

## **BIOCHEMICAL COUNTERACTION OF HEAT STRESS INJURY IN WHEAT UNDER NEW VALLEY DESERT CONDITIONS**

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**ABSTRACT:** *Two successive seasons were carried out during 2007/2008 and 2008/2009 at Agricultural Experimental Station of Desert Research Center (DRC) located in El-Kharga Oasis, El Wadi El Gadded Governorate to study the biochemical indicators which associated with counteraction of heat stress injury in wheat by using induced resistance .i.e. Calcium chloride, Phenylalanine, Methionine, Methanol, Ethephon and Salicylic acid as a foliar application as well as tap water as a control. The two genetic materials used were; Sids1 (heat tolerant) and Gemmeiza7 (heat sensitive). Results showed that all foliar application treatments appeared to be effective on all growth traits and grain yield. Sids1 exceeded Gemmeiza7 under most of foliar application treatments. Calcium treatment (0.8% CaCl<sub>2</sub>) was the best foliar application followed by Ethephon treatment (300 ppm) then Salicylic acid (25 ppm), which associated with heat tolerance in wheat cultivars. The other foliar application treatments coming in the second order. These results associated with increase in some biochemical constituents such as photosynthetic pigments, antioxidant enzymes (catalase and peroxidase) and/or decrease in other constituents such as malondialdehyde content which related to the counteraction of heat stress injury.*

*Analysis of zymogram gel superoxide dismutase (SOD) pattern revealed the presence of five bands for both wheat cultivars. Band number 5 is presented in all samples of both cultivars under all foliar application treatments included control. Bands (No. 3 and 4) were not presented in all the samples of Sids1 and Gemmeiza7 in case of control and all foliar application treatments (except ethephon treatment). Unique bands (No.3 and 4) were appeared in wheat plants treated with ethephon at rate 200 and 300 ppm. Also, there were detectable changes in band intensity for wheat cultivars grown under different foliar application treatments which associated with heat tolerance in plants. Also, data showed that 16 amino acids were detected including acyclic and cyclic amino acids. Acyclic amino acids contain: aliphatic unsubstituted amino acids (Glycine, Alanine, Valine, Leucine, and Isolucine) and aliphatic substituted: hydroxy (Serine,*

*Threonine), thio (Methionine), carboxy (Aspartic, Glutamic), diamino (Lysine) and guanidino (Arginine). Cyclic amino acids include: aromatic (Phenylalanine, Tyrosine), heterocyclic (Histidine) and imino acid (Proline). There was a marked increase in amino acids content in plants as a result to foliar application treatments and this is depending on the concerned amino acid, dose of foliar applications and wheat cultivars. Also, carboxy amino acids recorded the high amounts with all foliar application treatments and mostly higher than other amino acids possibly due to their being precursors for synthesis of most amino acids which associated with heat tolerance. The utilization from such field experiments:*

- *In study and evaluation of heat tolerance basics for wheat plants under El Wadi El Gedeed conditions which known as heat stress, with recommendation to use heat tolerance genotypes such as Sids1, which associated with biochemical constituents.*
- *The benefit from biochemical indicators which associated with heat tolerance to improve sensitive genotypes such as Gemmeiza7 by using induced resistance.*

**Key Words:** *Wheat, Heat stress, Biochemical counteraction, Antioxidant enzymes, Malondialdehyde, amino acids, Growth and Yield.*

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## **INTRODUCTION**

In Egypt, there is shortage in wheat production as it only covers about 64% of the local consumption. Therefore, the improvement of wheat productivity is being a native goal and a great attention should be paid to overcome or minimize the gap between production and consumption. This may occurred by expansion through reclaimed areas which represent the good hope of cultivated lands in increasing our agricultural production and subsequently in overcoming the deficiency in food requirements, as well as, increasing the vertical production through using chemical materials which are safe on human health and environment. Most of new reclaimed lands are subjected to heat and high solar radiation stress as in El Wadi El Gadedd (New Valley) region which considered as a good hope for agricultural expansion. It represents about 45 % of the total area of Egypt and has about 3.3 million Fad. Heat and solar radiation stress are the most serious factors limiting growth of several plants there.

Plants exposed to environmental stress factors, such as high light intensity and heat stress suffer from oxidative damage catalyzed by reactive oxygen species (ROS) e.g. superoxide radical  $O_2^-$ , hydrogen peroxide  $H_2O_2$  and hydroxyl radical  $OH^\cdot$ . ROS are known to be primarily responsible for impairment of cellular function and growth depression (Cakmak, 2007). Enhanced respiration rates relative to photosynthesis at high temperatures are more detrimental in  $C_3$  plants (wheat) than in  $C_4$  because the rates of

## **Biochemical counteraction of heat stress injury in wheat .....**

both dark respiration and photorespiration are increased in C<sub>3</sub> plants at higher temperatures (Taiz and Zeiger, 2002).

As a part of the photorespiratory pathway, H<sub>2</sub>O<sub>2</sub> is produced in the peroxisomes, where it can also be formed during the catabolism of lipids as a by-product of  $\beta$  oxidation of fatty acids (Wu *et al.*, 2007). Also, high temperature injury can result in considerable pre-harvest and post-harvest crop losses. One mechanism of injury involves the generation and reactions of ROS (Liu and Huang, 2000). Cellular increases in ROS can either act as secondary messenger that switch on cellular defense mechanisms, as in the hypersensitive response, or can cause cell dysfunction and act as drivers of cell death (Foyer and Noctor, 2005). In order to limit oxidative damage under stress condition plants have developed a series of detoxification systems that break down the highly toxic ROS (Larkindale and Huang, 2004).

Application of some chemical materials as foliar application is one of the most important way to reduce the adverse effect of heat stress on wheat plants. In this respect, Gong *et al.* (1998) reported that calcium may have a role in heat stress signaling. It has been shown that calcium signaling inhibitors and calmodulin inhibitors limited survival and increased electrolyte leakage from membranes after treatment. Also, application of salicylic acid (SA) has recently been reported to increase heat tolerance in plants, and associated with its protection against oxidative damages (Larkindale and Huang, 2004). Also, Hayat *et al.* (2010) found that SA is an endogenous plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development. According to Nonomura and Benson (1992)a,b who stated that application of methanol have been reported to increase yield in a number of C<sub>3</sub> but not C<sub>4</sub> crops. The Ethephon (2 chloroethyl phosphonic acid) is considered as a source of ethylene which is degraded when reaching the internal plant tissues releasing then ethylene, chlorate and phosphate ions. Ethylene is synthesized from methionine through a sequential action of the enzymes amino cyclopropane-1-carboxylic acid synthase and amino cyclopropane-1-carboxylic acid oxidase (Yueri *et al.*, 2002). Ethylene has been implicated in a number of stresses induced pathways (Foyer *et al.*, 1997).

The present study aims to study the biochemical indicators which associated with counteraction of heat stress injury in wheat plants by using induced resistance.

