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Full Length Research Paper

Studies on effects of salicylic acid and thiourea on biochemical activities and yield production in wheat (*Triticum aestivum* var. Gimaza 9) plants grown under drought stress

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Drought is a problem of water deficit in the soil. It causes restrictions of wheat plants growth and productivity. In the present approach, we explored new methods for drought managment. Wheat grains were presoaked in ~1 mmol salicylic acid (SA). Wheat was left to grow with 40% soil water holding capacity in pots until yielding stages. The leaves which were produced were two-times sprayed with 2.5 and 5 mmol thiourea (Th) before anthesis. Morphological and biochemical traits were analyzed on the heading stage in addition to yield and yield components. Results show a conspicuous anti-drought effect in pretreated-compared to untreated-wheat. For example, pretreated wheat with SA and/or Th, possessed a significant increase in carotenoids, antioxidant enzymes activities and some metabolites (growth promotors, photosynthetic pigments, carbohydrates, nitrogenous constituents and minerals). On the contrary, lipid peroxidation and H_2O_2 decreased in level under the effect of external treatments. Wheat general morphology and yield components were generally improved upon SA and/or Th pretreatments. These data reinforced further investigations to reveal the reason behind the anti-drought responses in treated wheat with low doses of SA and/or Th before and during exposure to a progressive drought.

Key words: Biochemical activity, foliar application, salicylic acid, thiourea, wheat.

INTRODUCTION

Drought is a phenomenon of insufficient water supply to plants. Like other abiotic stressors, drought affected initially water relations on the cellular level as well as whole plant (Beck et al., 2007). It influenced various physiological and biochemical processes, such as translocation, ion uptake, respiration, photosynthesis, carbohydrates, nutrient metabolism and hormones (Farooq et al., 2009), and ended with plant general growth retardations leading to death.

Drought also resulted in oxidative stress (Ozkur et al., 2009) which subsequently caused the formation of reactive oxygen species in chloroplasts and mitochondria (Fu and Huang, 2001). Wheat is one of the most important crops. Like other cereals, wheat is subjected to drought stress conditions on various growth stages. Fetching new strategies such as mass screening and breeding, marker-assisted selection, and exogenous application of hormones and osmoprotectants to seed or growing plants were yet considered necessary methods towards mitigation (Farooq et al., 2009). Analyses of different parameters of pretreated wheat such as morphological status, yield and yield components, hormonal level, pigment content, carbohydrates,

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Abbreviations: SA, Salicylic acid; **Th,** thiourea.

nitrogenous compounds, antioxidant enzymes, and mineral nutrition conveyed a better understanding of the anti-drought and other encountering mechanisms that were involved.

In this study, a new technique was invented to mitigate wheat grown under 40% soil water holding capacity for whole life cycle by presoaking grains in ~1 mmol salicylic acid (SA) and/or vegetative sprays before anthesis using 2.5 and 5 mmol thiourea (Th). Morphological, biochemical and yield analyses were determined to underline wheat responses under the combined effects of SA and/or Th and drought.

MATERIALS AND METHODS

Plant materials and growth conditions

Field experiments were carried out at 'the Research and Production Station in Nubariah; the affiliate of National Research Center-Dokki-Cairo' during the two successive seasons of 2009 to 2010 and 2010 to 2011. Pure strain of wheat (*Triticum aestivum* var. Gimaza 9) grains were purchased from the Agricultural Research Center, Egypt. Planting was done in plastic pots (37 cm in diameter and 40 cm in height) filled with 20 kg sandy soil. The physical and chemical properties of sandy-clay soil were as follows (%): sand, 90.08; silt, 0.66; clay, 9.26; moisture content, 16.57; water pressure 5.25 and other properties of the used soil were: pH, 8.2; electrical conductivity, 1.7; Na^+, 0.982; K^+, 0.31; Ca^+, 7.02; Mg^{++}, 4.02; Cl^-, 0.566; and HCO_3, 1.3.

The soil was fertilized with nitrogen/phosphorus/potassium at the rate of 80 kg N, 30 kg P_2O_5 and 30 kg K_2O / feddan and added in the form of Ammonium nitrate (33.5% N), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O), respectively. Superphosphate and potassium sulphate were added before planting; while, nitrogen was added at three equal intervals, the 1st before planting and the other two at three week intervals. Wheat grains were divided into two groups; the grains of the first group were primed in ~ 1 mmol SA, while the grains of the second group were primed in water for 12 h. After a complete emergence, wheat plants (15 day-old) were planted on the 30th and 29th of November and grown till they were 120 days old. The plants were irrigated daily with tap water for 15 days from time of planting to allow for the establishment of the plants. The applications in the pre-anthesis stages were processed by foliar applications using thiourea in one of the following concentration: 2.5 mmol (Th_1) or 5.0 mmol (Th_2) or their interaction ($SA+Th_1$) and ($SA+Th_2$).

Six groups of wheat were planted according to the application regime: the 1st group was treated with distilled water as control; for the 2nd group, grains were soaked in 1 mmol salicylic acid for 12 h; for the 3rd and 4th groups, plants were sprayed with the following concentrations of *Th* (2.5 and 5 mmol), respectively; for the 5th and 6th groups, grains were soaked in 1 mmol *SA* and the grown plants were sprayed with the following concentrations of *Th:* 2.5 and 5 mmol, respectively. The first and second foliar applications were carried out after 30 and 40 days from sowing, respectively.

Experimental sampling for each group was achieved after 60 days (heading stage). On harvest, 30 plants of each group were collected to investigate yield quantity and yield components as well as biochemical changes in the flag leaf were added to growth characters studies.

Growth characters

Shoot height: The heights of 30 shoot replicates were taken at random from each treatment and the measurements were carried out from above soil surface until the end of growing tip of the plants and recorded in centimeters.

Flag leaf area: The leaf area (cm^2) was determined by the method described in Quarrie and Jones (1979) using the following proposed equation: Leaf area = Length × Breadth × 0.75

Fresh and dry weight of shoot and root: Wheat plants from every treatment were weighed immediately after clipping and estimation of the fresh weight of both shoot and root was carried out. The fresh materials was dried at 80°C to constant dry weight. The fresh and dry weight of both shoot and root were calculated as g/plant.

Root length: All measurements were carried out from the above ground to the root tip and were recorded in centimeters.

