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ANTIOXIDANT RESPONSES IN WHEAT PLANTS (*TRITICUM AESTIVUM* L.) TREATED WITH THIOUREA

Radhwan A. Baqer[1], Hussein K. Al-Kaaby[2] and Luma H. Adul-Qadir[2]
[1]Ministry of Education, Directorate of Education, Dhi Qar, Iraq
[2]Department of Biology, College of Education for Pure Sciences, University of Basrah, Iraq

Abstract

The effect of seeds pretreatment with thiourea solution on salt stress tolerance in wheat plant (cultivar Ebba-99) was studied. Seeds pretreated with 6 mmol/L^{-1} of thiourea in addition to distilled water (control) for 6 hours, then the seeds was cultivated after during in pots contain on mixture river sand and peat. The seedling was exposed after two weeks from date of germination for induced salt stress with NaCl at concentrations (50, 100, 150 mmol) for 8 weeks. Salt stress caused significant decrease in growth of shoot height, leaf area, fresh and dry weights, relative water content (RWC) and significant increase activity of CAT, SOD and APX. The salt stress caused significant decrease in concentration of Ascorbic Acid while did not record significant difference in concentration both of Glutathione and Carotene, Lipid peroxidation (MDA), Proline accumulation significantly increased under salt stress conditions. Thiourea application by seeds soaking led to improvement in some of growth parameters, antioxidants system, reduction in MDA content and electrolyte leakage.
***Keywords*:** Seeds priming, Thiourea, Wheat (*Triticum aestivum* L.), Salt Stress, antioxidants compounds.

Introduction

The salinity is one of the most important abiotic stresses which limits the growth and productivity of agricultural crops. According to food and agriculture organization, there are more than 800 million hectares of agricultural lands strictly affected by the salinity which intimidates the capacity of agriculture on control of developing population increase (Munns and Tester, 2008). The elevated salinity affects on the plants by reducing water absorption ability of roots. Also, the high concentration of salts inside the plant itself can be toxic which leads to the inhibition of many physiological and biochemical processes such as absorption of nutrients, metabolism, damage in cellular membranes, ionic imbalance, changes in levels of phytohormones, repression of enzymatic activity and imbalance in photosynthesis. This finally leads to shortage in the productivity. These effects reduce the growth and development of plants and its survival (Hasegawa *et al*., 2000; Hussain *et al*., 2011).

The efforts which aimed to improving plant growth under saline stress by inheritance and breeding did not success because the trait of salinity tolerance is multigenic. Trait that is difficult to be transmitted using technique of genetic engineering (Flowers *et al*., 2000). In past few years, many strategies were implemented to improve the growth of plants under inappropriate environmental conditions including technique of pretreatment of seeds (seed priming) (Paparella *et al*., 2015) as favorable techniques that protect the plants from different biotic and abiotic stresses without affecting the preservation of seeds or the environment or human. This is safe and inexpensive strategy which enhances the natural defense mechanisms of plants before exposure to stress. That helps growth with least possible damage by creating what is called priming memory (Jones and Dangl, 2006). Thus, the pretreatment seed is smart, active and realistic choice for protection of plants and stimulates their growth more effectively under stress conditions (Goellner and Conrath, 2007).

Thiourea compound is one of the compounds used for treatment of seeds before breeding (agriculture). Alternatively, the thiocarbamide which contains carbon, nitrogen, sulphur and two of important functional groups, thiol (-SH) group and amino (-NH2) group. So, thiourea is an important molecule from the physiological perspective (Sahu *et al*., 2005; Wahid *et al*., 2017).

Many studies showed the role of exogenously application to thiourea in order to improve plants growth under various stress conditions. (Mahatma *et al*., 2009) showed that the soaking of seeds with thiourea leads to an improvement in growth of wheat plant exposed to saline stress condition. Another study done by (Anjum *et al*., 2011) demonstrated that adding of thiourea by foliar spray enhances growth of wheat plant under condition of salinity and high temperature. Also, the foliar spray improves the growth of maize plant with thiourea compound at concentration of 400 µm under saline stress condition (Sanaullah *et al*., 2016). So, the present study aims to evaluate the role of pretreatment of seeds with thiourea on tolerance of wheat plant under salt stress conditions.