## **MATERIALS AND METHODS**

### **I. Field experiments**

Two field experiments were carried out during 2007/2008 and 2008/2009 seasons at Agricultural Experimental Station of Desert Research Center

(DRC) located in El Wadi El Gadded Governorate (El Kharga Farm) to study the biochemical counteraction of heat stress injury in two wheat cultivars.

### **Plant materials growth conditions**

The grains of two wheat cultivars; Sids1 and Gemmeiza7 were obtained from the Field Crop Institute, Agriculture Research Center. In each season, wheat grains were sown in first week of November. The experiments were designed in split plot design with tree replicates. The plot was 6 m<sup>2</sup>, each plot was fertilized with super phosphate at the rate of 200 Kg/fad. before planting, potassium sulphate at the rate of 100 K<sub>2</sub>O Kg/fad. and ammonium nitrate at rate of 180 Kg/fad. The fertilizers were added in two equal doses after 30 and 60 days from sowing Mechanical and chemical analysis of soil and irrigation water are presented in Table (1).

### **Treatments**

The field experiment included two main factors (thirty six treatments):

#### **1. Wheat cultivars:**

Two wheat cultivars Sids1 (heat tolerant) and Gemmeiza7 (heat sensitive)

#### **2. Foliar application treatments:**

Six chemical treatments each with three concentrations in addition to control as follows:

- Tap water (control)
- Calcium chloride (CaCl<sub>2</sub>) at 0.2, 0.4 and 0.8 %.
- Phenylalanine (PA) at 20, 40 and 60 ppm.
- Methionine (Met) at 20, 40 and 60 ppm.
- Methanol (MOH) at 10, 20 and 30 %.
- Ethephon (Eth) (2 dichloroethyl phosphonic acid) at 200, 300 and 400 ppm.
- Salicylic acid (SA) at 25, 50 and 100 ppm.

Each treatment was sprayed on plants at a rate of 400 liter/Fad. after 45 and 65 days from sowing. All treatments were applied in the morning.

### **Plant sampling**

Two plant samples were taken during the experiment from each treatment. The first one was taken 75 days from sowing to determine photosynthetic pigments, antioxidant enzymes, malondialdehyde and amino acids as well as growth traits (plant height, fresh and dry weights/plant). The second sample was taken after harvesting to determine the following traits; plant height (cm) and grain yield (ton/fad.).

## **II. Chemical analysis**

### **Moisture**

The moisture content of wheat's shoot was determined according to A.O.A.C. (1995).

## Biochemical counteraction of heat stress injury in wheat .....

### Photosynthetic pigments

Chlorophyll (Chl) a, b and carotenoids were extracted and estimated according to A.O.A.C. (1975) and calculated according to the formula of Wettstein (1957).

### Lipid peroxidation level

The level of lipid peroxidation in the plant tissue was quantified by determination of malondialdehyde (MDA), a breakdown product of lipid peroxidation according to Health and Packer (1968) and modified by Zaho *et al.* (1994).

**Table (1): Mechanical and chemical analysis of the experimental soil and chemical analysis of underground irrigation water at El-Wadi El-Gedeed.**

**a) Mechanical analysis of the experimental soil.**

Characters	Values
Total sand (%)	51.05
Clay (%)	30.94
Silt (%)	18.01
Texture class	Sandy clay loam

**b) Chemical analysis of the experimental soil.**

Characters	Values
pH	8.12
E.C. (mmhos/cm)	2.44
Soluble cations (meq/L)	
Ca <sup>++</sup>	7.08
Mg <sup>++</sup>	2.15
Na <sup>+</sup>	16.04
K <sup>+</sup>	0.88
Soluble anions (meq/L)	
CO <sub>3</sub> <sup>=</sup>	-----
HCO <sub>3</sub> <sup>-</sup>	5.59
Cl <sup>-</sup>	14.39
SO <sub>4</sub> <sup>=</sup>	6.17

**c) Chemical analysis of irrigation water.**

Characters	Values
pH	7.38
E.C. (mmhos/cm)	1.23
Soluble cations (meq/L)	
Ca <sup>++</sup>	1.70
Mg <sup>++</sup>	1.01
Na <sup>+</sup>	9.11
K <sup>+</sup>	0.51
Soluble anions (meq/L)	
CO <sub>3</sub> <sup>=</sup>	-----
HCO <sub>3</sub> <sup>-</sup>	3.12
Cl <sup>-</sup>	5.96
SO <sub>4</sub> <sup>=</sup>	3.25

### **Soluble protein**

Soluble protein contents of wheat shoots were determined according to Lowry's method (Lowry *et al.*, 1951).

### **Antioxidant enzymes**

#### **Catalase activity (E.C 1.11.1.1.6)**

The extraction was performed according to Maxwell and Bateman (1967) with some modifications. The catalase (CAT) activity was determined as the change in absorbance at 240 nm, at *Spectronic Genesis.5* as: ( $\Delta \text{Abs}_{240}$ ) /mg protein/1min.

#### **Peroxidase activity (E.C 1.11.1.7)**

Peroxidase (POX) was determined by using O-Dianisidine method according to Worthington Biochemical Corp (1972). The change in absorbance was recorded at 460 nm for 3 minutes by *Spectronic 601* spectrophotometer ( $\Delta \text{Abs}_{460}$ ) /mg protein / 3 min.

#### **Superoxide dismutase isozyme (EC.1.15.1.1)**

The (SODs) were extracted from plant samples and separated by native polyacrylamide gel electrophoresis (PAGE) according to Stegman *et al.* (1985). After electrophoresis, the isozyme of interest was identified by incubating the gel in an appropriate substrate solution such that a colored product was produced at the site of the enzyme Wilson and walker (2000). The staining ingredients were mixed and poured over gel according to Siciliano and Shaw (1976).

### **Identification and determination of protein amino acids**

The hydrolyzed protein amino acids were determined according to the method described by Pellet and Young (1980). Amino acids composition was determined by amino acid analyzer apparatus model "Eppendorf-Germany LC 3000"

### **III. Statistical analysis**

Data were analyzed statistically according to the procedure outlined by Snedecor and Cochran (1982). Combined analysis over growing seasons was done when the homogeneity test was insignificant according to Gomez and Gomez (1984). Duncan's multiple range test was used for the comparison between means (Duncan, 1955).