Yield components

At harvest, all potted plants were subjected to yield component analyses. The following characters were determined: spikes number/plant, spike length/plant (cm), spikes weight/plant, grains number/spike, grains weight/plant, seed index, grain yield (kg/feddan), biological yield (kg/feddan), harvest index (= economic yield/straw yield) and crop index (= grain yield/biological yield) (Beadle, 1993).

Statistical analysis

Data of 30 measurements from two independent experiments were analyzed through ± SD values using SPSS statistics data document for Windows, version 17.0. and Excel program, 2003. Each experiment was statistically analyzed according to Snedecor and Cochran (1980). Least significant difference (LSD) at 5% level of probability was calculated to compare means of different treatments.

Physiological analyses

Biochemical analyses were carried out on fresh and dry materials for the taken samples as indicated.

Identification of endogenous hormones

10 g of fresh tissue per sample was homogenized with 80% (v/v) ethanol and stirred overnight at 4°C. The extract was filtered through a Whatman filter and the methanol evaporated under vacuum. The aqueous phase was adjusted to pH 2.5 with 1 N HCl, then partitioned with ethyl acetate 3 times, and finally passed through anhydrous sodium sulfate. After that the ethyl acetate phase was evaporated under vacuum, the dry residue containing acidic hormones (fraction I) was dissolved in 2.0 ml of methanol and stored in vials at 4°C. This fraction was used for auxins, gibberellins, and abscisic acid determinations. On the other hand, the aqueous phase fraction was adjusted to pH 8.0 with some drops of potassium hydroxide 1 M and partitionated four times with N-butanol (1/4 of its volume each time). For cytokinins determination, the n-butanolic phase (fraction II) was concentrated to 5 ml. Auxins, gibberellins, and abscisic acid were detected by high performance liquid chromatography (HPLC) isocratic UV analyzer, reverse phase C18 column (RP-C18 µ Bondapak, Waters). The column used included octadecylsilane (ODS) ultrasphere particle (5 µm), the mobile phases used were

acetonitrile-water (26:74 v/v) at pH 4.00; Flow rate: 0.8 ml/min, detection: UV 208 nm. The standard solution of the individual acid was prepared in the mobile phase and chromatographed.

HPLC of cytokinin substances: Cytokinin fractions (zeatin and zeatin riboside) were detected by HPLC isocratic UV analyzer: ODS reverse phase C18 colum; 20 min gradient from 0.1N acetic acid; pH 2.8 to 0.1 N acetic acid in 95% aqueous ethanol at pH 4. The flow rate was 1 ml/min; detection was UV 254 nm. Standards of zeatin and zeatin riboside were used (Müller and Hilgenberg, 1986).

Photosynthetic pigments

The photosynthetic pigments (chl *a*, chl *b* and carotenoids) were colorimetrically determined according to Metzner et al. (1965). A known fresh weight of wheat flag leaves was homogenized in 85% aqueous acetone for 5 min. The homogenate was centrifuged at 6000 rpm and the supernatant was made up to the volume of 25 ml with 85% acetone. The extract was measured against a blank of pure 85% aqueous acetone at three wavelength 663, 644 and 452 nm using Shimadzu 240 UV/VIS spectrophotometer. Taking into consideration the dilution made, the concentration of the pigment fractions was measured as µg/ml using the following equations:

Chlorophyll a = 10.3 E663-0.918E644 = µg/ml
Chlorophyll b = 19.7E644-3.870E663 = µg/ml
Carotenoids = 4.2 E452-(0.0264 chlorophyll a + 0.426 chlorophyll b) = µg/ml.

Estimation of carbohydrates

Extraction of plant tissues: Sugars were extracted by overnight submersion of dry tissue in 10 ml of 80% (V/V) ethanol at 25°C with periodic shaking. The extract was filtered and the filtrate was oven dried at 60°C then dissolved in a known volume of water to be ready for determination of soluble sugars (Homme et al., 1992). Total soluble sugars and sucrose were determined using modifications of the procedures of Yemm and Willis (1954) and Handale (1968) respectively.

Estimation of total soluble sugars (TSS): They were analyzed by reacting 0.1 ml of methanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for 10 min and the cooled samples were read at 625 nm using spekol spectrocolourimeter VEB Carl Zeiss.

Estimation of sucrose: Sucrose content was determined by first degrading reactive sugars present in 0.1 ml extract with 0.1 ml 5.4 N KOH at 97^{o}C for 10 min. 3 ml of freshly prepared anthrone reagent were then added to the cooled reaction product and the mixture was heated at 97^{o}C for 5 min, cooled and read at 620 nm using spekol spectrocolourimeter VEB Carl Zeiss.

Estimation of polysaccharides: The method used for estimation of polysaccharides in the present study was that of Thayumanavan and Sadasivam (1984). The plant tissue is treated with 80% ethanol to remove sugars, then starch is extracted with percholric acid. In hot acidic reaction, starch is hydrolysed into glucose and dehydrated to hydroxyl methyl furfural. This compound forms a green coloured product with anthrone reagent. Dry tissues (0.5 g) were homogenized in hot 80% ethanol to remove sugars, centrifuged and then the residue was retained. The residue was washed repeatedly with hot 80% ethanol till the washing did not give colour with anthrone reagent. The residue was dried well over a water bath. To the dry residue, 50 ml of distilled water and 6.5 ml of 52% perchloric acid were added, then extracted at 0°C for 20 min, centrifuged (2000 rpm, 5 min) and the supernatant was stored. The extraction was repeated using fresh perchloric acid, centrifuged (2000 rpm, 5 min) and the supernatant was cooled and increased in volume to 100 ml. In a clean test tube, 0.2 ml of the supernatant was pipetted and increased in volume to 1.0 ml with distilled H_2O. 4 ml of anthrone reagent were added to each tube. The mixture was heated for 8 min, in a boiling water bath, then cooled rapidly and the intensity of the green to dark green colour of a glucose units was measured at 630 nm using a spectrophotometer. Poly-saccharides content (starch) was determined from the standard curve of glucose.

Lipid peroxidation and hydrogen peroxide content

Lipid peroxidation: The level of lipid peroxidation was measured by determining the levels of malonadialdehyde (MDA) content using the method of Hodges et al. (1999). A leaf sample (200 mg) was homogenized in 10 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 x *g* for 10 min and 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°C for 30 min and then quickly cooled in an ice bath and centrifuged at 10000 x *g* for 10 min. The absorbance of supernatant was recorded at 532 nm by spekol spectrocolourimeter VEB Carl Zeiss. 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 nmol g^{-1} fresh weight.

