International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.01, pp 346-354, 2014-2015

Effect of thiourea and salicylic acid on antioxidant defense of wheat plants under drought stress

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Abstract: Plant posses efficient system for scavenging reactive oxygen species that protect them from destructive oxidative reaction. As part of this system antioxidative enzymes and some bioactive constituents are key elements in the defense mechanisms. Pot experiment was conducted to evaluate the potential of grain presoaking in salicylic acid (SA) (1mM) or foliar application of thiourea (TU) with two concentrations (2.5 or 5 mM) or their combinations in improving antioxidant defense system of wheat under normal irrigation and drought stress conditions. The effect of SA and TU treatments on bioactive constituents and antioxidant enzymes activity nearly had similar trend under normal irrigation and drought conditions. Thiourea and salicylic acid caused increases in total flavonoids and total phenol contents as compare with untreated control, especially by foliar application of 2.5 mM TU on SA-treated plants. It produced more than 30% increases in flavonoid and phenolics contents in wheat leaves grown under normal irrigation or water shortage conditions compared with corresponding control. All SA and TU treatments resulted in great reduction effect on putrescine, malondialdhyde and hydrogen peroxide contents in normal irrigated and drought stressed plants. These constituents exhibited more than 30% reduction due to application of 2.5 mM TU on SA-treated plants. Treating wheat plants with TU, SA and their combination resulted in great increases in the activity of superoxide dismutase (SOD) and catalase (CAT) enzymes accompanied by great reduction in peroxidase (POX) and ascorbic peroxidase (APX) activities. Maximum increase of SOD and CAT was observed by treating plants with TU at 2.5 mM for SA-pretreated plants either under normal irrigation (44% and 32%, respectively) or drought conditions (57.8% and 47.8%, respectively). It can be concluded that combined application of thiourea as seed treatment and foliar spray was more effective in improving the wheat performance by enhancing antioxidant compounds (phenolics and flavonoids), membrane stability, antioxidant enzymes (SOD and CAT) and by reducing hydrogen peroxide free radical

Keywords: *Salicylic acid, Thiourea, Drought stress, Antioxidant compounds, antioxidant enzymes.*

1. Introduction

Several reactive oxygen species (ROS) are continuously produced in plants as byproducts of aerobic metabolism. Even under optimal conditions, many metabolic processes produce ROS. The production of toxic derivatives/ROS is increased as a result of all types of environmental stresses[1]. Drought stress induced high production of ROS[2] and caused damages to mitachondria and chloroplast by increasing hydrogen peroxide (H_2O_2) concentration and lipid peroxidation (MDA) of the tissues[3]. To counteract the toxicity of ROS a complex antioxidant system, composed of both nonenzymatic and enzymatic constituents is present in all plant cells[1]. Antioxidant enzymes (e.g. superoxide dismutase, SOD; catalase, CAT; peroxidase, APX and ascorbic peroxidase, APOX) have been related to water deficiency and are considered the main components of antioxidative machinery for drought resistance in higher plants[4]. Additionally, phenolics and flavonoids constituents are considered as a cellular adaptive mechanism for scavenging ROS during stress and preventing subcellular damage[5].

Thiourea is a non-physiological thiol and has been employed by various researchers to impart stress tolerance and improve yield of crops like wheat [6], and maize[7]. Thiourea application improved the plant growth potential and photosynthetic efficiency. This was concomitant with the onset of early maturity and increased crop yield. All these effects could be related to ROS scavenging activity which has been first demonstrated in HL 60 cell lines[8]. Later this has also been proved in plants by demonstrating its ameliorative action towards drought [9] and salinity[10] stress that are known to cause oxidative damage

Salicylic acid is an endogenous plant growth regulator of phenolic nature has been found to generate a wide range of metabolic and physiological responses in plants[11]. Salicylic acid treatment was found to enhance the efficiency of antioxidant system in plants[12]. The exogenous application of Salicylic acid enhanced the activities of antioxidant enzymes (CAT, POX and SOD) when sprayed exogenously to the drought stressed tomato plants [13]. The priming of seeds with lower concentrations of Salicylic acid, before sowing, lowered the elevated levels of ROS due cadmium exposure and also enhanced the activities of various antioxidant enzymes in *Oryza sativa,* thereby protecting the plants from oxidative burst[14].

The present investigation was conducted to explore the effects of seed soaking treatments with salicylic acid and/or vegetative sprays before anthesis using thiourea on hydrogen peroxide, lipid peroxidation and some enzymatic and non-enzymatic antioxidants in drought stressed and unstressed wheat plants.