## **RESULTS AND DISCUSSION**

### **I. Growth traits**

Data in Table (2) clearly demonstrated that  $\text{CaCl}_2$  significantly increased all growth traits as compared with the control. But the maximum value of plant height was achieved at rate of 0.4 %. Also, the maximum value of fresh and dry weights was obtained by  $\text{CaCl}_2$  at rate 0.2 %. In this connection, Sids1 exceeded Gemmeiza7 in fresh and dry weights. Concerning the effect

### **Biochemical counteraction of heat stress injury in wheat .....**

of interaction between foliar application and wheat cultivars, data showed that the highest value of plant height was obtained by Sids1 after treatment with 0.4 %. Also, applied  $\text{CaCl}_2$  at rate 0.2% gave the maximum value of fresh weight and dry weight for Sids1 and Gemmeiza7, respectively. The enhancement in growth parameters of wheat plants after treatment with  $\text{CaCl}_2$  may be ascribed to: 1) calcium may be involved in plant tolerance to heat stress by regulated antioxidant metabolism or / and water relations (Jiang and Huang, 2001) 2) plays a major role in the initiation of many signal transduction processes in higher plant cells, including bud formation, polar growth, gas exchange regulation, secretion, movements and light and hormone regulated growth and development (Hepler and Wayne, 1985) 3) this nutrient actively influences one of the processes most vital to plant growth and nitrogen metabolism (López-Lefebre *et al.*, 2000) 4) increasing cell division (Tuteja and Mahajan, 2007) 5)  $\text{Ca}^{+2}$  accumulates as calcium pectate in the cell wall and binds the cells together, also  $\text{Ca}^{+2}$  required for pollen tube, growth and elongation (Sanders *et al.*, 2002) 6)  $\text{Ca}^{+2}$  required as a counter cation for inorganic and organic anions in the vacuole and as an intracellular messenger in the cytosol (Mahajan and Tuteja, 2005).

Foliar application of PA and Met significantly enhanced growth parameters in wheat plants. In this regard, the maximum value of plant height was obtained by applied PA and Met at rate of 40 ppm. Also, the same dose of PA gave the highest value of fresh weight. In this connection, there was significant effect between the two wheat cultivars in dry weight after treatment with PA. Regarding the effect of interaction, data showed that the highest values of plant height were obtained by Sids1 and Gemmeiza7 after treatment with 40 ppm Met and 40 ppm PA, respectively. Applied PA at 20 ppm recorded the highest value of fresh weight for Gemmeiza7 and dry weight for Sids1. But the highest values of fresh weight and dry weight were achieved by Gemmeiza7 at rate of 60 ppm Met. These results were compatible with those obtained by Abd El-Aziz *et al.* (2009). The stimulative effect of amino acids (PA and Met) on growth parameters may be attributed to: 1) amino acids produced a high quality of inflorescences (Abd El-Aziz and Balbaa, 2007) 2) the role of amino acids in increase the content or activity levels of endogenous promoters particularly gibberellins and IAA (Wilkins, 1989).

Data in Table (2) showed that MOH treatments appeared to be effective on growth parameters. In this respect, the maximum values of plant height, fresh weight and dry weight were obtained by applied MOH at rate of 20 %. Concerning the effect of interaction, data elucidated that the highest values of plant height and dry weight were obtained by Gemmeiza7 at rate of 30 % MOH. Also, the same cultivar recorded maximum value of fresh weight after treatment with 20% MOH. The effect of MOH on growth parameters is well documented by Madhaiyan *et al.* (2006).

**Table (2): Growth traits as affected by foliar application, wheat cultivars and their interaction at 75 days from sowing.**

Foliar application	Plant height (cm)			Fresh weight (gm)			Dry weight (gm)		
	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean
<b>Calcium chloride (%)</b>									
Control	84.33 d	78.73 e	81.53 C	131.40 c	110.30d	120.90 C	28.92 c	22.56 d	25.74 B
0.2	104.30 b	96.82 c	100.60B	180.10 a	174.80 a	177.50 A	32.41 b	36.31 a	34.36 A
0.4	110.60 a	102.10 b	106.30A	140.40 b	128.10 c	134.20 B	21.43 d	22.16 d	21.79 C
0.8	102.10 b	102.10 b	102.10B	134.10c	142.20 b	138.10 B	24.47 d	31.00 bc	27.73 B
Mean	100.30A	94.96 B		146.50A	138.90B		26.80 A	28.01 A	
<b>Phenylalanine (ppm)</b>									
Control	84.33 d	78.73 e	81.53 D	131.40bc	110.30d	120.9 C	28.92 c	22.56 e	25.74BC
20	93.32 b	91.07 bc	92.19 B	121.0cd	153.9 0a	137.5 B	41.94 a	34.43 b	38.18A
40	91.87 bc	103.10 a	97.51 A	143.6ab	151.10a	147.4 A	28.05 c	27.65 cd	27.85 B
60	88.10 cd	90.50 bc	89.30 C	115.00d	115.00d	115.0 C	23.21 e	24.46 de	23.83 C
Mean	89.40 A	90.86 A		127.70A	132.60A		30.53 A	27.27 B	
<b>Methionine (ppm)</b>									
Control	84.33 c	78.73 d	81.53 C	131.40bc	110.30cd	120.90AB	28.92 b	22.56 cd	25.74 A
20	88.94 b	88.84 b	88.89 B	123.4b-d	103.70 d	113.50 B	21.27 d	25.23 c	23.25 B
40	93.27 a	91.97 ab	92.62 A	128.10bc	135.70ab	131.90 A	20.73 d	20.97 d	20.85 C
60	90.74 ab	91.13 ab	90.93AB	110.7cd	154.70a	132.70 A	22.02 d	33.14 a	27.58 A
Mean	89.32 A	87.67 A		123.40A	126.10A		23.23 A	25.47 A	
<b>Methanol (%)</b>									
Control	84.33 d	78.73 e	81.53 C	131.40 b	110.30 d	120.90 C	28.92 a	22.56 bc	25.74AB
10	102.7ab	102.30ab	102.5 A	139.70 a	114.80 d	127.30 B	24.43 b	24.60 b	24.51 B
20	101.4 b	103.60ab	102.5 A	140.40 a	142.30 a	141.30 A	22.34 bc	30.89 a	26.61 A
30	93.84 c	105.30 a	99.58 B	124.20 c	114.30 d	119.30 C	20.40 c	30.91 a	25.65AB
Mean	95.57 A	97.47 A		133.90A	120.90A		24.02 A	27.24 A	
<b>Ethephon (ppm)</b>									
Control	84.33 d	78.73 e	81.53 C	131.40ab	110.30cd	120.90AB	28.92 b	22.56 cd	25.74 B
200	91.51 b	98.01 a	94.76 A	104.00cd	144.10 a	124.00AB	18.21 e	20.67 d	19.44 C
300	86.49 cd	85.41 cd	85.95 B	98.65 d	130.60ab	114.70 B	17.50 e	23.30 c	20.40 C
400	80.50 e	88.32 c	84.41 B	118.60bc	141.20a	129.90 A	29.15 b	34.91 a	32.03 A
Mean	85.71 A	87.62 A		113.20 B	131.60 A		23.44 A	25.36 A	
<b>Salicylic acid (ppm)</b>									
Control	84.33 d	78.73 e	81.53 C	131.40 c	110.30 e	120.90 C	28.92 c	22.56 e	25.74 C
25	88.43 c	90.33 a-c	89.38 B	131.60 c	169.00a	150.30 A	25.27 d	44.70 a	34.98 A
50	88.19 c	90.17 bc	89.18 B	125.20 d	143.80 b	134.40 B	25.23 d	34.82 b	30.02 B
100	92.48 ab	92.89 a	92.68 A	129.10cd	146.30 b	137.70 B	21.41 e	25.07 d	23.24 D
Mean	88.36 A	88.03 A		129.30 B	142.30 A		25.21 B	31.78 A	

Values followed by the same letter (s) are not significantly different at  $p < 0.05$ . \*Gem7=Gemmeiza7

From data listed, it could be noticed that plants applied with Eth showed promotive effects on growth parameters. The maximum value of plant height was achieved by the lowest rate of ethephon at 200ppm. However, the maximum values of fresh weight and dry weight was obtained by Eth at 400 ppm. In this concern, Gemmeiza7 recorded the higher value of fresh weight than Sids1. Concerning the effect of interaction, the highest values of plant height and fresh weight was obtained by Gemmeiza7 after treatment with 200 ppm Eth. Whereas, the highest value of dry weight was recorded by



## **Biochemical counteraction of heat stress injury in wheat .....**

Gemmeiza7 at rate of 400ppm Eth. These results were compatible with Hussein (2007) on barley. Also, Ethephon mode of action acts *via* liberation of ethylene, which is absorbed by the plant and interferes in the growth process (Thomson, 1993).