Hydrogen peroxide (H_2O_2) content: H_2O_2 content was determined using the method of Velikova et al. (2000), in which fresh samples of leaf tissue (100 mg) was extracted with 5 ml of 0.1% TCA and centrifuged using Labofuge 2000 centrifuge at 12000 *g* for 15 min. Then, 0.5 ml of supernatant was mixed with 0.5 ml of 10 mM phosphate buffer (pH = 7) and 1 ml of 1 M potassium iodide. The absorbance was determined at room temperature at 390 nm with a Unicam UV–visible double beam spectrophotometer from Helios company. The amount of H_2O_2 was calculated using the extinction coefficient 0.28 $\mu m^{-1}cm^{-1}$ and expressed as nmol/g fresh weight.

Estimation of nitrogenous constituents

Method of extraction: The method used in this investigation was essentially that des-cribed by Hassanein (1977) using dried plant tissues. A known weight of the dried powdered tissue was transferred to a glass mortar followed by 20 ml distilled water. The tissue was ground thoroughly and occasionally over a period of an hour. The mixture was then quantitatively transferred to a boiling tube, and maintained at 80ºC for 15 min. The insoluble residue was removed by filtration and the filtrate was made up to a certain volume and used for estimation of amino-N and total soluble nitrogen fractions. Total nitrogen was determined directly using the dry powdered plant tissues.

Estimation of amino – N: The buffer was prepared by adding 20 ml distilled water and 5 ml glacial acetic acid to 27 g sodium acetate and completed to 75 ml with distilled water (pH = 5.3-5.4), then 1.5 ml sodium cyanide (490 mg/L) was added. For preparation of ninhydrin reagent, 10 mg cadmium acetate was dissolved in glacial acetic (0.2 ml + 0.8 ml distilled water), 200 mg ninhydrin was added and the solution made up to 10 ml by 50% acetone.

Estimation of total soluble nitrogen: The total soluble-N was

determined by the conventional micro-kjeldahl method (Pirie, 1955). An aliquot of the extract was taken into digestion flask followed by 0.5 g catalyst (k_2SO_4, 80 g; $CuSO_4$.5 H_2O 20 g; SeO_2, 0.3 g) as reported by Yemm and Folkes (1953). This was followed by the addition of 1 ml water and 2 ml concentrated ammonia-free H_2SO_4. The contents of the flask were heated in a micro-heater for 8 h. The digest was quantitatively transferred into the Markham micro–Kjeldahl with the least amount of ammonia-free distilled water and then 15 ml of 40% NaOH solution was added. A strong current of steam was then passed and the ammonia was distilled into a measured volume of 2% boric acid. The ammonia was then titrated against exactly N/70 HC1 using bromocresol green/methyl red indicator till a faint red end point was obtained. After correction for the reagent blanks, the titration figures were converted into mg nitrogen using the following formula: 1 ml N/70 HCI = 0.2 mg N.

Estimation of total nitrogen: Total-N was determined by the conventional micro–Kjeldahl method. A sample of 0.05 g of the dry powdered plant tissue was weighed into a digestion flask. Sulphate mixture followed by 1 ml ammonia-free water and 3 ml of ammonia-free H_2SO_4 were added. The sample was then incinerated, ammonia distilled off and nitrogen determined as mentioned before. The protein-N content was calculated from the difference between total-N and total soluble-N.

Assay of enzymes activities

***Enzyme extraction*:** MuKherjee and Choudhuri (1983) described sample preparation. A fresh leaf sample (250 mg) was 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) at pH 6.8. The homogenates were centrifuged at 20000 x *g* for 20 min. The supernatant was made up to a known volume with the same buffer and used as "enzyme preparation" for the assay of different enzymes activities.

Super oxide dismutase (SOD, EC 1.12.1.1) assay: SOD activity was measured according to the method of Dhindsa et al. (1981). 3 ml of the 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 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 Spekol Spectrocolourimeter VEB Carl Zeiss.

One unit of SOD activity was defined as the amount of the enzyme that caused 50% inhibition of NBT to blue formazan.

Peroxidase (POD, EC 1.11.1.7) assay: POD activity was assayed using a solution containing 5.8 ml of 50 mM phosphate buffer at 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 spectrophotometrically by spekol spectrocolourimeter VEB Carl Zeiss within 60 s at 470 nm and 25°C (Bergmeyer, 1974). 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°C (Kong et al., 1999). The blank sample was made by using buffer instead of enzyme extract. The enzyme activities were assayed.

Ascorbate peroxidase (APX): Apx assay was performed using the method of Koricheva et al. (1997) with few modifications. 10 ml of the solution contained 5.5 ml of 50 Mm phosphate buffer at pH 7.0, 0.5 ml of the enzyme extract, 1.0 ml and 20 mM L-ascorbic acid. The decrease rate in absorbance as ascorbate oxidased was monitored at 290 nm with a UV-Vis spectrophotometer(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.

Catalase (CAT, EC 1.11.1.6) assay: CAT activity was assayed according to the method of Chen et al. (2000). 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 s with a Spekol Spectrocolourimeter VEB Carl Zeiss 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 s at 25°C (Kong et al., 1999).

In case of enzyme assay, volume at zero time was taken as blank and the activity of the enzyme/g fresh weight/hour was expressed as (Δ x Tv x 60 min) / t x v x f. wt where, Δ is the absorbance of the sample after incubation minus the absorbance at zero time, TV is the total volume of filtrate, t is the time (minutes) of incubation with substrate and V is the total volume of filtrate taken for incubation and f. wt. is the fresh weight used (Fick and Qualset 1975).

Determination of mineral concentrations

The dried plants powder was digested in a mixture of concentrated nitric acid, sulphuric acid and perchloric acid at the ratios 10: 1: 4, respectively, made to a constant volume with distilled water according to the method of Chapman and Pratt (1978) with certain modification. A 0.5 g ground powder of plant material (oven dried at 80°C) was placed in a 100 ml digestion flask which has been previously washed with acid and distilled water; 10 ml of the previous acids mixture were added. The samples were digested on electric heater until dense white fumes appeared and finally the solution become clear. The samples were left to cool and diluted with distilled water and quantitatively transferred into a 100 ml volumetric flask. The volume was made up to a known volume with distilled water. Filtration was carried out using filter paper whatman No. 42 and the filtrate was kept in tightly screwed brown bottles. The acid digest of the plant matter was analyzed for determination of potassium (K), calcium (Ca), magnesium (Mg) and phosphorus (P) according to the following methods:

Phosphorus: Phosphorus content in the digested samples was determined calorimetrically by ascorbic acid method described by Murphy and Riley (1952). Results were expressed as mg/g dry weight of flag leaf. An aliquot, 125 ml of 5 N sulphuric acid was mixed with 37.5 ml of ammonium molybdate (4%). An aliquot, 25 ml of ascorbic acid solution and 12.5 ml of potassium antimonyl tartarate solution were added. This reagent was freshly prepared. An aliquot (1 ml) of the digested solution was transferred into a 50 ml volumetric flask, mixed with 20 ml reagent, and then the volume was diluted to the mark with distilled water. The optical density of the solution was measured spectrophotometrically at 620 nm. Concentration of phosphorus was obtained from standard curve for different concentration of standard potassium dihyhydrogen phosphate solution, and then calculated as mg/g dry weight.