2. Materials and Methods

2.1. Growth conditions

Pot experiment was carried out at Research and Production Station in Nubariah; the affiliate of National Research Center-Dokki-Cairo, Egypt. Wheat grains (*Triticum aestivum* var. Gimaza 9) were purchased from the Agricultural Research Center, Egypt. Wheat grains were incubated for 12 h in distilled water and/or in salicylic acid (1mM). The seeds were planted on November 15th 2010 in plastic pots (37 cm in diameter and 40 cm in height) filled with 20 kg sandy soil. After complete emergence, thinning was carried out to leave 10 seedlings per pot. Then, pots were divided into two sets, in the first set plants were grown under normal irrigation condition (irrigation every 5 days). Plants in the second set were exposed to drought stress (irrigation every 10 days). Each set was divided into 6 groups. In the 1st group, grains were presoaked in distilled water as 'control'. In the 2nd group, grains were presoaked in 1 mM salicylic acid (SA) for 12 hrs. In the 3rd and 4th groups; plants were sprayed with the following concentrations of thiourea (TU): 2.5 and 5 mM, respectively. For the 5th and 6th groups, grains were presoaked in1 mM SA and the grown plants were sprayed with the following concentrations of TU: 2.5 and 5 mM, respectively. Twice foliar applications of TU were carried out after 30 and 40 days from sowing. Treated plants were subjected to similar fertilization practices. Samples were taken after 90 days from sowing (anthesis stage) and subjected to analysis.

2.2. Enzyme preparation and assays

Extract for determination of antioxidant enzyme activities were prepared as described[15]. A fresh flag leaf samples (250 mg) were frozen in liquid nitrogen and finely ground by pestle in a chilled mortar, the frozen powder was added to 10 ml of 100 mM phosphate buffer (KH_2PO_4/K_2HPO_4) pH 6.8. The homogenates were centrifuged at 20000 xg for 20 minutes. The supernatant was made up to a known volume with the same buffer and used as "enzyme preparation" for the assay of different enzyme activities.

2.2.1. Superoxide dismutase (SOD, EC 1.12.1.1) assay

SOD activity was measured according to the method[16]. Three ml of mixture contained 13 mM methionine, 0.025 mM of p – nitro blue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate and 0.5 ml enzyme extract. Reaction was started by adding 0.002 mM riboflavin and placing the tubes below two 15 Watt (W) fluorescent lamps for 15 min. The reaction was stopped by switching off light and covering the tubes with black cloth. The tubes without enzyme developed maximal colour. A non – irradiated complete reaction mixture served as blank. The absorbance was measured at 560 nm, using spectrophotometer (Shimadzu). One unit of SOD activity was defined as the amount of the enzyme that causing 50 % inhibition of NBT to blue formazan under the experimental condition.

2.2.2. Peroxidase (POX, EC 1.11.1.7) assay

POX activity was assayed using a solution containing 5.8 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the enzyme extract and 2.0 ml of 20 mM H_2O_2. After addition of 2.0 ml of 20 mM pyrogallol, the rate of increase in absorbance as pyrogallol was determined by spectrophotometer (Shimadzu) within 60 second at 470 nm and 25^oC [17]. One unit of enzyme activity was defined as the amount of the enzyme that catalyzed the conversion of one micromole of H_2O_2 per minute at 25^oC[18]. The blank sample was made by using buffer instead of enzyme extract. The enzyme activities were expressed by U /hr /g FW.

2.2.3. Catalase (CAT, EC 1.11.1.6) assay

CAT activity was assayed according to the method of [19]. The reaction mixture with final volume of 10 ml containing 40 µl enzyme extract was added to 9.96 ml H_2O_2 phosphate buffer (pH 7.0) (0.16 ml of 30 % H_2O_2 to 100 ml of 50 mM phosphate buffer). CAT activity was determined by measuring the rate change of H_2O_2 absorbance in 60 second with spectrophotometer (Shimadzu) at 250 nm. The blank sample was made by using buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme that reduced 50 % of the H_2O_2 in 60 second at 25^oC [18].

2.2.4. Ascorbate peroxidase (APX, EC 1.11.1.11) assay

APX assay was performed using the method of [20] with few modifications. 10 ml of solution contained 5.5 ml of 50 mM phosphate buffer pH 7.0, 0.5 ml of the enzyme extract, 1.0 ml of 20 mM H_2O_2, 1.0 ml 20 mM EDTA and 2.0 ml of 20 mM l-ascorbic acid. The decrease rate in absorbance as ascorbate oxidized was monitored at 290 nm with spectrophotometer (Shimadzu) (ε= 2.8 mM^{-1} cm^{-1}). One unit of enzyme activity was calculated as the amount of the enzyme that catalyzed the conversion of micromole of H_2O_2 per minute at 25 ˚C.