Also, SA significantly enhanced plant growth traits in wheat plants. The maximum value of plant height was obtained by applied SA at 100 ppm. Whereas, the maximum value of fresh and dry weights was achieved by SA at 25 ppm. In this connection, Gemmeiza7 exhibited the superior value at fresh weight and dry weight than other cultivar. Concerning the effect of interaction, data showed that the highest values of fresh weight and dry weight were obtained by Gemmeiza7 after the treatment with 25 ppm SA. But the highest value of plant height was obtained by Gemmeiza7 when SA applied at rate 100 ppm. The aforementioned results were in agreement with those which were obtained by Singh and Usha (2003) and El Shraiyy and Hegazi (2009). Also, the enhancement in growth traits after treatment with SA may be ascribed to: 1) accumulation of hormones in wheat seedlings (Shakirova *et al.*, 2003) 2) the positive role of SA in improving hormonal regulation and improvement of leaf turgidity by causing stomatal closure, decreasing the rate of transpiration, increasing relative water content (El Hakem, 2008) 3) regulating some chemical contents such as total soluble proteins, total phenols, proline, total soluble carbohydrates and sugars (El Shraiyy and Hegazi, 2009) 4) generate a wide range of metabolic and physiological responses (Hayat *et al.*, 2010) 5) plays an important role in regulating a member of plant physiological processes (Arfan *et al.*, 2007).

## **II. Chemical determinations**

### **1. Photosynthetic pigments**

Photosynthetic pigments in leaves are recorded in Table (3). It is clear that, CaCl<sub>2</sub> treatments significantly increased photosynthetic pigments in leaves as compared with the control. The maximum value was achieved by CaCl<sub>2</sub> at rate 0.8 % (except Chl a at rate 0.4%). There was a significant effect in some photosynthetic characters which declared that Sids1 explored higher values of Chl b, (a+b) and total pigments than Gemmeiza7. As to the effect of interaction, data showed that the highest values of Chl a, carotenoids and total pigments were obtained from Gemmeiza7 after treatment with CaCl<sub>2</sub> at rate 0.8%. While, Sids1 plants treated with 0.4% recorded the maximum values of Chl b and Chl (a+b). In this regard, the effect of CaCl<sub>2</sub> on increment of photosynthetic pigments content under heat stress is well documented by Fu and Huang (2003).

The enhancement in photosynthetic pigments in wheat leaves after treatment with CaCl<sub>2</sub> may be ascribed to: 1) the effect of such substance on increasing the biosynthesis of these pigments and the protection of the photosynthetic apparatus from damage by heat stress (Zhao and Tan, 2005) 2) some of NAD kinase, which associated with the chloroplast, was

dependent on calcium and light activated 3) NADP product served as the terminal electron acceptor for photosystem I (Jarrett *et al.*, 1982).

Results showed that PA and Met significantly enhanced the increment of photosynthetic pigments in wheat leaves. Under foliar application of PA, Gemmeiza7 had a higher Chl b, carotenoids, Chl (a+b) and total pigments than other cultivar. While, Sids1 exceeded Gemmeiza7 in Chl b after treatment with Met. In this accord, the interaction effect was also proposed that the highest values of Chl a, b, carotenoids, Chl (a+b) and total pigments were obtained by Gemmeiza7 when PA applied at rate 40 ppm. Also, the maximum values of Chl a, carotenoids, Chl (a+b) and total pigments were obtained by Gemmeiza7 after treatment with Met at rate 20ppm. The increment in photosynthetic pigments in wheat leaves after treatment with PA and Met may be due to the enhancement of pigment biosynthesis (Abd El-Aziz *et al.*, 2009). The positive effect of amino acids on enhancing photosynthetic pigments may be due to: 1) help to increase chlorophyll concentration in plants leading to higher degree of photosynthesis (Hahlbrock and Scheel, 1989) 2) Met serves as a methyl group donor in various plant tissues (Cleland, 1963) 3) the succinyl COA (Kerb's cycle intermediate) and the amino acid glycine, initiate the biosynthetic pathway leading to chlorophyll formation (Abd El-Aziz *et al.*, 2009)

Data in Table (3) clearly showed that MOH significantly enhanced photosynthetic pigments. The maximum value was achieved by MOH at rate 10%. The higher Chl a, (a+b) and total pigments values were obtained by Gemmeiza7 and this revealed a significant effect achieved by this cultivar, but the other remaining photosynthetic pigments parameters had no significant effect between the two cultivars. The interaction effect can be deduced from tabulated data, the highest values of Chl a, (a+b) and total pigments were produced from Gemmeiza7 after treatment with 10% MOH. The interpretation of MOH role in enhancement the plants under stress conditions is that: 1) reduce photorespiration ( $C_3$  plants) and increasing efficiency of carbon utilization (Nonamura and Benson, 1992b) 2) their protective function and have a particular role in the protection of leaf cells from photooxidative damage (Neill and Gould, 2003) 3) affects the expression of hundreds of genes and that multiple detoxification and signaling pathways are activated may contributed to increase in photosynthetic pigments (Downie *et al.*, 2004).

Application of Eth decreased Chl a content. But plants treated with 400 ppm recorded the highest values of Chl b, (a+b) and total pigments. In this regard, there was a significant effect between the two cultivars exhibited by Gemmeiza7. As to the effect of interaction, data exhibited that the highest value of Chl a was achieved by Gemmeiza7 when Eth applied at rate 300ppm. Also, the same cultivar recorded the highest values of Chl (a+b), carotenoids and total pigments after treatment with 200ppm.