Materials and Methods

The study was conducted in the plant physiology laboratory in the department of biology - College of Education for pure sciences - University of Basra to study the effect of seeds pretreatment with Thiourea on stress salt tolerance in wheat plants (*Triticum aestivum* L.) cv. Ebba 99.

Seeds pretreatment with thiourea, NaCl-induced Salt stress.

Wheat seeds was obtained from Seeds validation center - Najaf governorate. Seeds were pre-treated (soaking) with different concentrations of thiourea (1, 2, 4, 6, 8 mmol) in addition to control (distilled water) for three periods (3, 6, 9, hours) in order to obtain the optimum concentrations and time after germination of pre-treated seeds for seven days. This was achieved according to the following criteria:

germination percent, flag leaf length, seedlings fresh weight and root length. Taking into consideration the result of Preliminary experiment, seeds were pre-treated with optimum concentration 6 mmol of thiourea in addition control treatment(distilled water) for 6 hours. Salt stress was induced with different concentrations (50, 100, 150 mmol) of sodium chlorine salt for 8 weeks of culturing.

Vegetative Parameters

Height and fresh weight (FW) of the shoots was measured and after that shoots were oven-dried over a period of 48 h at 70ºC to a stable weight for the determination of dry weight (DW). Leaf area (LE) (cm^2) was determined according to Robertson and Giunta, (1994). Relative water content (RWC) was measured according to Barr and Weatherly, (1962).

Biochemical Parameters

Cell membranes damage was estimated by Measurement the electrolyte leakage according to the method described by Yadav *et al*., (2016). Lipids peroxidation was estimated by the content of malondialdehyde (MDA) whose concentration was estimated by John and Steven, (1978) method. Free proline concentration was estimated according to Bates *et al*. (1973) method.

Enzymes assays: estimate was carried out using the extract of the shoot as the enzymes source. The activity of Catalase (CAT) (EC 1.11.1.6) was estimated according to Hadwan and Kadhum, (2018) method. Super oxide dismutase (SOD/ Cu-Zn) (EC:1.15.1.1) activity was estimated using a simple and rapid method that mainly depends on the enzyme ability to inhibit pyrogallol oxidation (Marklund and Marklund,1974). Ascorbate peroxidase (APX) activity (EC 1.11.1.11) was evaluated according to the method described by Nakano and Asada, (1981).

Determination of antioxidant compounds: Ascorbic acid concentration was estimated according to Moldovan *et al*. (2011) method. Carotene concentration was measured by spectrophotometric method according to the method described previously (Kirk and Allen, 1965). Glutathione (GSH) concentration was determined according to the method described by Noctor *et al*. (2012).

Statistical analysis

The data were subjected to the analysis of variance (ANOVA), and the means were compared via the Least Significant Difference (L.S.D) test at level 0.05 by using GenStat program.

Results

Effect of NaCl and its interaction with thiourea on the vegetative growth properties

The increase in salt levels cause a significant decrease in shoot height, leaf area, fresh and dry weight of shoot and relative water content (RWC) at two concentrations (100, 150 mmol/L^{-1}) NaCl compared with control treatment. Whereas, the pretreatment of seeds with a solution of thiourea at concentration of 6 mmol for 6 hours before planting leads to an improvement in the parameters of vegetative growth (height shoot, leaf area, fresh weight of the shoot and (RWC) of wheat growing under salt stress conditions in comparison with the formation of plants seeds and untreated growth under the same conditions of salt stress.

Effect of NaCl and its interaction with thiourea on enzymatic antioxidants

A significant increase in the activity of enzyme antioxidants (CAT, SOD) was observed with an increase in level of salt stress precisely at concentration of 150 mml of NaCl compared with control. The APX enzyme demonstrated difference of the activity levels, as a significant increase in its effectiveness was observed at concentration of 50 mmol NaCl alone compared to control. Whereas, the pretreatment of seeds with thiourea and treatment of the NaCl at the two concentrations 100 and 150 mmol resulted in a significant increase in the activity of CAT and SOD enzymes compared with the control treatment. Also, The treatment of the seeds with a thiourea solution resulted in an improvement in the activity of the APX enzyme in plants grown under conditions of salt stress.

