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Article

The Role of Hydrogen Peroxide in Enhancing the Antioxidant System of Wheat *Triticum aestivum* L. Growing under Conditions of Salt Stress

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ABSTRACT

This study was conducted on wheat growing during the 2020 growing season to discover the effect of soaking wheat seeds with a hydrogen peroxide solution on the increased ability of the crop to withstand salt stress. The hydrogen peroxide factor involved using six concentrations (0, 0.5, 1, 1.5, 2 and 2.5) m mol, while the salt concentration depended on salt water irrigation.(Na^+cl^-) salt when the concentration(75) m.mol.during the growing season, a number of features such as the relative water content of the leaves.Total chlorophyll, soluble sugar, carotene, H2O2 content, protein, glutathione (GSH). The results of the malondialdehyde (MDA) content and K/Na ratio showed that increasing levels of hydrogen peroxide at some 2 m.mol limit reached the highest levels.H2O2 content and thus enhanced proline activity. Glutathione (GSH) malondialdehyde (MDA) is an indication that has helped to improve plants' ability to resist saline stress.

Keywords: Hydrogen peroxide, Sodium chloride, Antioxidant, Wheat, Salt Stress

INTRODUCTION

Wheat is a major agricultural crop that leads to grain crops directly linked to human nutrition. Wheat can be grown in various soils, but it is preferred in soils with low salt content, such as exposure to wheat plants. Higher salt concentrations may expose them to severe growth weakness and thus lower yield due to saline stress. Hydrogen peroxide (H2O2) is a vital oxygen metabolite used as a courier in the cellular signal pathways needed for growth. The stresses such as salt induce oxidative stress in plants caused by the generation and accumulation of superoxides (O_2^-), hydrogen peroxide(H_2O_2)and hydroxyl radicals (OH^-), which are commonly known as reactive oxygen species (ROS) [1]. The growth of the wheat crop may not be affected by slightly lower salt levels, or it may not be affected at all. At the same time, the production begins to decline

until it reaches zero. With an increase in salt concentration. Due to exposure to severe inhibition and death at a concentration ranging between (100 - 200) mmol/liter sodium chloride salt [2]. Reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical (OH^-) and superoxide root ($O2^-$) are produced during aerobic metabolism. However, their production rate increases under different stress conditions, including salt stress [3].

Free radicals can act as signaling molecules at low concentrations to stimulate gene expression of cellular response and defense genes [4]. Many studies have indicated the positive effect of treatment with hydrogen peroxide in the field of increasing the tolerance to salts, such as increasing the rate of photosynthesis and improving water relations through the maintenance of the leaves on their swelling pressure under a condition of high salinity due to the increase in the effectiveness of the plant's antioxidant system [4]. As saline stress reduces the osmotic potential of the soil solution, the water content of plant tissues is reduced. Under normal growth conditions, there is a balance between what is produced and destroyed by reactive oxygen species because of the necessity of their occurrence and participation in metabolic processes under stressful saline conditions. Oxidative stress is induced due to a lack of production control. Moreover, the accumulation of active oxygen roots; this study is intended to test the ability of hydrogen peroxide to increase the effectiveness of plant antioxidants in salt stress.

MATERIAL AND METHODS

Preparation of soil and the experimental factors

This study was conducted in the Faculty of Agriculture and Marshlands-University of Thi-Qar fields during the wheat agricultural season. For the year 2020, by using 2 kg pots containing 2 peat moss and 1 sand, the physicochemical properties of the soil are as follows:

pH:7.4, O.M:2.67%, EC:8.13milimos. $^{cm-1}$, C.E.C: 1.48meq/100 g soil.

In this study, one factor is soaking wheat seeds containing various concentrations of hydrogen peroxide solution at six levels (0, 0.5, 1.1.5, 2 and 2.5 mmol) for 9 hours. These seeds were planted after being air-dried at a laboratory temperature of 5 seeds per pot; the seeds were watered with the nutrient. Solution alone until the completion of the germination process and the completion of the early growth of the seedlings.