Potassium: Potassium content was determined using flam photometer model (JENWAY Pf P7). The results were expressed as mg/g dry weight.

Table 1. Growth morphology (shoot height, flag leaf area, fresh weight and dry weight) of potted wheat plants grown under drought stress on the heading stage and actions of grain presoak using salicylic acid (SA) and/or foliar application using thiourea (Th) or their interactions (SA+Th).

Treatment	Shoot height (cm)	Shoot Leaf area (cm^2)	Shoot fwt ($mg.g^{-1}$)	Shoot dwt (g)	Root length (cm)	Root fwt ($mg.g^{-1}$)	Root dwt ($mg.g^{-1}$)
Normal condition							
Control	61.25±2.45	16.52±2.30	10.70±1.20	2.61±0.60	16.38±9.0	0.68±0.18	0.194±0.16
Th_1	69.5±3.76	17.18±2.31	12.55±0.77	3.14±0.51	17.20±9.27	0.79±0.21	0.233±0.02
Th_2	65.5±5.35	17.93±4.77	15.20±0.51	4.15±0.64	20.25±10.45	0.80±0.25	0.313±0.13
SA	70±4.54	18.09±3.73	15.95±1.27	5.07±0.16	20.75±11.18	0.90±0.96	0.33±0.12
$SATh_1$	73±2.64	24.18±4.91	22.76±0.90	5.40±0.90	24.50±12.24	1.09±0.50	0.478±0.04
$SATh_2$	71±4.0	20.43±2.76	18.67±2.01	5.23±0.43	22.75±12.22	0.96±0.18	0.374±0.05
L.S.D at 0.05	1.46	1.09	1.2	0.64	0.98	0.35	0.19
Drought condition							
Control	58.75±4.27	10.40±1.87	9.50±1.47	2.02±0.72	11.55±8.43	0.50±0.17	0.19±0.06
Th_1	59.38±3.56	12.27±2.97	12.16±0.38	2.71±0.54	12.90±8.60	0.56±0.13	0.195±0.08
Th_2	63.75±5.40	13.04±5.0	14.96±1.95	3.6±0.78	15.19±8.87	0.62±0.17	0.249±0.16
SA	65.5±3.77	16.08±2.07	16.44±6.84	4.12±0.31	15.56±9.9	0.72±0.03	0.297±0.03
$SATh_1$	68.67±3.56	18.98±1.04	20.26±3.08	4.66±1.28	18.38±8.56	0.88±0.13	0.421±0.03
$SATh_2$	66.75±1.50	17.35±4.84	17.92±6.84	4.34±0.45	17.06±8.2	0.73±0.19	0.314±0.02
L.S.D at 0.05	2.07	1.54	1.69	0.89	1.30	0.50	0.26

Values are expressed as means±SD.

Magnesium and calcium: Calcium and magnesium contents were determined by using Atomic Absorption Spectrophotometer; Perkin Elemer model 1100. The results were expressed as mg/g dry weight.

RESULTS

Morphological characters of wheat plants grown in pots under drought stress were determined on the heading stage in 60 day old plants. It was clearly shown that all morphological aspects were regulated in response to drought stress and values above the control values in pretreated wheat was obtained. For example, salicylic acid/Thiourea ($SA+Th_1$) pretreatments of control plants resulted in significatly high values for shoot height (cm), flag leaf area (cm^2), shoot fresh weight/mg dry weight (dwt), shoot dry weight/mg dwt, root length (cm), root fresh weight/mg dwt and dry weight/mg dwt as follows: 73, 24.18, 22.76, 5.4, 24.5, 1.1 and 0.478, respectively (Table 1). On the other hand, the morphological characters were down regulated in $SA+Th_1$ drought-stressed wheat as follows: 68.67, 18.98, 20.26, 4.66, 18.38, 0.88 and 0.421, respectively. Morphological characters of pretreated wheat with the other treatments (SA, Th_1 and Th_2) had surpassed that of the control but with values less than those pretreated with $SA+Th_1$ as indicated in Table 1.

Yield and yield components were analysed for their drought survival within preprotection using different treatments. All data collected were up-regulated in the pretreated stressed as well as unstressed wheat. The highest values of spike length, spike number, spike weight per plant, grain number per plant, grain weight per plant, weight of 100 grains, biological yield, straw, grain yield, crop index and harvest index in $SA+Th_1$ pretreated wheat are listed in normal and stressed-wheat (Figure 1). Generally, values of yield components in pretreated wheat using SA, Th_1 and Th_2 and $SA+Th_2$ were relatively lower than those in $SA+Th_1$pretreated-wheat.

Hormonal changes were determined using HPLC technique. Values of gibberellins (GA3), Indole-3-Acetic acid (IAA), abscisic acid (ABA), zeatin (Z) and zeatin ribiside (ZR) in control and stressed wheat were as follows: 47.77, 37.6, 3.02, 2.08, 28.14, 36.35, 0.037, 0.029, 0.032 and 0.015 µg/100 g fwt, respectively. These values were increased in pretreated wheat with $SA+Th_1$ and the values in control and stressed wheat were: 110.66, 98.44, 5.04, 3.81, 4.3, 13.96, 0.096, 0.059, 0.076 and 0.04 µg/100 g fwt for GA3, IAA, ABA, Z and ZR, respectively (Table 2). All treatments had registered higher values above the control and stressed wheat values in untreated wheat.