**Biochemical counteraction of heat stress injury in wheat .....**

**Table (3):**

**Table (3). Cont.**

## **Biochemical counteraction of heat stress injury in wheat .....**

It is observed that high doses from Eth exhibited the lower values of photosynthetic pigments, while the low doses lead to the opposite trend. The effect of Eth in decreasing pigments is well documented by Yonghua *et al.* (1995). On the other hand, Hussein (2007) showed that Eth resulted in an increment in Chl a, b and carotenoids contents in barley leaves. The enhancement of photosynthetic pigments in leaves of wheat plants after treatment with Eth may be ascribed to: 1) effect of Eth on stomata and mesophyll cells. 2) effect of Eth on photosynthetic rate, stomatal conductance, carbonic anhydrase activity and 1 amino cyclopropane 1 carboxylic acid synthase activity and ethylene production (Khan, 2004). 3) ethylene affected CO<sub>2</sub> assimilation and the plant responded depending on the tissue concentration (Mattoo and White, 1991).

It is evident from the data presented in Table (3) that SA at rate 100ppm gave the maximum value of photosynthetic pigments. There was a significant effect between the two cultivars but, Gemmeiza7 have a higher concentration of Chl a, b, (a+b) and total pigments than other cultivar. Regarding the effect of interaction, data showed that the highest values of Chl a, b, a+b, carotenoids and total pigments were obtained by Gemmeiza7 after treatment with 100ppm SA. The latter results are completely closer to Larque Saavedra (1978), Amin *et al.* (2008) and Khan *et al.* (2010). The accumulation of photosynthetic pigments as a result of SA is due to increase in photosynthetic efficiency as reflected by increasing in chlorophyll a, b and carotenoids contents in wheat plants (Amin *et al.*, 2008).

### **2. Lipid peroxidation content (malondialdehyde)**

From the data presented in Table (4) it's clear that CaCl<sub>2</sub> alleviated the MDA toxic product to plant formed under heat stress conditions, but the minimum value was obtained at rate 0.8%. In this connection, the lowest MDA content was produced from Sids1 as compared with the other cultivar. Concerning the effect of interaction, data showed that Sids1 recorded the lowest MDA value at rate 0.4%. The effect of CaCl<sub>2</sub> on alleviating MDA content is well documented by Fu and Huang (2003) and Soumen (2007). The role of calcium in controlling membrane structure and function may be ascribed to: 1) calcium by binding to phospholipids, stabilizes lipids bilayers and thus provides structural integrity to cellular membranes (Burstrom, 1968) 2) calcium is necessary to maintain the integrity and selective ion transport of the plasma membrane (Hanson, 1960) 3) calcium involved in oxidative signal transduction concomitant with the regulation of antioxidant enzymes under heat stress conditions (Coria *et al.*, 1998).

Data in Table (4) clarified that PA and Met significantly reduced the accumulation of MDA toxic product in wheat leaves (except 60 ppm Met). The lowest value was obtained after treatment with 60 ppm PA and 40 ppm Met. In this respect, Sids1 plants sprayed with PA and Met have lower content of MDA than other cultivar. As to the effect of interaction, data showed that Gemmeiza7 recorded the lowest level of MDA at 20ppm PA. Also, plants

applied with 40ppm Met gave the lowest value in leaves of Sids1. The effect of Met in alleviating the MDA toxic product in leaves may be due to ethylene which is synthesized from Met through a sequential action of the enzymes amino cyclopropane-1-carboxylic acid synthase and amino cyclopropane-1-carboxylic acid oxidase (Yueri *et al.*, 2002). In the same trend, Larkindale and Knight (2002) declared that plants have evolved mechanisms to cope with the problems caused by high temperatures. 1-aminocyclopropane-1-carboxylic acid (a precursor to ethylene) could protect plants against heat induced oxidative injury.

**Table (4): Antioxidant enzymes and lipid peroxidation in shoots as affected by foliar application, wheat cultivars and their interaction at 75 days from sowing.**

Foliar application	Catalase activity $\Delta_{240}$ /mg protein/ 1min			Peroxidase activity $\Delta_{460}$ /mg protein/ 3min			Malondialdehyde content ( $\eta$ mol / g fresh wt.)		
	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean
<b>Calcium chloride (%)</b>									
Control	0.029 e	0.103 e	0.066D	9.05 f	8.90 f	8.975 D	107.60 a	109.70a	108.60 A
0.2	0.571 c	0.052 e	0.311C	12.53 c	11.50 d	12.02 B	95.13 c	98.58 b	96.85 B
0.4	0.332 d	1.036 a	0.684A	19.41 a	18.15 b	18.78 A	40.63 f	99.34 b	69.98 C
0.8	0.803 b	0.132 e	0.468B	11.47 d	10.49 e	10.98 C	65.01 d	52.11 e	58.56 D
Mean	0.434 A	0.331 B		13.11 A	12.26 B		77.09 B	89.93 A	
<b>Phenylalanine (ppm)</b>									
Control	0.029 e	0.103 d e	0.066D	9.05 bc	8.90 b-d	8.97 B	107.60 a	109.70 a	108.60 A
20	0.283 c	0.580 b	0.431B	5.167 d	6.617 cd	5.89C	75.74 d	54.74 g	65.24 D
40	0.563 b	0.020 e	0.292C	10.90 b	10.25 bc	10.57 B	69.42 e	81.32 b	75.37 B
60	1.290 a	0.163 c d	0.726A	31.96 a	30.94 a	31.45 A	67.14 f	79.00 c	73.07 C
Mean	0.541 A	0.216 B		14.27 A	14.18 A		79.97 A	81.19 A	
<b>Methionine (ppm)</b>									
Control	0.029 f	0.103 e f	0.066D	9.05 d	8.90 d	8.975 C	107.60 c	109.7 bc	108.60 B
20	0.923 a	0.240 d	0.582A	22.69 b	28.16 a	25.42 A	86.24 d	88.83 d	87.54 C
40	0.388 c	0.570 b	0.479B	12.61cd	16.92 c	14.76 B	69.02 f	73.33 e	71.18 D
60	0.528 b	0.147 d e	0.338C	15.06 c	13.95 c	14.51 B	111.9 b	160.1 a	136.0 A
Mean	0.467 A	0.265 B		14.85 B	16.98 A		93.70 B	108.0 A	
<b>Methanol (%)</b>									
Control	0.029 e	0.103 d e	0.066C	9.05 e	8.90 e	8.975C	107.60 b	109.70 a	108.60 A
10	0.522 b	0.511 b	0.517A	19.32 a	14.11 bc	16.71 A	93.53 d	104.30 c	98.91 B
20	0.542 b	0.282 c	0.412B	13.03cd	15.82 b	14.43 B	75.98 f	79.48 e	77.73 C
30	0.653 a	0.133 d	0.393B	15.13 b	11.64 d	13.38 B	68.35 h	71.18 g	69.76 D
Mean	0.437 A	0.257 A		14.13 A	12.62 B		86.36 B	91.16 A	
<b>Ethephon (ppm)</b>									
Control	0.029 e	0.103 d	0.066C	9.05 cd	8.90 cd	8.975 B	107.60d	109.70 c	108.60 B
200	0.089 d	0.330 a	0.210A	6.147 e	7.887 de	7.017 C	139.90 b	142.50 a	141.20 A
300	0.193 b	0.124 cd	0.159B	10.34 c	9.027 cd	9.682 B	89.78 f	93.57 e	91.68 C
400	0.175bc	0.092 d	0.134B	17.75 b	22.62 a	20.19 A	83.76 g	78.36 h	81.06 D
Mean	0.122 A	0.162 A		10.82 A	12.11 A		105.2 A	106.0 A	
<b>Salicylic acid (ppm)</b>									
Control	0.029 f	0.103 e	0.066C	9.05 c	8.90 c	8.975 C	107.60 b	109.70 a	108.60 A
25	0.195 d	0.330 c	0.262B	7.753 d	7.32 d	7.535 D	100.60 c	97.79 d	99.18 B
50	0.132 e	0.029 f	0.081C	11.33 b	10.91 b	11.12 B	43.84 f	48.69 e	46.26 C
100	0.827 a	0.733 b	0.780A	16.97 a	17.79 a	17.38 A	28.41 h	29.56 g	28.98 D
Mean	0.296 A	0.299 A		11.28A	11.23 A		70.10 A	71.43 A	