Two weeks after the growing date, the treatment was done with the nutrient solution added to the sodium chloride salt at a concentration of (75) m.mol.and the wheat with this concentration. There were about twelve waterings during the growing season, and the period between the waterings varied about seven days. Moreover, about 70 days after the date of cultivation, the following measurements were taken as follow:

Relative water content of leaves (RWC)

The relative water content of the leaves was determined after isolation and taking their fresh weight (FW) directly using a sensitive balance. Then they were cut into pieces (5-10) cm, placed in test tubes containing distilled water and left in darkness for 4 hours. After the samples were extracted, the water was disposed of using filter paper and the weights were measured again. That represents their saturation weight (turgid weight) (TW), and then their dry weight (DW) was calculated after drying in the electric oven.at 700ºC during a 48-hour period, the relative water content was estimated using the following equation:

RWC= ((FW - DW)/ (TW – DW))* 100

Total chlorophyll

The total chlorophyll in the leaves of the wheat plant was determined according to [5]. The sample of plant leaves (0.5) gm was ground through a ceramic mortar with 80% acetone until the green color of the leaves disappeared, the extract has been filtered, and then the absorbance has been measured. By using a spectrophotometer at a wavelength of 663 nm for chlorophyll a and 645 nm for chlorophyll b, the total chlorophyll was calculated using the following equation:

Total chlorophyll (mg/L)= (20.2*D645) + (8.02*D663)

Total soluble sugar content

Six methods established the total soluble sugar content of wheat leaves.

Carotene

A 100 g sample was ground in a ceramic mortar with 20 ml of acetone to a concentration of 80% and put into a centrifuge at a speed of 3000 revolutions per minute for 15 minutes. The filtrate was removed and reextracted by adding 5 ml of acetone each time until the filtrate was lost in color. Then, the filtrate was collected and used to estimate the carotene concentration; the absorbance was measured at wavelengths 480, 645 and 663 nm using a spectrophotometer. The following equation estimated the carotene content:

Carotene ($mg.gm^{-1}$)= ((A480 + A663 0.114) – (A645 0.638))/ W ×1000 ×a)

a. The wavelength within the spectrophotometer cell.

v= The volume of the sample.

w= The fresh weight of the sample.

Enzyme extraction and assay

Approximately (0.5g) of the wheat leaf sample was homogenized in a cold 0.1M phosphate buffer (pH = 7.5). The protein content of the samples was determined using a bovine albumin method [7].

H_2O_2 content

The amount of hydrogen peroxide was calculated using a standard curve method for extract and measured OD at 390nm [8].

Proline

A leaf sample weighing 0.5gmwas crushed with sulphosalicylic acid at a concentration of 3% and filtered by filter paper (Whatman no.2), then 2 cm^3 was taken from the filtrate and mixed with 2 ml ninhydrin and 2 cm^3 of glacial acetic acid, then incubated in a water bath at 100ºC for one hour and then put the mixture in an ice bath, then extracted with 4 ml of toluene until the dye-carrying phase is separated from the aqueous phase, the resulting color was measured using a spectrophotometer at the wavelength 520 nanometers according to the method of [9].

Glutathione (GSH)

Glutathione (GSH) was estimated according to the method of [10] using a spectrophotometer at a wavelength of 412 nm.

Malondialdehyde content (MDA)

It was estimated according to the method of [11] using a 100 microliter of the sample and a spectrophotometer at a wavelength of 532 nm.

$K^+$and Na^+ estimation

The concentration of K^+ and Na^+ was measured according to the method [17]. One 200 mg sample was ground with 7 ml of concentrated nitric acid and 3 ml of hydrofluoric acid for 20 minutes employing the microwave, then the sample and

the acid were placed in appropriate containers and closed firmly and heated. Using a microwave oven, the temperature of each container was increased to 160 5oC for a period not exceeding 6 minutes. It was left there for 10 minutes to complete the digestion process. Then, the contents were filtered utilizing a centrifuge and analyzed by the inductively coupled plasma mass spectrometer.

Statistical Analysis

All samples were processed in triplicate with averages calculated. Statistical analysis of this experiment was completed using SPSS software (version 20). Two-way ANOVA was used in data analysis, and significance was determined at $P \leq 0.05$. Only the hydrogen peroxide concentrations were considered constant in this study. Data were expressed as mean with standard error ± SD [12].

RESULTS

The results of ANOVA showed significant differences ($p<0.05$) in the relative water content of leaves Figure 1, total soluble sugar content Figure 3, carotene Figure 4, H_2O_2 content Figure 5, proline Figure 6, glutathione (GSH) figure 7 and malondialdehyde content (MDA) figure 8; these traits were affected by using different levels of hydrogen peroxide, where the control treatment was gave the highest levels of relative water content of leaves (RWC) and total soluble sugar content (86.21%, 53.45 $mg.g^{-1}$)respectively. In comparison, the seeds that were soaked with hydrogen peroxide at 2.5 m.mol were given the lowest values in the same traits in addition to carotene (52.83%, 39.93 $mg.g^{-1}$and 10.52$\mu g.g^{-1}$FW) respectively.