Pigment content on the heading stage was determined in flag leaf of control and drought-stressed wheat. Chl*a*, Chl*b* and carotenoids values in control were 1.25, 0.49 and 0.58 mg/g fwt, respectively, whereas 1.07, 0.40 and 0.54 mg/g fwt were the same values reported in drought-stressed plants (Table 3). All pretreatments resulted in an

abrupt increase in the pigment content particularly when SA+Th_1 was used. The values of pretreated wheat with SA+Th_1 were: 1.55, 0.65 and 0.82 in control plants and 1.32, 0.62 and 0.68 mg/g fwt in drought-stressed wheat, respectively.

Carbohydrate content

The level of carbohydrate modulation was similar to the previously described metabolic components. Compared to other pretreatments (Table 3), levels of total soluble sugars (TSS), sucrose, starch and total carbohydrate were highly accumulated in SA+Th_1 pretreated control plants (14.35, 9.9, 141.9 and 166.2 mg/g dwt, respectively) and in stressed wheat (16.2, 11.7, 134.1, 162.0 mg/g dwt, respectively).

Lipid peroxidation, H_2O_2, and antioxidant enzymes

The level of lipid peroxidation and H_2O_2 decreased in wheat plants with different pretreatments particularly SA+Th_1 as the lowest values were recorded in the control wheat (10.4 and 9.7), respectively and 15.6 and 11.54 was recorded in drought-stressed wheat, respectively (Table 3).

Amino N, soluble-N, protein and Total N

The nitrogenous constituents in wheat plant grown under drought or normal conditions were altered with treatments. The increase in nitrogenous fractions were significantly increased with SA and/or Th treatments particularly SA+Th_1. The highest values of nitrogenous constituents were acheived in the control as follows: 3.55, 13.8, 44.6 and 58.4 mg/g for amino-N, soluble-N, protein and total N, respectively. The nitrogenous constituents in SA+Th_1-drought stressed wheat following the same order were as follow: 2.9, 8.6, 23.5 and 32.0 mg/g, respectively (Table 4).

Antioxidant enzymes

The level of antioxidant enzymes was checked in wheat plants grown under normal and drought conditions. Data showed a sharp decrease in both levels of guaiacol peroxidase and ascorbate peroxidase antioxidant enzymes (POD and APX) in the pretreated wheat compared to untreated control and drought stressed-wheat (Table 4). The antioxidant enzyme; SOD was increased successively in value in pretreated control and drought stressed-wheat. The highest levels of SOD and CAT were found in SA+Th1 pretreated control wheat: 68.4 and 158.1 mg/g, respectively, and in drought stressed-wheat the values were 54.4 and 89.1, respectively (Table 4).

Mineral content

Mineral composition and mineral content were estimated in wheat plants pretreated with SA and/or Th before subjected to drought stress. Levels of P, K, Ca and Mg increased generally with the pretreatments and their values in control wheat were as follow: 8.3, 22.6, 8.0 and 1.8 mg/g dwt, respectively. On the other hand, among the rest of the pretreatment, drought stressed-wheat pretreated with SA+Th1 acheived the highest mineral up-regulation as follows: 6.9, 18.5, 6.9 and 1.7 mg/g dwt, respectively (Table 5).

DISCUSSION

In the current approach, we examined the effect of new applications applied to wheat grown with water stress to ascertain whether pre-soaking grains in low dose of SA (~1 mmol) prior culture and/or Th spraying of leaves before anthesis stage would alleviate the drought symptoms in wheat plants. Our results are in agreement with recent studies which reported that spraying leaves with hydrogen peroxide decreased the physiological drought symptoms through series of molecular and biochemical pathways, which lead to biosynthesis of oligosaccharides known to assist plants tolerating drought stress (Yushi et al., 2011). Drought is an important stress factor targeting plant growth through its serious impact on cell enlargments, growth expansion and elongation (Shao et al., 2008). Here, all data showed drought deleterious effects on water stressed wheat. They also proved that different treatments resulted in significantly alleviated drought symptoms, which appeared as up regulation of general morphological characteristics particularly the synergestic effect of SA and Th_1 on plant growth (Table 1). The reduction in wheat height was encountered by foliar application using different treatments. It is well known that a decline in cell enlargement and leaf senescence is pronounced in plants under water stress (Bhatt and Srinivasa Rao, 2005). Therefore, SA and Th treatments likely mitigate these symptoms from plant's cell and tissues. Flag leaf effects were associated with the reduced cell expansion which in turn resulted from a declined mitotic division and low turgor pressure (Shao et al., 2008). In contrast with previous studies (Sacks et al., 1997; Wullschleger et al., 2005), the root growth in wheat subjected to drought in this study was significantly reduced although the root dry weight was not decreased.
Effect of drought on yield and yield components was studied as the increase in both grain number and individual grain weight are of key importance in yield production (Soriano et al., 2002). It was speculated that drought influenced stem elongation and hence affected spike characters per unit area and grain yield (Day and