2.3. Determination of hydrogen peroxide (H_2O_2)

Hydrogen peroxide content was determined using the method[21], in which fresh samples of leaf tissue (100 mg) was extracted with 5 ml of 0.1 % trichloroacetic acid (TAC) and centrifuged at 12000g for 15 minutes. Then 0.5 ml of supernatant was mixed with 0.5 ml of 10 mM phosphate buffer (pH=7) and 1 ml of 1M potassium iodide. The absorbance was determined at 390 nm. The amount of H_2O_2, read using the extinction coefficient 0.28 μm^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

2.4. Determination of lipid peroxidation (MDA)

The level of lipid peroxidation was measured by determining the levels of malondialdhyde (MDA) content using the method[22]. A flag leaf sample (200 mg) was homogenized in 10 ml of 5 % trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 xg for 10 min to 2.0 ml aliquot of the supernatant 4.0 ml of 0.5 % thiobarbaturic acid (TBA) in 20 % TCA was added. The mixture was heated at 95^OC for 30 min and then quickly cooled in an ice bath and centrifuged at 10000xg for 10 min the absorbance of supernatant was recorded at 532 nm by spectrophotometer (Shimadzu). The value for non – specific absorption at 600 nm was subtracted. The MDA content was calculated using its absorption coefficient of 155 n mol^{-1} cm^{-1} and expressed as n mol (MDA) g^{-1} fresh weight.

2.5. Determination of total phenols and total flavonoids

A known weight of wheat flag leaves was extracted by overnight submersion of dry tissue in 10 ml of 80 % (v/v) ethanol at 25^oC with periodic shaking, then filtered through a glass funnel. The residue was re-extracted and filtered twice with 80% ethanol, and then the filtrate was made up to a known volume.

Total phenols content was determined calorimetrically according to the method reported[23]; using Folin-Ciocalteau phenol reagent. For estimation, 1 ml of ethanol extract was mixed with 10 drops of concentrated hydrochloric acid, heated rapidly in boiling water bath for 10 minutes, cooling, then 1 ml of Folin-Ciocalteau reagent and 1.5 ml of 14% sodium carbonate were added. The mixture was made up to 5 ml by distilled water, shaken well, and then kept in a boiling water bath for 5 minutes. The developed color was measured at 650 nm against a reagent blank using spectrophotometer (Shimadzu). Total soluble phenolic compounds were calculated as mg g^{-1} dry weight using standard curve with pyrogallol.

Total flavonoids content was determined[24]. Appropriate dilutions of sample extracts (2 ml) were reacted with 0.2 ml of 5% sodium nitrite, after 5 mints, followed by reaction with 0.2 ml of 10% aluminium chloride to form a flavonoid–aluminium complex. Solution absorbance at 510 nm was immediately measured and compared to that of catechin standards.

2.6. Determination of polyamine compounds by HPLC

The extraction method was essentially similar to that adopted[25]. The dry tissue (0.3 g) of flag leaves at anthesis stage was ground in a mortar in the presence of 5% cold HClO4 at 48,000 g x 20 minutes by the centrifuge. Standard and plant extracts were benzoylated[26].

HPLC was performed on a system consisting of two solvent metering pumps (Shimadzu) programmed with a microprocessor controller, and the solvents were mixed under pressure by automixer (Shimadzu). The benzoylated samples were injected through autosampler (Knauer), into a fixed 25 µl loop syring loading injector for loading onto reverse phase C18 column (RP-C18 µ Bondapak, Waters) . The column used included octadecylsilane (ODS) ultrasphere particle (5-µm). Samples were eluted at room temperature through 250 x 4.6 mm column with a programmed aqueous 0.2 % acetic acid: methanol (v/v) solvent gradient, changing from 60 to 95 % in 25 minutes at a flow rate 1 ml/min. Elution was completed by 45 minutes. The column was washed with 100% methanol and re-equilibrated with 60% methanol for the next sample was injected. The samples detected at 254 nm.

2.7. Statistical analysis

Data was statistically analyzed[27]. Least significant difference (LSD) at 5% level of probability was calculated to compare means of different treatments.