Using 2 m mol hydrogen peroxide gave the highest ranges of H2O2 and malondialdehyde (MDA) content (2.54, 1.58) µmol.g-1FW, respectively. At the same time, the lowest values were in the check (1.63, 0.85) µmol.g-1FW respectively. The seeds that are soaked with hydrogen peroxide at 1.5 m.mol gave plants the highest values of carotene, proline and glutathione. (GSH).(18.32, 84.09) µg.g-1FW and 3.60 µmol.g-1FW, respectively, while the lowest values were for hydrogen peroxide at 0.5 for proline (49.25) µg.g-1FW and control for glutathione (1.75) µmol.g-1FW.

The saline treatment of wheat plants has increased the Na and Cl- content in the plant's cells and thus prevents the absorption of nutrients such as K.

The result of the ANOVA analysis showed that the different levels of hydrogen peroxide do not affect total chlorophyll and Figures 2 and 9 of the K/Na ratio. Correlation analysis indicates that there is a significant positive correlation ($p<0.01$) between H2O2 content and proline, glutathione (GSH) and malondialdehyde activity. (MDA) (r= 0.90**, r= 0.95**, r= 0.90**) respectively.This result indicates that the seeds of wheat plants were treated with hydrogen peroxide.at moderately high levels, which increases the capacity of the wheat plant.to prevent the adverse effects of salt stress by improving the antioxidant action and tolerance to salt stress.