Effect of NaCl and its interaction with thiourea on non-enzymatic antioxidants

The results in Table (2) illustrate that treatment of plants with NaCl at concentrations 50, 100 and 150 mmol do not significantly affect the carotene and glutathione concentrations rates compared with the control treatment. Also, treatment of plants with NaCl at two concentrations of 100 and 150 mmol resulted in a significant decrease in the concentration of ascorbic acid in comparison with the control. While, the pretreatment of seeds with a solution of thiourea before planting caused a significant increase in the concentration of carotene in plants treated with NaCl at a concentration of 100 mmol as well as an improvement in the concentration of ascorbic acid with the same concentration of NaCl.

Effect of NaCl and its interference with thiourea on integrity indicators of cell membranes

The observations of Table (2) show a significant increase in the content of malondialdehyde (MDA) when treating plants with NaCl at all concentrations compared with control. Moreover, a significant increase in the percentage of electrolytic leakage in plants treated with NaCl in concentrations of 100 and 150 mmol. Also, the treatment of plants developing of seeds pretreated with an optimal solution of thiourea resulted in a significant decrease in the MDA content when treated with NaCl 150 mmol compared with NaCl alone at the same concentration.

Table 1 : Effect of NaCl (50, 100 and150) mmol/L^{-1} and its interaction with seeds pretreatment at (6) mmol/L^{-1} concentration of thiourea on the vegetative parameters and cellular membranes integrity indicators.

Treatments	Shoot height (cm. Plant $^{-1}$)	Leaf Area (cm^2. Plant $^{-1}$)	Shoot FW (g. Plant $^{-1}$)	Shoot DW (g. Plant $^{-1}$)	RWC (%)	MDA (µmmol.g^{-1}.fw)	Electrolyte leakage (%)
Control	43.3±3.51	10.82±1.21	1.36±0.10	0.38±0.036	81.3±5.15	0.624±0.1	15.61±1.4
50 mmol NaCl+ Thiourea	40.67±3.05	11.92±0.76	1.48±0.33	0.373±0.03	77.6±6.91	0.836±0.128	17.85±1.76

50 mmol NaCl only	42 ±1.0	10.4 ±1.15	1.29±0.11	0.29±0.055	76.5±8.85	0.998±0.177	18.97±2.04
100 mmol NaCl+ Thiourea	38.67±2.51	9.88 ±1.64	1.35±0.36	0.337±0.041	74±5.2	1.15±0.117	20.74±2
100 mmol NaCl only	35.3±2.08	8.64 ±0.29	1.06±0.19	0.283±0.02	68.5±6.7	1.425±0.069	22.38±1.06
150 mmol NaCl+ Thiourea	35.9±1.76	8.57 ±1.61	1.10±0.10	0.277±0.025	71.8±8.56	1.372±0.197	22.15±2.4
150 mmol NaCl only	33±1.0	7.17 ±0.91	0.81±0.15	0.223±0.049	66.8±10.16	1. 815±0.31	24.33±2.373
L.S.D at level 0.05	**4.05**	**2.05**	**0.304**	**0.1**	**10.9**	**0.37**	**3.51**

Table 1 : Effect of NaCl (50, 100 and150) mmol/L^{-1} and its interaction with seeds pretreatment at (6) mmol/L^{-1} concentration of thiourea on the enzymatic and non-enzymatic antioxidants .

Treatments	CAT ($U.min^{-1}.g^{-1}$.FW)	SOD ($U.min^{-1}.g^{-1}$.FW)	APX ($U.min^{-1}.g^{-1}$.FW)	Carotene ($\mu g.g^{-1}$.FW)	GSH ($\mu mol.g^{-1}$.FW)	Ascorbic acid ($\mu g.g^{-1}$.FW)	Proline ($\mu g.g^{-1}$.FW)
Control	0.109±0.043	35.8±6.19	0.646±0.14	7.12±2.25	3.10±0.21	64.1±5.53	48.0±2
50 mmol NaCl+ Thiourea	0.208±0.084	38.1±7.96	1.11±0.24	11.02±3.32	3.70±0.07	56.7±3.94	75.7±5.5
50 mmol NaCl only	0.269±0.11	37.2±2.53	1.341±0.39	6.12±2.69	3.82±0.62	55.3±5.92	69.3±7.5
100 mmol NaCl+ Thiourea	0.343±0.081	50.2±26.16	0.961±0.12	18.02±8.5	3.737±0.106	62.0±8.20	105.7±7.09
100 mmol NaCl only	0.351±0.083	42.2±9.27	0.695±0.148	11.58±3.15	3.87±0.45	51.6±4.65	104.3±8.5
150 mmol NaCl+ Thiourea	0.308±0.132	85.2±6.97	0.697±0.17	13.35±5.11	3.51±0.3	49.4±7.76	109.0±15
150 mmol NaCl only	0.389±0.06	60.3±9.15	0.465±0.10	10.59±1.41	3.60±0.16	44.9±8.84	112.3±11.59
L.S.D at level 0.05	**0.1569**	**21.05**	**0.3737**	**7.659**	**0.925**	**11.64**	**21.29**