3. Results and Discussions

3.1. Effect of drought stress on bioconstituents and antioxidant enzymes activity

The obtained data (Table 1) showed that drought stress resulted in significant increases in total flavonoids and total phenols in flag leaves of wheat plants. These results might be due to the function of flavonoids and phenolics, as members of the antioxidant family, on reducing potentials and accessibility of radicals under oxidative stress[28] and promoting plant protection[29] through lipid peroxidation prevention[30].

Table 1. Effect of drought stress on enzymatic and non-enzymatic constituents in wheat flag leaves

Parameter	Flavonoid	Phenols	Polyamines(µg g/g)			H_2O_2	MDA	Enzyme activity (unit/ h/g)			
Treatment	(mg/g)	(mg/g)	Put	Spd	Spm	(nmol /g)	(nmol /g)	SOD	CAT	POX	APX
Unstressed	1.14[b]	1.60[b]	4.76[b]	2.39[b]	11.5[b]	16.7[b]	16.6[b]	30.0[b]	61.2[a]	515[b]	117[b]
Stressed	1.28[a]	1.79[a]	6.54[a]	3.73[a]	23.8[a]	21.1[a]	21.9[a]	37.2[a]	55.2[b]	704[a]	127[a]

Values with the same letters in a column are not significantly different at P < 0.05 by the least significant difference (LSD) test.

A pronounced increase in endogenous polyamine components, Put, Spd and Spm was observed in flag leaves of wheat plants exposed to drought stress compared with normal irrigated plants (Table 1). Polyamines are implicated in the control of cell growth and development in eukaryotic cells and the response to a wide range of abiotic stress conditions[31]. During drought tolerance, putrescine was able to bind to antioxidant enzymes, such as superoxide dismutase, or to be conjugated to small antioxidant molecules, allowing them to permeate to the sites of oxidative stress within the cells [31].

As shown in Table (1), wheat plants responded to drought stress by inducing marked increase in H_2O_2 and MDA contents. Similar results were obtained[32]. Under stress, accumulation of ROS including H_2O_2 caused oxidative damage in plants and produced MDA, the biomarker of membrane lipid peroxidation in the cellular environment [33,34]. Drought exhibited increased SOD or POX or APX activities and decreased CAT activity in wheat leaves as compared to normal irrigated plants (Table 1). Plant posses' efficient system for scavenging ROS that protect them from destructive oxidative reaction[8]. As part of this system antioxidative enzymes are key elements in the defense mechanisms. Many changes have been detected in the activities of antioxidant enzymes in plants under stress[1].

3.2. Effect of SA and TU on total flavonoids and total phenols contents

Table 2. Effect of salicylic acid (SA) and thiourea (Th) treatments on flavonoids, phenols and polyamine compounds in wheat flag leaves, under normal irrigation and drought stress conditions.

Treatments	Total flavonoids	Total phenols	Polyamine compounds		
			Put	Spm	Spd
	Normal condition				
Control	1.14±0.02	1.60±0.09	4.76±0.09	11.52±0.30	2.39±0.10
Thiourea1	1.23±0.03	1.66±0.08	4.40±0.10	2.50±0.10	2.21±0.08
Thiourea2	1.38±0.03	1.85±0.11	4.40±0.20	1.23±0.10	1.86±0.07
salicylic acid	1.50±0.02	1.95±0.12	3.78±0.10	14.66±0.50	5.84±0.05
Th1+SA	1.70±0.04	2.10±0.15	1.85±0.10	5.00±0.10	8.75±0.10
Th2+SA	1.59±0.03	2.06±0.20	2.81±0.20	2.72±0.07	3.86±0.05
$LSD_{5\%}$	0.27	0.15	0.52	0.71	0.4
Drought condition					
Control	1.28±0.03	1.79±0.20	6.54±0.20	23.84±1.10	3.73±0.07
Thiourea1	1.32±0.02	1.82±0.23	5.10±0.20	3.97±0.09	2.91±0.09
Thiourea2	1.42±0.03	2.12±0.25	6.05±0.10	22.26±1.0	2.91±0.09
salicylic acid	1.69±0.03	2.16±0.30	4.95±0.20	40.36±1.20	1.56±0.05
Th1+SA	1.78±0.04	2.37±0.32	4.95±0.10	9.82±0.80	2.38±0.07
Th2+SA	1.66±0.04	2.31±0.40	2.60±0.10	4.32±0.05	14.40±0.20
$L.S.D_{5\%}$	0.15	0.087	0.52	2.9	1.4

