{N}$	52.67$_{L}$	**35.42$_{G}$**
Icamber	42.67$_{N}$	69.33$_{H}$	85.33E	102.7$_{C}$	**75.00$_{C}$**
Cham$_{3}$	60.67$_{JK}$	77.33$_{G}$	100.7$_{C}$	120.7$_{B}$	**89.83$_{B}$**
Doma$_{1}$	11.33$_{T}$	18.67$_{S}$	31.33$_{Q}$	42.67$_{N}$	**26.00$_{H}$**
Icaverve	62.67$_{IJ}$	80.67$_{F}$	118.7$_{B}$	130.7$_{A}$	**98.17$_{A}$**
Treatment average	**36.42$_{D}$**	**49.83$_{C}$**	**71.96$_{B}$**	**85.83$_{A}$**	**-**
LSD (0.01)	**Salinity levels**	4.265			
	Genotypes	1.437			
	Interaction	2.874			
CV (%)	2.16				

The same letters at the level of columns and lines indicate no significant differences at the 0.01 significance level.

3-3- Effect of salt stress on the activity of the enzyme superoxide dismutase (SOD): The activity of the SOD enzyme was significantly higher at the highest salt level (72.32 units/mg protein), while it was significantly lower in the control and the lowest salt level (48.91, 50.09 units/mg protein, respectively) (Table 3). The SOD enzyme activity was significantly higher in Doma1 (77.68 units/mg protein), while it was significantly the lowest in the Aghamatales (41.52 U/mg protein/mg protein) (Table3). The mean SOD enzyme activity was significantly higher in the two cultivars Bouhoth11 and Doma1 at the highest salt level without significant differences between them (91.49, 89.19 units/mg protein, respectively), while it was significantly lower in the control for the Aghamatales genotype (31.16 units/mg protein) (Table 3). SOD is among the enzymatic antioxidants, as the first line of defense against oxidative stress in plants, as it plays an important role in the dismutation of free superoxide radicals ($O_2^{\bullet-}$) into H_2O_2 and molecular oxygen (O_2), causing the reduction of superoxide concentration [31]. Its activity is increased in response to many abiotic stresses, and the tolerance of plant species or genotypes to oxidative stress depends on the quantity and activity of this enzyme. These results are consistent with that of [32], which found an increase in the activity of this enzyme with an increase in the level of salt stress in wheat plants.

Table 3: Effect of salt stress levels on SOD enzyme activity in the durum wheat genotypes.

Genotypes	Salinity levels (mM NaCl)				Genotypes average
	0	50	100	150	
Bezater	45.65$_{Q}$	53.12$_{MNO}$	61.50$_{HI}$	72.26$_{E}$	**58.13$_{D}$**
Cham$_{5}$	31.16$_{U}$	35.73$_{T}$	44.14$_{QR}$	55.03$_{LMN}$	**41.52$_{F}$**
Aghamatales	55.43$_{KLM}$	39.39$_{S}$	66.25$_{F}$	78.63$_{C}$	**59.92$_{C}$**
Bouhoth$_{11}$	57.68$_{JK}$	48.80$_{P}$	52.93$_{NO}$	70.64$_{E}$	**57.51$_{D}$**
Icamber	56.86$_{KL}$	63.42$_{GH}$	80.03$_{BC}$	91.49$_{A}$	**72.95$_{B}$**
Cham$_{3}$	44.14$_{QR}$	43.02$_{R}$	48.99$_{P}$	59.44$_{IJ}$	**48.90$_{E}$**
Doma$_{1}$	35.67$_{T}$	42.09$_{R}$	52.03$_{O}$	61.89$_{H}$	**47.92$_{E}$**
Icaverve	64.66$_{FG}$	75.14$_{D}$	81.72$_{B}$	89.19$_{A}$	**77.68$_{A}$**

Treatment average	48.91c	50.09c	60.95$_{B}$	72.32$_{A}$	-
LSD (0.01)	Salinity levels	3.370			
	Genotypes	1.211			
	Interaction	2.421			
CV (%)	1.92				

The same letters at the level of columns and lines indicate no significant differences at the 0.01 significance level.