Values followed by the same letter (s) are not significantly different at  $p < 0.05$ . \*Gem7=Gemmeiza7

## **Biochemical counteraction of heat stress injury in wheat .....**

From the obtained data, MOH significantly alleviated the effect of heat stress and production of MDA in leaves, but the most effective treatment was 30% MOH. In this connection, there was a significant effect appeared achieved by Sids1. Concerning the effect of interaction, data showed that the minimum value was obtained by Sids1 treated with 30% MOH. Also, application of Eth in low levels decreased MDA content. The lowest MDA content was obtained after treatment with 400ppm. Results observed that 200ppm Eth accumulated the highest MDA value and this result is compatible with Yonghua *et al.* (1995). In this regard, Larkindale and Knight (2002) showed that Eth a source of ethylene alleviated the thiobarbituric acid reactive substances and increased survival. Concerning the effect of interaction, data showed that the lowest value of MDA was produced by Gemmeiza7 with 300ppm.

Application of SA significantly enhanced the alleviation of MDA content. It was decrease with increasing levels of SA. The lowest value of MDA content was obtained by Sids1 after treatment with 100 ppm. The effect of SA in alleviating MDA is well documented by and Agarwal *et al.* (2005) and Shi *et al.* (2006). SA may switch on pathways that result in preventing of oxidative damage or repair that damage, also it acts as a potential non enzymatic antioxidant as well as plant growth regulator, which plays number of plant physiological processes (Larkindale and Knight, 2002 and Arfan *et al.*, 2007).

### **3. Antioxidant enzymes**

#### **3.1. Catalase and Peroxidase**

Data in Table (4) demonstrated that  $\text{CaCl}_2$  significantly enhanced CAT and POX activities in wheat leaves. But the maximum values were achieved by applied  $\text{CaCl}_2$  at rate 0.4 %. In this concern, there was a significant effect between the two cultivars, where Sids1 exhibited the superior value in the activity of both enzymes. As to the effect of the interaction, data displayed that the highest value of CAT activity was obtained by Gemmeiza7 after treatment with 0.4%. Also, the highest value of POX activity was obtained by Sids1 under the same conditions. These results are agreed with Chen *et al.* (2004) and Kolupaev *et al.* (2005). The enhancement in antioxidant enzymes activities after treatment with  $\text{CaCl}_2$  may be attributed to: 1) Ca may be involved in regulated antioxidant metabolism and helped maintain higher activity, this associated with reduces in  $\text{H}_2\text{O}_2$  and alleviate the damage to cell membranes. 2) Ca treatment resulted in a transient increase in cytosolic  $\text{Ca}^{+2}$  concentration during heat stress and may alleviate heat injury and enable plant cells to better survive (Gong *et al.*, 1998). 3) Ca also may switch on pathways that result in prevention of oxidative damage or repair of that damage (Larkindale and Knight, 2002).

It is apparent from data in Table (4) that all treatments of PA and Met promotive the activity of antioxidant enzymes in leaves as compared with the control. The maximum values of CAT and POX activities were achieved by PA

at rate 60 ppm and application of Met at rate 20 ppm. In this concern, there was a significant effect between the two cultivars, where Sids1 recorded the highest value of CAT activity after treatment with PA and Met. While, Gemmeiza7 achieved the superior value of POX activity after treatment with Met. Regarding the effect of interaction, data showed that the highest values of CAT and POX in PA treatment were recorded by Sids1 after treatment with 60 ppm. While, the highest value of CAT activity was obtained by Sids1 after treatment with Met at rate 20ppm. Also, the same dose of Met gave the maximum value of POX activity in leaves of Gemmeiza7. There is no previous work which can clarify the mode of action of each Met or PA effect on the defense mechanism (antioxidant enzymes) in plants but in a few words, it is speculated that Met is the precursor of ethylene in plant which can be implicated in a lot of defense mechanisms in plants against oxidative injury so it may be have the same behavior and effect. In the same line, PA which is a precursor of the SA biosynthesis in plant has the same trend of heat stress impedance and oxidative stress amelioration.

Table (4) shows that application of MOH significantly increased antioxidant enzymes CAT and POX activities in leaves. But the maximum values were achieved at rate 10%. In this concern, there was a significant effect between the two cultivars in POX activity, where Sids1 recorded the higher POX activity than Gemmeiza7. As to the effect of the interaction, data displayed that the highest value of CAT activity was obtained by Sids1 after treatment with 30%. Also, the same cultivar recorded the highest value of POX activity when MOH applied at rate 10%. The enhancement effects of MOH may be contributed to the positive effect of this volatile organic compound in growth parameters, photosynthetic pigments and the amelioration of MDA toxic product content.

Application of Eth significantly increased catalase activity in wheat leaves. This was true for POX activity only under high level of Eth. The highest value of CAT activity was achieved by Gemmeiza7 when Eth applied at rate 200 ppm. However, the maximum value of POX activity was recorded by Gemmeiza7 after treatment with 400 ppm. These results are well established by Larkindale and Huang (2004). Concerning SA, it appeared to be effective on CAT and POX activities in leaves. The maximum values of both enzyme activities were achieved after applied SA at rate 100ppm for both cultivars. The aforementioned results were in agreement in some extent with Shi *et al.* (2006) and Saleh *et al.* (2007)

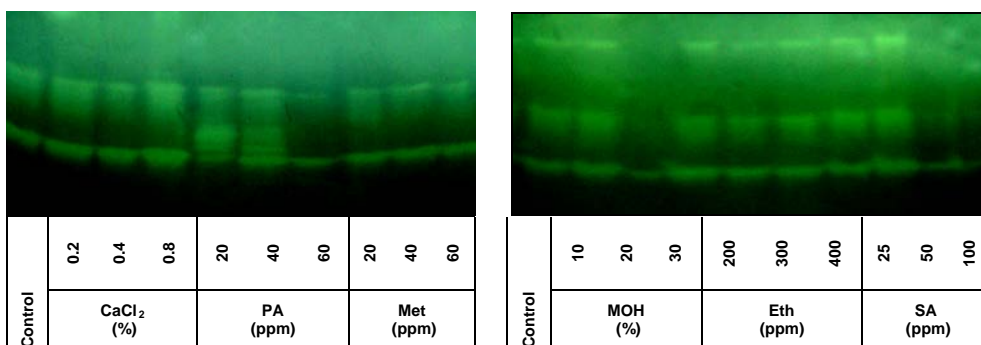
### **3.2. Superoxide dismutase**

Analysis of zymogram gel SOD pattern revealed the presence of 5 bands for the two wheat cultivars Table (5) and Fig. (1&2). Band number 5 is presented in all samples of the both cultivars under all treatments and the control. In contrast, bands (No. 3 and 4) were absent in all samples of Sids1 and Gemmeiza7 in case of control and all foliar application treatments (except Eth treatment). Unique bands (No.3 and 4) were observed due to