Foliar application of TU with two concentrations (2.5 and 5 mM) or grain presoaking in SA (1mM) or their combinations caused increases in total flavonoids and total phenol contents as compare with untreated control (Table 2). Such enhancement effects of SA and TU was observed for drought stressed and unstressed plants. SA treatment alone produced more enhancement effects on flavonoids and phenolics than TU individual treatment, under either normal irrigation or drought conditions. Combination between TU and SA resulted in the most enhancement effects, especially when combined low dose of TU with SA. Presoaking of wheat grains in SA followed by foliar spray with TU at 2.5 mM resulted in higher increment in flavonoids and phenolics contents (49% and 31%, respectively) for normal irrigated plants and for drought stressed plants (39% and 32%, respectively). Similar enhancement effects of SA and TU on flavonoid and phenol in drought stressed and non-stressed wheat plants were observed[9]. The accumulations of phenolic compounds and the phenylalanine ammonia lyase (PAL) activity in cell culture of Salvia *miltiorrhiza* were stimulated 8h after the treatment with SA. The elicitation effect of SA on phenolic accumulations correlated with the PAL activity[35].

3.3. Effect of SA and TU on polyamine compounds

Data in Table (2) indicated that TU and their combination with SA treatments tended to reduce polyamines, especially Put and Spm compounds in the flag leaves of drought stressed and unstressed wheat plants.

Concentration of Put in flag leaves of wheat plants achieved marked reduction due to SA, TU and their combination treatments compared with control. The most reduction effect on Put content was obtained by application of 2.5 mM TU on SA pre-treated plants (61%) for normal irrigated plants and by application of 5 mM TU on SA pre-treated plants (60%) for drought stressed plants. As for Spm, except the enhancement effect of SA alone, all TU and their combination with SA treatments caused great reduction effects, ranged between 56.6% and 89% for normal irrigated plants and between 58.9% and 83.3% for drought stressed plants. Also for Spd compound, pronounced decrease was observed in flag leaves of normal irrigation conditions in the response of foliar application of TU at 2.5 mM and 5 mM. . But, under this favorable irrigation condition, using SA alone and their combination with TU at 2.5 mM or 5 mM resulted in great enhancement effects on Spd contents. Under drought condition, except the enhancement effect for application of 5 mM TU on SA pre-treated plants, all TU and SA treatments caused remarkable reduction in Spd contents.

In partial agreement with our results, reference[36] found that SA treatments enhanced Spd as well as total polyamines in sweet basil and marjoram plants. They suggested that SA treatments have higher adaptive capacity to stress, originating from promoting polyamines synthesis and better osmotic adjustment. The

decrement of Put in response to treatments with SA and/or THU in normal irrigated plants and drought stressed ones might be due to the conversion of Put into different polyamines (Spd) and/or amino acids. Similar results were obtained on rice plants[37]. However, another researches showed that the reduction of Put level under water stress was related directly to the decrease in arginine decarboxylase activity, but the decrease in Spd and Spm under these conditions could be closely related to the decrease in Put level. Polyamines in general and Put in particular could be reduced *via* the stimulation of certain enzymes designed to break them down[38]. These enzymes are diamine oxidase and/or polyamine oxidase. Also, this could be attributed to the inhibition of certain enzymes which responsible for their synthesis from their precursors; arginine or ornithine [31]. Similar results were obtained, where the decrement of polyamines might be attributed to the activity of polyamine oxidase, a degradative enzyme[39].

3.4. Effect of TU and SA on hydrogen peroxide and lipid peroxidation

Hydrogen peroxide (H_2O_2) in wheat flag leaves was shown to be significantly decreased due to TU, SA and their combination treatments, under normal irrigation or under drought stressed conditions compared with corresponding control (Table 3).

Table 3. Effect of salicylic acid (SA) and thiourea (Th) treatments on hydrogen peroxide, malondialdhyde and antioxidant activities in wheat flag leaves, under normal irrigation and drought stress conditions.