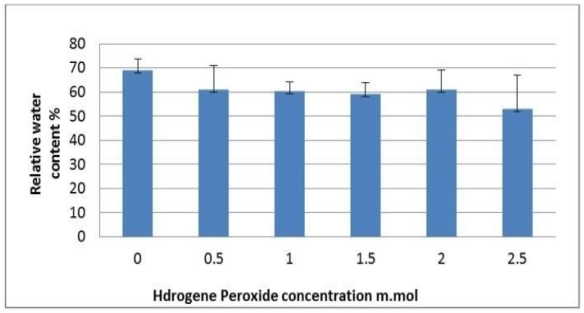

Fig (1): Effect of hydrogen peroxide on relative water content

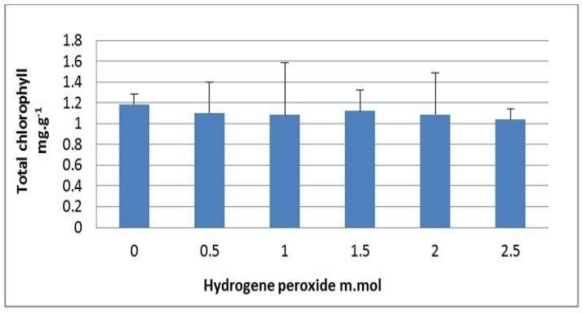

Fig (2): Effect of hydrogen peroxide on total chlorophyll content

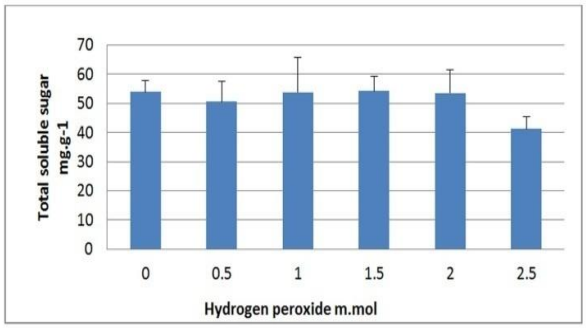

Fig (3): Effect of hydrogen peroxide on total soluble sugar content

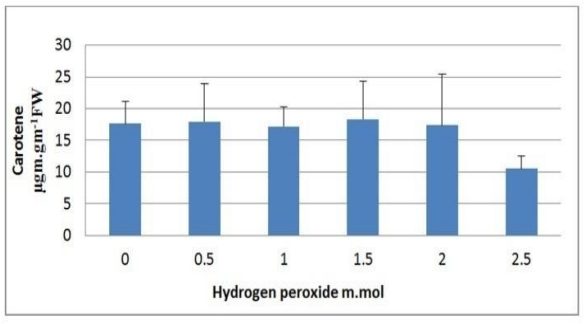

Fig (4): Effect of hydrogen peroxide on carotene content

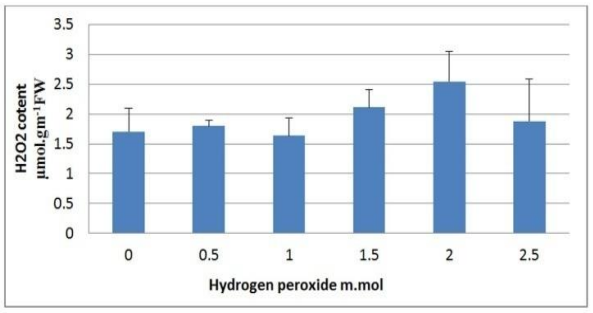

Fig (5): Effect of hydrogen peroxide on H_2O_2 content

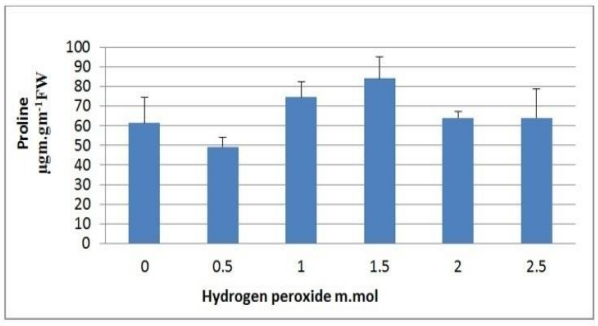

Fig (6): Effect of hydrogen peroxide on proline content

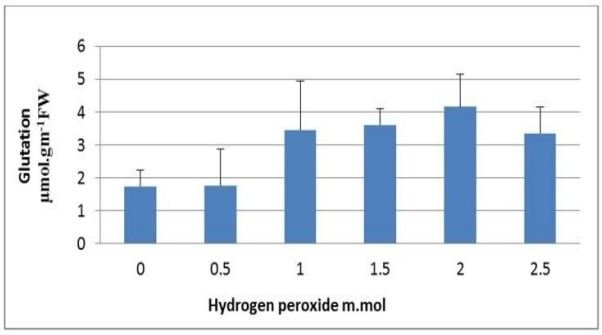

Fig (7): Effect of hydrogen peroxide on Glutation (GSH) content

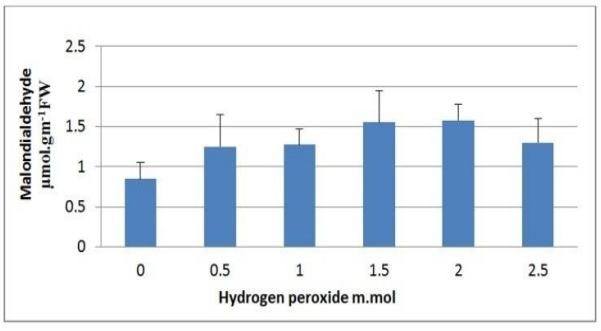

Fig (8): Effect of hydrogen peroxide on Malondialdehyde (MDA) content

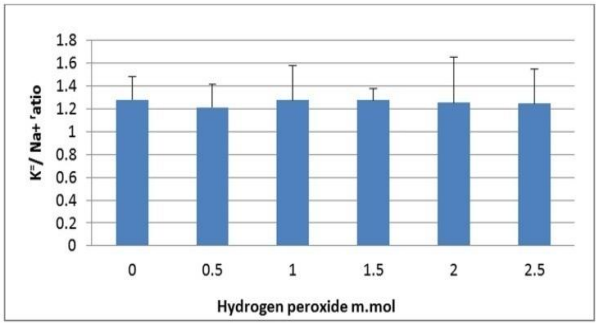

Fig (9): Effect of hydrogen peroxide on K^+/Na^+ ratio

DISCUSSION

Hydrogen peroxide plays a major role in activating the plant's defense system, which improves the plant's growth and tolerance to oxidative stress [13]. In this study, the decline in relative water content is the total soluble sugar level. Due to the effect of saline stress, the average chlorophyll content is due to the disruption of the plant's water relations and hormonal balance. The unbalance of respiration and photosynthesis and the weakening of the water absorption process from the root and, thus, the decrease in the plant's overall growth rate is consistent with [14]. It was increasing malondialdehyde, carotene, glutathione and proline. It is evidence that salt stress has increased the production rate of reactive oxygen species with oxidative damage to cell membranes and other biological molecules [15]. As a consequence, there is a defect in the processes of cellular oxidation and reduction, which is the role of hydrogen peroxide in the regulation of the action of antioxidants by signaling its molecule. It eliminates the toxicity of ROS oxygenated radicals where glutathione acts as a catalyst in catalytic responses to reduce the hydrogen peroxide molecule to a water molecule, and beta-carotene is considered the most active to protect cellular components from damage to active oxygen, such as proline changes the osmosis of the cells by accumulating them inside the cells without damaging them. This confirms that hydrogen peroxide enhances the action of antioxidants such as carotene and glutathione under conditions of salt tension [16].

CONCLUSION

This study showed that natural growth, that is to say, without environmental stress, like the effect of salts, reaches the highest rates.in the relative water content of the plant, and therefore, the water relations in the plant are in the best condition, also revealed as soaking wheat seeds with moderate levels of hydrogen peroxide can achieve an increase in the efficiency of antioxidant enzymes and, therefore, increase the capacity of the plant to resist saline stress.

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