3-4- Ascorbate peroxidase (APX) enzyme activity: Results showed significant differences ($P \leq 0.01$) in the activity of the ascorbate peroxidase (APX) enzyme between the different salt (NaCl) levels, genotypes, and the interaction between them. It was noticed that the activity of the enzyme (APX) was significantly higher at the highest salt level (338.0 µmol/min/mg protein), while it was significantly lower in the control (237.9 µmol/min/mg protein) (Table 4). The activity of the enzyme (APX) was significantly higher in the cultivar Doma1 at the highest salt level (495.5 µmol/min/mg protein), while it was significantly the lowest in the control treatment for Cham5 (122.3 µmol/min/mg protein) (Table 4). Increased activity of APX was observed in a large number of plant species under *in vitro* abiotic stress conditions [33], and ascorbate peroxidase is a very important enzyme in the dumping of free radicals, as it works to reduce the concentration of H_2O_2 resulting from increased activity of SOD. These results are consistent with the findings of [34], who found an increase in the activity of this enzyme with an increase in salt stress in the wheat plants.

Table 4: Effect of salt stress levels on APX enzyme activity in the studied durum wheat genotypes.

Genotypes	Salinity levels (mM NaCl)				Genotypes average
	0	50	100	150	
Bezater	204.0$_{R}$	222.5$_{P}$	294.7$_{L}$	330.2$_{I}$	**262.9$_{E}$**
Cham$_5$	166.8$_{V}$	177.2$_{U}$	234.1$_{O}$	258.2$_{N}$	**209.1$_{F}$**
Aghamatales	260.5$_{N}$	276.6$_{M}$	299.8$_{K}$	329.7$_{I}$	**291.7$_{D}$**
Bouhoth$_{11}$	296.8$_{L}$	300.6$_{K}$	335.1$_{H}$	382.3$_{F}$	**328.7$_{C}$**
Icamber	325.2$_{J}$	365.9$_{G}$	408.7$_{C}$	485.9$_{B}$	**396.4$_{B}$**
Cham$_3$	141.9$_{X}$	162.5$_{W}$	183.5$_{T}$	217.0$_{Q}$	**176.2$_{G}$**
Doma$_1$	122.3$_{Y}$	160.2$_{W}$	193.5$_{S}$	205.2$_{R}$	**170.3$_{H}$**
Icaverve	385.5$_{E}$	400.6$_{D}$	400.6$_{D}$	495.5$_{A}$	**420.5$_{A}$**
Treatment average	**237.9$_{D}$**	**258.3$_{C}$**	**293.8$_{B}$**	**338.0$_{A}$**	-
LSD (0.01)	**Salinity levels**	3.005			
	Genotypes	1.411			
	Interaction	2.821			
CV (%)	0.46				

The same letters at the level of columns and lines indicate no significant differences at the 0.01 significance level.

3-5- Catalase enzyme activity (CAT): The average activity of the enzyme CAT was significantly higher at the highest salt concentration (38.77 mmol/min/mg protein), while it was significantly lower in the control (without NaCl) (24.72 mmol/min/mg protein) (Table 5). This is due to the variation in the concentration of Hydrogen peroxide. The mean CAT enzyme