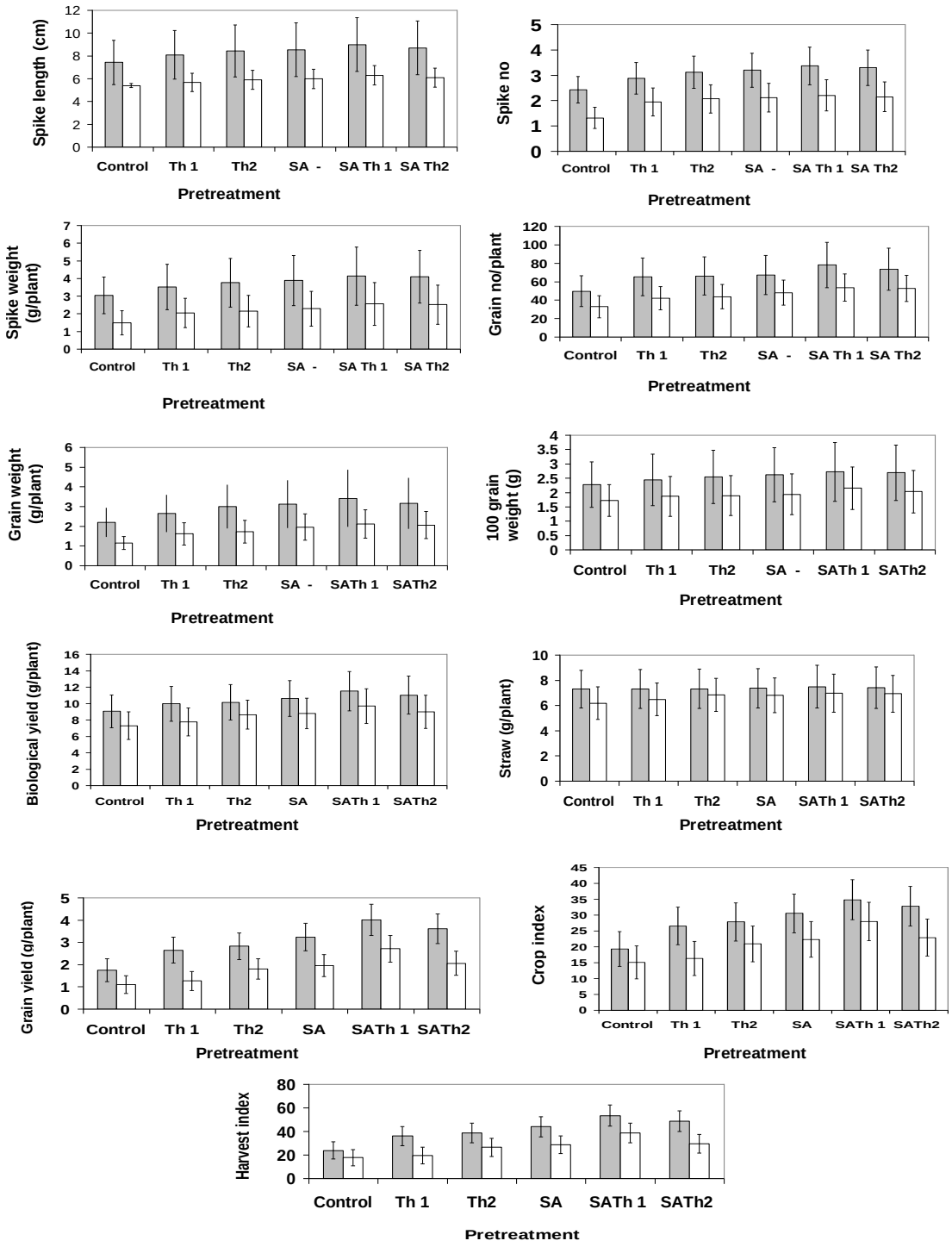

Figure 1. Yield components of potted wheat plants grown under drought stress and actions of grain presoaked using salicylic acid (SA) and/or foliar application using thiourea (Th) or their interactions (SA+Th).

Intalap, 1970). Furthermore, drought between tillering and flowering was reported to cause a reduction in the following components: grain protein yield, number of spike/m^2, number of grain/spike, and number of grain/m^2 (Entz and Fowler, 1990). Other researchers showed a significant fertility drop in spikes (40 to 60%) upon wheat plant's exposure to drought (Giunta et al., 1993). In addition, the present results show significant decrease in crop yield under the moderate drought stress (Figure 1). Treatments with SA and/or Th highly alleviated the

Table 2. Hormonal changes (µg/100 g fwt)of flag leaf of potted wheat plants grown under drought stress on the heading stage and actions of grain presoaked using salicylic acid (SA) and/or foliar application using thiourea (Th) or their interactions (SA+Th).

Treatment	GA3 Normal	GA3 Drought	IAA Normal	IAA Drought	ABA Normal	ABA Drought	Z Normal	Z Drought	ZR Normal	ZR Drought
Control	47.77±3.2	37.57±6.1	3.02±0.03	2.08±0.2	28.14±2.1	36.35±2.6	0.037±0.001	0.029±0.01	0.032±0.01	0.015
Th_1	51.50±6.4	46.0±7.8	3.68±0.1	2.80±0.1	19.42±2.5	29.95±2.6	0.044±0.01	0.043±0.01	0.038±0.02	0.017
Th_2	71.90±5.7	66.79±12.7	3.81±0.5	2.86±0.5	17.49±2.5	27.66±2.8	0.063±0.01	0.046±0.02	0.047±0.01	0.019
SA	80.0±7.4	76.00±41.7	3.95±0.1	2.90±0.1	10.95±1.6	22.74±1.4	0.068±0.01	0.052±0.01	0.058±0.01	0.029
$SATh_1$	110.6±10.6	98.44±11.8	5.04±0.2	3.81±0.2	4.30±0.8	13.94±1.4	0.096±0.02	0.059±0.01	0.076±0.02	0.04
$SATh_2$	95.90±12.4	95.00±61.3	4.72±0.1	3.00±0.2	9.05±1.5	18.12±2.1	0.076±0.01	0.054±0.02	0.071±0.01	0.032

Values are expressed as means±SD. Z, Cytokinins fraction zeatin; ZR, cytokinins fraction zeatin riboside.

Table 3. Biochemical analyses of flag leaf of potted wheat plants grown under drought stress on the heading stage and actions of grain presoak using salicylic acid (SA) and/or foliar application using thiourea (Th) or their interactions (SA+Th) as indicated in the table.

Treatment	Chl *a* (µg)	Chl *b* (µg)	Caroteniods (µg)	T.S.S (mg/g^{-1} dwt)	Sucrose (mg/g^{-1} dwt)	Starch (mg/g^{-1} dwt)	T.C (mg/g^{-1} dwt)	MDA ($nmol\ g^{-1}$ fwt)	H_2O_2 ($nmol\ g^{-1}$ fwt)
Normal condition									
Th_1	1.25±0.1	0.49±0.01	0.58±0.09	9.80±0.1	6.50±1.0	110.0±5.2	126.30±9.0	16.16±1.1	14.88±1.1
Th_2	1.28±0.3	0.54±0.02	0.6±0.05	10.30±1.0	7.25±1.0	112.50±6.1	130.10±13.0	14.08±2.1	14.36±2.1
SA	1.31±0.2	0.56±0.01	0.64±0.02	11.40±1.3	7.40±0.5	116.70±4.6	135.50±11.0	14.08±2.1	12.98±1.1
$SATh_1$	1.32±0.02	0.58±0.02	0.68±0.01	12.90±1.4	8.00±1.0	119.00±2.6	139.90±12.0	13.44±1.5	11.30±1.2
SATh2	1.55±0.1	0.65±0.02	0.82±0.02	14.35±2.1	9.90±2.0	141.90±6.0	166.20±14.0	10.40±2.1	9.70±1.8
$SATh_2$	1.5±0.3	0.61±0.01	0.79±0.01	13.65±1.7	8.40±0.6	139.10±8.0	161.20±10.4	12.48±1.2	10.34±2.1
L.S.D at 0.05	0.07	0.04	0.06	0.95	0.49	2.33	5.01	0.86	0.69
Drought condtion									
Control	1.07±0.02	0.40±0.01	0.54±0.01	12.80±1.0	7.85±1.3	82.50±5.3	103.20±12.3	17.84±2.5	17.89±1.3
Th_1	1.14±0.01	0.50±0.02	0.56±0.02	13.10±1.5	8.55±1.1	92.80±3.9	114.50±11.8	17.36±1.7	16.07±1.2
Th_2	1.16±0.04	0.53±0.01	0.6±0.01	13.30±2.1	9.05±1.1	111.30±1.7	133.90±16.2	16.48±0.9	16.04±1.0
SA-	1.18±0.01	0.56±0.01	0.62±0.02	13.50±1.6	9.30±1.2	122.10±3.6	144.30±18.2	15.84±1.5	15.50±1.3
$SATh_1$	1.32±0.04	0.62±0.01	0.68±0.01	16.20±1.5	11.7±1.4	134.10±2.5	162.00±14.8	15.60±2.1	11.54±1.1
$SATh_2$	1.29±0.01	0.58±0.01	0.64±0.01	15.80±2.1	11.0±1.5	126.3.7±4.5	152.80±16.2	15.68±2.1	14.71±1.4
L.S.D at 0.05	0.10	0.06	0.08	1.33	0.69	3.31	7.09	1.22	0.97