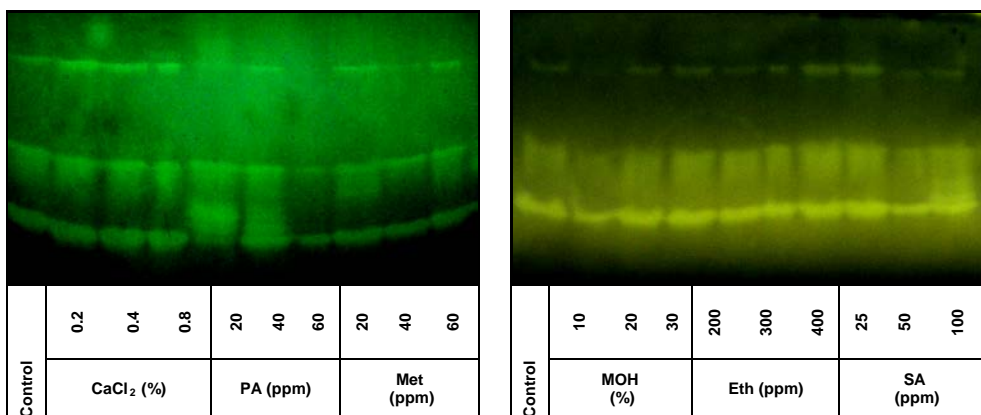


**Biochemical counteraction of heat stress injury in wheat .....**

treated plants with Eth at rate 200 and 300 ppm (except band No.4 for Gemmeiza7 when treated with 200ppm Eth). Also, unique band (No.3) was observed in Gemmeiza7 after treatment with 400 ppm. On contrary, bands (No.1 and 2) were disappeared in Gemmeiza7 when Eth applied at rate 400ppm. In the same direction, band number 1 in Sids1 and Gemmeiza7 was disappeared after treatment with Eth at rate 400 and 200 ppm, respectively. In this regard, bands (No.1 and 2) were absent in case of Sids1 when CaCl<sub>2</sub> applied at rate 0.4% and Met at rates 40 & 60ppm. In addition, there were detectable changes in band intensity for both cultivars grown under different treatments. Bands intensity for Sids1 (No. 1, 2 and 5) was increased after treatment with CaCl<sub>2</sub> and PA (except No.1 and 2 with 0.4% CaCl<sub>2</sub>). Also, there was slight increase in band intensity (No.5) when Gemmeiza7 treated with CaCl<sub>2</sub> (0.2 and 0.8%) and PA (20ppm). These results were agreed with those obtained by Zai *et al.* (2001) and Kolupaev *et al.* (2005).



**Fig.(1):Zymogram of superoxide dismutase banding pattern in shoots of Sids1 as affected by foliar application at 75 days from sowing.**



**Fig.(2):Zymogram of superoxide dismutase banding pattern in shoots of Gemmeiza7 as affected by foliar application at 75 days from sowing.**

**Table (5): Profile of superoxide dismutase isozyme pattern in shoots of two wheat cultivars as affected by foliar application at 75 days from sowing.**

Band number	Band intensity																		
	Treatments																		
	Control	Calcium chloride (%)			Phenylalanine (ppm)			Methionine (ppm)			Methanol (%)			Ethephon (ppm)			Salicylic acid (ppm)		
	0.2	0.4	0.8	20	40	60	20	40	60	10	20	30	200	300	400	25	50	100	
<b>Sids 1</b>																			
1	1.7	2.1	0	2.1	2.0	1.9	1.8	1.8	0	0	1.0	1.3	1.0	1.3	1.2	0	1.3	1.3	1.4
2	1.8	2.5	0	2.6	2.5	2.7	2.5	2.8	0	0	1.7	1.7	1.6	1.7	1.7	2.0	1.9	1.8	1.9
3	0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	2.1	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1	2.2	0	0	0	0
5	2.7	3.0	3.1	2.8	3.0	3.0	2.9	2.9	3.2	3.3	2.8	2.5	2.4	2.4	2.3	2.6	2.5	2.5	2.6
<b>Gemmeiza7</b>																			
1	1.3	1.3	1.3	1.3	1.3	1.3	1.2	1.2	1.2	1.3	1.5	1.6	1.5	0	1.5	0	1.6	1.6	1.6
2	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.0	1.0	1.0	1.6	1.6	1.6	1.5	1.6	0	1.6	1.7	1.7
3	0	0	0	0	0	0	0	0	0	0	0	0	0	1.6	1.6	1.7	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	0	0
5	1.0	1.1	1.0	1.1	1.1	1.0	1.0	1.0	1.0	1.1	1.9	1.8	1.8	1.8	1.8	1.8	1.9	1.9	1.9

In Table (5) application of Met had a slightly positive effect on bands intensity of Sids1 (No.1, 2 and 5) at rate 20ppm only, but the other foliar applications (20 and 40ppm) exhibited only the appearance of one band (No.5) which had high intensity. In spite of that, Met had no effect on the second cultivar Gemmeiza7 (except band No.5 with 60ppm). Additionally, bands intensity (No.1, 2 and 5) was increased after treatment with Met, Eth and SA on plants of Gemmeiza7 (except bands No.1 & 2 with 400 ppm Eth and band No.1 with 200ppm Eth). In this respect, band intensity for Sids1 (No.2) was increased when Eth applied at rate 400 ppm and SA applied at rates 25 and 100ppm. Also, band number 5 for the same cultivar took the same trend after treatment with 10% MOH. These results agreed with Choi *et al.* (2004). The induce effect of SA on SOD was mentioned by He *et al.* (2005).

#### 4. Amino acids

Table (6 and 7) indicated the 16 amino acids were detected including acyclic and cyclic amino acids. Acyclic amino acids contain: aliphatic unsubstituted amino acids (AUAA) such as (Glycine, Alanine, Valine, Leucine, and Isolucine) and aliphatic substituted (ASAA) such as hydroxy (Serine, Threonine), thio (Methionine), carboxy (Aspartic, Glutamic), diamino (Lysine) and guanidino (Arginine). Cyclic amino acids contain: aromatic (Phenylalanine, Tyrosine), heterocyclic (Histidine) and imino acid (Proline).