activity was significantly higher in Doma1 (45.64 mmol/min/mg protein), while it was significantly lower in Cham5 (19.76 mmol/min/mg protein) (Table 5). The average CAT enzyme activity was significantly higher in the two cultivars Bouhoth11 and Doma1 at the highest salt level without significant differences between them (57.34, 54.56 mmol/min/mg protein, respectively), while it was significantly lower in the control for Cham5, Aghamatales, and Cham3 genotypes (17.09, 17.64, 17.83 mmol/min/mg protein, respectively), and at the lowest salinity level (50mM NaCl) for the genotypes Cham5, Aghamatales and Cham3 (18.50, 19.66, 19.67 mmol/min/mg protein respectively) (Table 5). Various environmental stresses induce increased CAT activity under *in vitro* conditions [35], which protects cells from the harmful effects of H_2O_2 accumulation during fatty acid oxidation and photorespiration. H_2O_2 is also one of the products of the transformation of the superoxide-free radical $O_2^{\bullet-}$ by the enzyme SOD. Catalase enzyme is distinguished from the rest of the enzymes by having the highest metabolic rates; only one molecule can convert millions of Hydrogen peroxide molecules into water (H_2O) and molecular oxygen (O_2) per second. These results are in agreement with the findings of [19], who found an increase in the activity of this enzyme with an increase in salt stress in the wheat plants.

Table 5: Effect of salt stress on CAT enzyme activity in the studied durum wheat genotypes.

Genotypes	**Salinity levels (mM NaCl)**				**Genotypes average**
	0	**50**	**100**	**150**	
Bezater	21.48_{LMNO}	25.03_{IJK}	33.51_{F}	37.74_{E}	$\mathbf{29.44_{E}}$
Cham5	17.64_{PQ}	19.66_{NOPQ}	23.83_{JKL}	27.44_{HI}	$\mathbf{22.15_{F}}$
Aghamatales	24.45_{IJKL}	29.18_{H}	36.89_{E}	40.99_{D}	$\mathbf{32.88_{D}}$
Bouhoth11	30.13_{GH}	33.41_{F}	37.07_{E}	43.24_{CD}	$\mathbf{35.96_{C}}$
Icamber	32.40_{FG}	37.37_{E}	45.00_{BC}	57.34_{A}	$\mathbf{43.03_{B}}$
Cham3	17.83_{PQ}	19.67_{NOPQ}	22.17_{KLMN}	25.67_{IJ}	$\mathbf{21.33_{F}}$
Doma1	17.09_{Q}	18.50_{OPQ}	20.25_{MNOP}	23.22_{JKLM}	$\mathbf{19.76_{G}}$
Icaverve	36.77_{E}	43.32_{CD}	47.93_{B}	54.56_{A}	$\mathbf{45.64_{A}}$
Treatment average	$\mathbf{24.72_{D}}$	$\mathbf{28.27_{C}}$	$\mathbf{33.33_{B}}$	$\mathbf{38.77_{A}}$	-
LSD (0.01)	**Salinity levels**	2.280			
	Genotypes	1.565			
	Interaction	3.129			
CV (%)	4.60				

The same letters at the level of columns and lines indicate no significant differences at the 0.01 significance level.

4. Conclusion:

Exposing durum wheat plants to salt stress causes an increase in the activity of antioxidant enzymes (SOD, APX, CAT), which increases the level of tolerance to salt stress. The increase in the intensity of salt stress caused an increase in some oxidation parameters (H_2O_2 and MDA), which indicate the extent of the damage caused by oxidative stress, compared to the non-saline medium. The studied wheat genotypes varied in the extent of their response to salt stress, as the average activity of antioxidant enzymes (SOD, APX, CAT) was significantly higher for Bouhoth11 and Doma1 cultivars, while the average leaf content of parameters such as H_2O_2 and MDA was significantly higher in the cultivar Icaverve. These results indicate that *in vitro* evaluation technology can be used as a rapid and effective technique to assess the genetic variation for salinity stress tolerance in durum wheat, depending on the response of different

growth parameters to the applied salt stress, due to its ease and saving time and effort. It is recommended to study the effect of salt stress on the investigated genotypes at the whole plant level within lysimeters or in the field and compare their performance with the laboratory results, to determine the credibility of the *in vitro* screening tool. The salinity tolerant genotypes can be used in breeding and genetic improvement programs for the durum wheat crop.

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