Discussion

The increase in the concentration of salts in the medium of growth negatively affects on plant growth through two stages of the negative effect of salt stress. First stage is represented by the osmotic effect of the salts surrounding the root and the reduction of the ability of the root cells to absorb water and mineral nutrients. Second stage includes the toxic effect as a result of prolonged accumulation of salts causing damage and death of leaves (Munns, 2005). In addition to the reduction in other growth indicators such as plant height, leaves area, fresh and dry weights of the root and shoot, the increase in the concentration of salts in the medium of plant growth causes a significant decrease in growth. Consequently in yield and quality of the crop, salinity causes stunting to the plant and changes in levels of organism. Furthermore, the plant suffers from other stresses, such as water stress and oxidative stress (Brugnoli and Björkman, 1992). Guo *et al*. (2015) observed a decrease in the growth of leaves and roots of wheat seedlings under salt stress conditions compared with seedlings that were not exposed to salt stress, and in a similar study achieved by Zou *et al*. (2016) found reduction in shoot length and the root system as well as the dry weight after (10) days of salt treatment at a concentration of (100) mM of NaCl.

The results above showed the positive effect of the pretreatment of seeds with thiourea before planting on growth characteristics of vegetative as it caused an improvement in growth of shoot height, leaf area, fresh and dry weights and the (RWC) of wheat grown under different levels of NaCl. Perhaps, this is due to the important role of the thiorea compound with distinctive properties. This refers to the great physiological importance of three functional groups, thiol and two groups of amino which could positively be reflected on the growth of plants grown under salt stress conditions (Robak and Marcinkiewicz, 1995; Sahu *et al*., 2005; Wahid *et al*., 2017). Another study done by Abdelkader *et al*. (2012) indicated the role of foliar spray with a thiourea solution in concentrations of (2.5 and 5) mmol that led to the improvement growth of wheat plant under water stress conditions as well as non-stressful conditions. The results obtained by Kaya *et al*. (2015) showed an increase in the fresh and dry weights and the water potential of the leaves of two cultivars of the maize plant developing from the seeds pretreated with thiourea 400 and 500 mg/l or treated by foliar spray with the same concentrations and developing under salinity conditions of NaCl at a concentration of 100 mM compared to plants untreated with thiourea and developing under conditions of salt stress.

Even under optimal conditions of growth, many metabolic processes produce the reactive oxygen species (ROS) such as the hydroxyl, peroxide, and superoxide radicals, and it is a common phenomenon in plants, but the frequency of their production increases when plants are exposed to environmental stresses such as salinity and drought stress their accumulation causes lipids oxidation, damage Proteins, DNA damage, cellular membranes and a decrease in the chlorophyll content, so plants have developed defensive systems to eliminate free radical toxicity (ROS) that include increased activity of antioxidant enzymes such as CAT and SOD as well as increased levels of the non-enzymatic antioxidant system (Faize *et al*., 2011; Gill and Tuteja, 2010). Numerous studies indicated changes in the activities of antioxidant enzymes (CAT, SOD, and POD) in plants growing under salinity conditions (Kaya *et al*., 2013). Heidari, (2009) observed a decrease in the activity of the APX enzyme in wheat leaves exposed to different levels of NaCl (100, 200) mM for 20 days after treatment. The increase in the activity of CAT and SOD antioxidant enzymes when wheat plants are exposed to salinity conditions may be due to an increase in salt stress led in an increased production of ROS which in turn attacks cellular components causing damage to proteins, peptides, nucleic acids and cellular membranes (Zhang *et al*., 2005; Kafkas *et al*., 2009).