Values are expressed as means±SD. T.S.S, total soluble sugars; T.C, total carbohydrates; MDA, lipid peroxidation.

Table 4. Nitrogenous constituents and antioxidant enzymes of flag leaf of potted wheat plants grown under drought stress in the heading stage and actions of grain presoaked using salicylic acid (SA) and/or foliar application using thiourea (Th) or their interactions (SA+Th).

Treatment	Amino N	Soluble N	Protein N	Total N	SOD (unit/g)	POD (unit/g)	CAT (unit/g)	APX (unit/g)
Normal condition								
Control	2.69±0.01	8.4±0.01	27.6±1.02	36±4.9	40.3±2.4	431.4±20.2	73.5±2.4	142.8±21.3
Th_1	3.09±0.02	8.7±0.01	27.7±2.3	36.4±2.8	44.2±7.4	364.8±12.5	77.1±1.5	92.4±21.3
Th_2	3.34±0.01	9.9±0.01	32.5±2.1	42.4±3.5	48.4±2.5	251.4±21.5	91.2±2.2	81.6±21.7
SA	3.42±0.01	11.4±0.01	38.2±2.2	49.6±5.7	49.6±5.6	248.4±17.8	97.2±3.2	76.2±14.7
$SATh_1$	3.55±0.01	13.8±0.02	44.6±2.1	58.4±4.7	45.4±5.3	166.0±19.4	158.1±2.5	45.0±21.5
$SATh_2$	3.44±0.01	12.9±0.02	41.5±2.4	54.4±4.6	52.4±6.4	202.9±21.6	111.9±3.2	61.8±21.6
L.S.D at 0.05	0.15	0.29	0.52	0.66	2.32	2.15	0.67	1.04
Drought condition								
Control	2.12±0.06	6.1±1.5	19.5±2.1	25.6±2.5	45.0±3.6	581.4±21.5	58.8±3.6	160.6±21.5
Th_1	2.29±0.02	6.6±1.0	20.6±2.6	27.2±2.5	53.1±3.7	492.6±22.5	62.7±3.7	130.0±22.6
Th_2	2.69±0.01	7.1±1.3	21.7±1.5	28.8±2.5	54.6±7.3	473.6±21.4	63.6±2.4	91.2±22.7
SA	2.74±0.01	7.5±1.2	22.5±1.4	30.0±3.2	61.2±5.6	409.2±23.5	67.8±2.8	86.4±21.4
$SATh_1$	2.90±0.03	8.6±1.9	23.5±1.7	32.0±2.1	68.4±3.5	263.2±11.56	89.1±3.7	58.2±12.0
$SATh_2$	2.84±0.01	7.7±1.2	23.4±2.4	31.2±2.4	66.6±2.7	382.8±1.56	84.6±6.3	66.6±11.4
L.S.D at 0.05	0.21	0.41	0.73	0.94	1.64	3.04	0.94	1.47

Values are expressed as means±SD.

Table 5. Nutritional value [phosphorus, potassium, calcium and magnesium (mg/g dwt)] of flag leaf of potted wheat plants grown under drought stress in the heading stage and actions of grain presoaked using salicylic acid (SA) and/or foliar application using thiourea (Th) or their interactions (SA+Th).

Treatment	P	K	Ca	Mg
Normal condition				
Control	3.3±0.38	18.5±0.04	5.3±0.07	1.3±0.02
Th_1	4.0±0.22	18.9±0.03	5.7±0.03	1.3±0.02
Th_2	5.4±0.43	19.1±0.01	5.8±0.01	1.6±0.02
SA	5.5±0.14	19.2±0.05	6.9±0.05	1.7±0.05
$SATh_1$	8.3±0.43	22.6±0.03	8.0±0.01	1.8±0.02
$SATh_2$	7.3±0.51	20.5±0.02	7.6±0.04	1.7±0.04
Drought condition				
Control	2.9±0.01	12.5±0.02	4.8±0.01	1.2±0.06
Th_1	2.8±0.04	13.9±0.09	5.3±0.02	1.4±0.02
Th_2	3.7±0.07	14.2±0.06	5.5±0.03	1.4±0.03
SA	4.3±0.07	16.3±0.03	5.8±0.04	1.5±0.06
$SATh_1$	6.9±0.05	18.5±0.04	6.9±0.03	1.7±0.01
SATh2	5.2±0.08	16.9±0.04	6.1±0.02	1.6±0.03

Values are expressed as means±SD.

drought stress paticularly the interaction SA+Th_1. Further investigations are required to underline the physiological and biochemical pathways changes in wheat yield upon pretreatments with these low substances. Hormonal changes in relation to drought stress was determined and are listed in Table 3. It was believed that endogenous plant hormone levels are useful in evaluating crop plant's resistance to drought stress (Simpson et al., 1979).