Parameter	H_2O_2	MDA	Antioxidant enzyme activity (unit/h/g FW)			
Treatment	(nmol/g FW)	(nmol/g FW)	SOD	CAT	POX	APX
Normal condition						
Control	16.7±1.3	16.64±1.3	30.0±1.6	62±3	515±19	117±5
Thiourea1	15.8±1.2	15.68±1.2	36.6± 1.8	63±3	459± 17	56± 3
Thiourea2	13.5±1.0	15.60±1.1	37.0±1.9	68±3	433±14	45±3
salicylic acid	13.1±1.1	15.28±1.0	38.4±2.1	69±3	430±14	42±2
Th1+SA	11.7±0.9	14.72±0.8	43.2±2.4	97±5	195±7	34±2
Th2+SA	12.5±1.1	15.20±0..9	40.2±2.2	80±4	419±12	35±2
$LSD_{5\%}$	1.9	1.8	3.9	6	27	7
Drought condition						
Control	21.1±1.6	21.9±1.6	37.2±1.4	55±2	704± 25	127± 6
Thiourea1	17.2±1.4	21.3±1.3	38.4±1.6	58±2	509±20	78±4
Thiourea2	14.6±1.3	18.7±1.2	39.1±1.8	58±2	463± 19	77±3
salicylic acid	14.5±1.2	15.4±1.2	41.7±1.8	65±3	457±17	70±3
Th1+SA	12.4±1.1	13.1±1.0	49.1±1.9	82±3	269±11	56±2
Th2+SA	13.0±1.6	14.1±1.1	43.2±1.9	74±3	433±16	66±3
$LSD_{5\%}$	2.7	2.6	3. 9	3	50	12

Sever reduction effect in H_2O_2 was observed for plants grown under drought stress. Presoaking of wheat grains in SA followed by foliar spray with TU at 2.5 mM resulted in higher reduction effect for normal irrigated plants (30.3%) or for drought stressed plants (41.5%). Reduction effects of SA on H_2O_2 free radical were previously mentioned by many investigators [1,9]. In this connection, the different effects of SA on protective enzyme activities were found associated with H_2O_2 metabolism were reported[40].

The concentration of malondialdhyde (MDA) as a product of lipid peroxidation was reduced in flag leaves of wheat plants when treated with TU, SA and their combinations under normal irrigation and drought conditions (Table 3). Presoaked of wheat grains in SA at 1 mM and foliar spray of obtained plants with 2.5 mM TU produced the maximum reduction effect of MDA under normal irrigation (11.5%) and for drought conditions (40.1%). In line with our results, it was reported that exogenous application of SA and TU had 22 % less lipid peroxidation in wheat leaves as compared to 5 dS/m salinity alone, which is in agreement with its role in quenching ROS and protect the cells from lipid peroxidation[1]. Also, reference[41] reported that thiourea application on wheat ameliorated the heat-induced damages by stimulating the total antioxidant activity through decrease in lipid peroxidation and membrane injury.

3.5. Effect of SA and TU on antioxidant enzymes activity

As shown in Table (3), the effect of SA and TU on antioxidant enzymes activity had similar trend under normal irrigation and drought conditions. Treating wheat plants with TU, SA and their combination resulted in significant increases in the activity of SOD and CAT enzymes as compare with corresponded untreated plants. Increases in the SOD activity ranged between 22% and 44% for normal irrigated plants as well as between 3.2% and 32% for drought stressed plants. While, the increase in CAT activity ranged between 1% and 57.8% for normal plants and between 4.3% and 47.8% for drought stressed plants. Maximum increase of SOD and CAT was observed by treating plants with TU at 2.5 mM for SA-pretreated plants either under normal irrigation or drought conditions. On the contrary, TU, SA and their combinations caused great inhibition effect in POX and APX activities for plants grown under normal irrigation and drought stress conditions. Maximum inhibition effects were recorded by foliar application of TU for SA-pretreated plants. Applying TU at 2.5 mM on SA pretreated plants inhibited POX and APX with 62.2% and 70.8%, respectively relative to control for normal irrigated plants and with 61.8% and 55.7%, respectively relative to control of drought stressed plants. Even under optimal conditions, many metabolic processes produce ROS, plant possess efficient system for scavenging ROS that protect them from destructive oxidative reaction[8]. As part of this system antioxidative enzymes are key elements in the defense mechanisms. Since SA is necessary for the induction of antioxidant defenses, it has been shown to be essential for the plant protection against oxidative stress[42]. The enhancement effect of exogenous SA on SOD and CAT activities was observed[43] for ginger plants grown under optimal growth condition and for tomato plants grown under salinity stress condition[44]. Enhancement of SA-induced SOD and CAT activities coupled with reduction in POX and APX in flag leaves of wheat plants indicate that H_2O_2 produced by SOD is scavenged by CAT [45]. There are very few reports on the effects of thiols on the antioxidant defense system. It was shown that due to thiol (TU, dithiothreiotol and thioglycolic acid) pretreatment, an increase in the activities of antioxidant enzymes was observed during water stress in wheat [46].

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