A study of Nathawat *et al*. (2007) the role of pretreatment of wheat plants seeds with compounds containing thiol group at a concentration of 6.6 mM for 6 hours and their effect on the growing wheat plant under conditions of PEG-induced water stress at 10% and for a period of 5 days, it led to an improvement in the activity of anti-oxidant enzymes (CAT, APX, GST, GR) compared to plants produced from seeds untreated and subject to water stress conditions. Hassanein *et al*. (2015) observed an increase in the activity of CAT and SOD enzymes, increase in the content of flavonoids and phenols compounds and a significant decrease in the activity of POX and APX enzyme in wheat seedlings producing from treatment of seedlings through foliar spray with thiourea at a concentration of 2.5, 5 mmol grown under normal conditions or dehydration conditions compared to plants untreated with thiourea. Sanaullah *et al*. (2016) found an increase in the activity of antioxidant enzymes (CAT, SOD, APX, POX) when adding thiourea at a concentration of 400 µL to the growth medium of two cultivars of maize plant subject to NaCl salinity conditions of 120 mM, since The increase in the effectiveness of antioxidant enzymes, including CAT, SOD, APX and other antioxidants, could be explained by treating plants with NaCl and seeds pretreating with a thiourea solution, possibly stimulating the genetic expression of the genes responsible for producing these enzymes.

The addition of a thiourea solution at a concentration of 0.25 mM to the medium of growth of two cultivars of maize plant exposed to cadmium stress at a concentration of 1000 µmol led to an improvement in the synthesis of osmoprotectants (proline, glycine betaine and soluble sugars), phenols, riboflavin, anthocyanins and ascorbic acid that act as antioxidants while cadmium stress alone caused significant deficiency in ascorbic acid and riboflavin, increased MDA content, soluble sugars, proline, and glycine betaine as well as increased cellular permeability in both cultivars (Parveen *et al*., 2018).

The disruption in the physiological functions that salinity stimulates occurs in the cells and tissues of the leaf as a result of an increase in the accumulation of toxic ions (chlorine and sodium ions) causing an increase in the production of ROS that cause damage to the cell membranes and thus affect their integrity as the oxidation of lipids in the cellular membranes changes much of the chemical and physiological properties of the double layer of lipids membranes and thus increase their permeability (Ozturk *et al*., 2012; Noctor *et al*., 2014). The results of the study shown (Table 2) showed a significant increase in the oxidation of lipids (MDA content) and an increase in electrolytic leakage when treatment with NaCl at concentrations 100 and 150 mmol compared with control treatment, and this was confirmed by several studies. Farooq and Azam, (2006) the effect of salinity on different cultivars of wheat plant found that salinity caused an increase in cellular membranes damage. This study suggested that the decrease in cellular membranes stability is a reflection of the lipids oxidation process caused by the ROS. Similarly, the results of Sairam *et al*. (2002) and Zou *et al*. (2016) indicated an increase in the MDA content in wheat seedlings exposed to salt stress. MDA represents the final product of the lipid peroxidation in cellular membranes and is one of the indicators the important of plant tolerance to stress (Rao and Sresty, 2000). In related of seeds pretreatment with thiourea and its role on cellular membranes integrity indexes of wheat plant grown under salt stress. An significant decrease in the MDA content observed and the percentage of electrolytic leakage compared to the treatment of NaCl alone at the same concentration (Table 3).

Salinity stress causes an increase in the production of ROS, which production to oxidative stress, that causing damage to photosynthetic pigments and oxidation of cellular membranes, proteins and nucleic acids (Yordanov *et al*., 2000). Therefore, plants had anti-oxidant systems represented by enzymatic antioxidants and non-enzymatic that scavenger free radicals and keeps them at an optimal concentration (Xu *et al*., 2008).We have proven through the results of the study that showed an increase the activity of enzymatic antioxidants as well as a significant improvement non-enzymatic antioxidants (carotene and ascorbic acid) when thiourea interference with NaCl in comparison with treatment of NaCl alone. The increase in the activity of antioxidant enzymes (CAT, SOD, APX) and other antioxidants, could explain that the treatment of plants with NaCl and the pretreatment of seeds with thiourea solution may have stimulated the genetic expression of the genes responsible for the production of these enzymes and a reflection of this in a decrease in the MDA content.

Conclusion

In conclusion, thiourea can be applied by seeds soaking to increase the salt tolerance of wheat plant. Seeds pretreated with thiourea resulted in a significant improvement in the plant growth, antioxidants system and reducing cellular membranes damage.

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