Simultaneous increase in two promoter hormones (GA3 and Z) was obtained in pretreated wheat with SA and/or Th under normal or drought conditions. The level of increase was conspicuously above the control level. The other promoters (IAA and ZR) had increased non-significantly, particularly in wheat pretreated with SA+Th1 as well. These results could explain the yield propagation as was reasoned to the resumed stem elongation under drought conditions under the effect of GA3. On the other hand, the growth inhibitor hormone (ABA) was decreased in level in pretreated wheat with SA and/or Th (Table 2). ABA was increased with the decrease of water potential of plant cell (Kannangara et al., 1981). Compared to control wheat, values of ABA were decreased to half of its value, whereas the ABA content was decreased below the half in SA+Th1 pretreated wheat under drought and normal conditions, respectively. These results show the effective applications of SA and Th to alleviate drought stress (Table 2).

Pigment composition and pigment content showed that typical oxidative stress leads to a significant Chl *a, b* and carotenoids decrease. The symptoms often explained as either slow synthesis or fast breakdown (Smirnoff, 1993). Although in some cases, the pigment breakdown was not associated with a reduction in the photochemical efficiency (El-Sheery and Cao, 2008) but contributed to an increased ratio of carotenoids to total chlorophylls (Liu et al., 2011). Beta-carotene can protect chlorophyll and prevent photoinhibition. It is closely associated with photosynthetic apparatus and quenches singlet oxygen efficiently. It was also reported that 25% increase of carotenes was associated with plant's acclimation to low water potential (Stuhlfauth et al., 1990).

In agreement with previous findings, our data show that pigment content generally decreased in in control plants at the heading stage owing to drought stress. Pigment content increased and improved level when grains were presoaked in SA before the foliar application using Th (Table 3). In drought stressed-wheat, the ratios of chl_a/chl_b and car/chl $_{a+b}$ increased on heading (data not shown) due to decrease of peripheral light-harvesting complexes and carotenoids under drought conditions (El Sheery and Cao, 2008). These results were also in concomitant with previous studies that showed an increased chl_a/chl_b ratio within intensified drought on wheat on premature stages (Demmig-Adams and Adams, 1996).

Carbohydrates have role in regulating the osmotic pressure in plants and as important defense substances, alleviate protoplasm coagulation under various stress factors (Vassiliev and Vassiliev, 1936).

Other studies reported significant effects of drought on altering the composition and ratio between water soluble and ethanol soluble carbohydrates; a shift was obtained as a level of water soluble carbohydrates had decreased in the expense of increased ethanol soluble carbohydrates due to fructans (a water soluble carbohydrate) hydrolysis (Virgona and Barlow, 1991). Based on the previous, and to characterize drought stress alleviation more definitely, it seemed necessary to analyse the carbohydrates.

On the heading stage, the total soluble carbohydrates (TSS- Table 3) were significantly increased from control (9.8 mg/g) to the stressed wheat (12.8 mg/g). Alternatively, the starch was decreased from 110 to 82.5 mg/g. The NSS/TSS ratio had decreased in stressed compared to control wheat. Generally, it was suggested that a decreased carbohydrate content under stress conditions is referred to the reduction of pigment and photosynthesis due to low expression of enzymes involved in photosynthesis under drought conditions (Bayramov et al., 2010). Simultaneously, the yield losses likely occurred due to a reduction in starch production and accumulation (Fowler, 2003).

Antioxidant enzymes have important role as they are frequently involved with a wide range of abiotic stresses. Drought stress is known to generate reactive oxygen species (ROS) which are recognized at the cellular level and scavenged through increased antioxidative systems (Ramachandra et al., 2004). Excessive ROS production can cause oxidative stress, which damages plants by oxidizing photosynthetic pigments, membrane lipids, proteins and nucleic acids (Yordanov et al., 2000). Non-enzymatic and enzymatic antioxidant optimized the levels of active oxygen species. Non-enzymatic antioxidants including β-carotenes, ascorbic acid (AA), α-tocopherol, reduced glutathione (GSH) whereas the enzymatic antioxidants were included superoxide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (Xu et al., 2008).

The nitrogenous constituents often decreased in corn shoot under drought stress as previously reported (Pinheiro et al., 2004; Knipp and Honermeier, 2006). The present data revealed slight decrease in amino nitrogen and soluble nitrogen under drought stress; on the other hand a significant decrease in protein-N and total nitrogen was observed (Table 4). The nitrogenous constituents contributed in building blocks of plant and since the wheat is sensitive to drought damage which affected cell elongation (Heinigre, 2000) and cell division controlled with sets of enzymes and nitrogenous compartments, the decline in these constituents under drought stress was a key importance of the down regulation in wheat plants overall metabolic activity and growth. Different pretreatments (SA and/or Th_1) had alleviated the damage consequences with one way or another. Neverthelese, the direct/indirect reasons for this response was not yet explored, further investigations are still required to explain the mechanism of action of SA and/or Th_1 in wheat.

Mineral composition (P, K, Ca, Mg) and mineral-nutrient relations were affected by drought stress during the life time course of wheat plants through their effects on nutrient availability, transport and partitioning in plants.

Except for K level in drought stressed wheat, pretreatments with SA+Th_1 had increased minerals availability and hence minerals accumulation above the control level even under drought stress (Table 5).

The percent of minerals accumulation was known to decrease upon drought stress (Peuke et al., 2010). Phosphorus accumulation acheived 65% decrease in stressed wheat and increased again with the pretreatments to a level surpassed the control which suggested that the translocation of P to shoots which was severely restricted even under mild drought stress (Rasnick, 1970) could be enhanced in wheat upon SA and/or Th pretreatments. Potassium availability and accumulation to plants was reported to acheive 68% decrease with the decrease of water content in soil (Kuchenbuch et al., 1986). In addition wilting in plants was often referred to a possible K^+ deficiency (Beringer and Trolldenier, 1978). Unlike other minerals, calcium had decreased 'non-significantly' under drought conditions (Yuncai and Schmidhalter, 2005). The accumulation of calcium in flag leaf of potted wheat grown under water stress had reached 91% compared to control and this percent had even increased in particularly SA+Th_1 pretreated wheat (Table 5). Whether Mg^{2+} deficiency was relevant to water stress was not yet explored, although Hu and Schmidhalter (2001) reported that Mg^{2+} concentration in wheat leaves was reduced under conditions of water deficit. In agreement, our data reported a non-significant decrease in Mg^{2+} concentration with water stress and a general increase with the pretreatments. The reason(s) behind this physiological upregulation required additional molecular and biochemical studies.

Conclusion

The present investigation was dedicated to highlight results of grain presoak in SA and/or foliar application using Th in wheat grown in pots with water stress conditions. Variable analyses showed that pretreatments and drought stress interactions had improved morphological the biochemical traits in wheat. These results were perspective for the continuation in this field and reinforcing further studies.

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