##### a. Acyclic amino acids

CaCl<sub>2</sub> increased the AUAA except valine, and ASAA except serine, methionine, lysine and arginine with 0.2% in shoots of Sids1. The highest values of acyclic amino acids were obtained at high rate. In the same trend, CaCl<sub>2</sub> enhanced all acyclic amino acids in shoots of Gemmeiza7, except glutamic acid with 0.2% and (aspartic & arginine) with 0.8%.

***Biochemical counteraction of heat stress injury in wheat .....***

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**Table (6)**

**Table (7)**

## **Biochemical counteraction of heat stress injury in wheat .....**

Meanwhile, the highest values of acyclic amino acids in Gemmeiza7 were recorded after treatment with  $\text{CaCl}_2$  at rate 0.4 %. However, application of PA enhanced the increment of AUAA except valine, and ASAA concentrations except arginine with 20ppm in Sids1. Also, there was increasing in the content of all acyclic amino acids in shoots of Gemmeiza7 after treatment with PA, except (aspartic & glutamic) with 40 ppm and arginine with (40 & 60 ppm). However, acyclic amino acids reached the highest values by applied PA at rate 60 ppm for Sids1 and 20 ppm for Gemmeiza7. All treatments of Met promote the biosynthesis of AUAA except valine, and ASAA except arginine in shoots of Sids1. Meanwhile, the low level of Met (20 ppm) promote the biosynthesis of acyclic amino acids in shoots of Gemmeiza7. Under foliar application of MOH, acyclic amino acids content in shoots of both cultivars responded positively with the high rate, except glutamic acid in Gemmeiza7.

Data cleared that Eth treatment raised the concentration of AUAA except valine, and ASAA in shoots of Sids1 as compared with control (without Eth). The same trend was observed in the foliar application of Eth at 200 and 400 ppm where there was an increment of most amino acids (AUAA and ASAA) obtained by Gemmeiza7. All foliar application of SA showed a marked increase in AUAA except valine in Sids1 cultivar. Also, the same phenolic compound elucidated a marked increase in ASAA except lysine and arginine with 25 and 50 ppm as compared with the control. Concerning Gemmeiza7, AUAA were increased after treatment with SA at rate 25 and 100 ppm. Also, ASAA such as serine, threonine and lysine took the same trend. Other ASAA appeared to be decreased or increased depending on the concerned amino acid and doses of foliar application (SA).

### **b.Cyclic amino acids**

Data in Table (6 and 7) showed that  $\text{CaCl}_2$  increased aromatic, heterocyclic and imino amino acids content in both cultivars, except PA and histidine with 0.2% in shoots of Sids1. Application of PA appeared to be effective on increase the content of all cyclic amino acids in wheat plants, except histidine with 20 ppm in Sids1 and tyrosine with 60 ppm in Gemmeiza7. Results declared that application of Met showed a marked increase in aromatic and imino amino acids in Sids1, while the heterocyclic amino acid showed an opposite trend. Also, there was a marked increase in all cyclic amino acids content in Gemmeiza7 after treatment with Met, except PA and histidine with 60 ppm. These results were compatible with Abd El-Aziz *et al.* (2009) and El Saber *et al.* (2010). Data in the same tables showed that MOH increased heterocyclic and imino amino acids concentration in both cultivars, except histidine with 20 ppm. Regarding aromatic amino acids, MOH at rate of 10% showed a marked decrease of PA content in shoots of Sid1, also decreased tyrosine content in Gemmeiza7 at rate of 20 % and 30%. Concerning Eth, it was raised the content of aromatic, heterocyclic and imino amino acids (except PA with 300ppm) in shoots of Sids1, as

compared with the control. Also, all doses of Eth had a positive effect on proline biosynthesis in Sids1. But Eth at rate of 300ppm enhanced the decrement of PA and histidine in Gemmeiza7, also Eth at 400 ppm decreased tyrosine content as compared with the control.

Data in Table (6 and 7) showed that SA appeared to be effective on accumulation of amino acids content i.e. aromatic and imino acid (proline) in Sids1. But SA at rates 25 and 50ppm decreased the accumulation of heterocyclic amino acid (tyrosine). In addition, there was a marked increase in all cyclic amino acids content in Gemmeiza7 after treatment with SA, except PA and histidine with 50 ppm. Furthermore, the increment of amino acids in plant by SA might be due to this substance affects the enzymatic activity and translocation of the metabolites to onion bulb (Amin *et al.*, 2007). In general, data in Table (6 and 7) showed that carboxy amino acids recorded the high amounts with all foliar application treatments and mostly higher than other amino acids possibly due to their being precursors for synthesis of most amino acids Amer (1989). These results were compatible with Sakhabutdinova *et al.* (2003), Deef (2007) and Hussein *et al.* (2007).

### **III.Plant height and grain yield**

Data in Table (8) demonstrated that CaCl<sub>2</sub> enhances plant height and grain yield under heat stress conditions. There was a significant effect between the two wheat cultivars. Sids1 exhibited higher value of plant height than Gemmeiza7. Concerning the effect of the interaction, data showed that the highest value of plant height was achieved by Sids1 after treatment with 0.4%. But the highest value of grain yield was achieved by Sids1 after applied CaCl<sub>2</sub> at rate 0.8% as compared with the control. The obtained results were in harmony with that obtained by Kumar and Minhas (2001) and Kumar *et al.* (2007).

Foliar application of PA and Met enhances plant height and grain yield. There was a significant effect between the both cultivars. Gemmeiza7 recorded the higher grain yield than Sids1 after treatment with PA, but Sids1 exhibited higher value in grain yield than Gemmeiza7 after treatment with Met. Concerning the effect of the interaction, data showed that, the highest value of plant height was achieved by Gemmeiza7 when PA applied at rate 40 ppm, but Sids1 gave the highest value in such parameter after applied Met at rate 40ppm. Also, the maximum values of grain yield were achieved by Gemmeiza7 with 60 ppm PA and Sids1 with 60ppm Met. The above results are compatible with Gamal El-Din and Abdel Wahid (2005)

Application of MOH enhances plant height and grain yield. In addition, Gemmeiza7 exhibited the superior value of plant height and grain yield than the other cultivar. Concerning the effect of the interaction, data showed that the highest value of plant height was achieved by Gemmeiza7 after treatment with MOH at rate 30 % followed by the same cultivar with 20%.

***Biochemical counteraction of heat stress injury in wheat .....***

**Table (8): Plant height and grain yield as affected by foliar application, wheat cultivars and their interaction at harvest.**

Foliar application	Plant height (cm)			grain yield (Kg / fad)		
	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean
<b>Calcium chloride (%)</b>						
Control	84.36 e	85.06 e	84.71 C	961.3 f	761.3 g	861.3D
0.2	105.90 b	98.69 d	102.30 B	1070 e	1380 d	1225 C
0.4	112.80 a	101.00 cd	106.90 A	1480 c	1547 b	1513 B
0.8	103.50bc	102.30b-d	102.90 B	1730 a	1510 bc	1620 A
Mean	101.60 A	96.75 B		1310 A	1300 A	
<b>Phenylalanine (ppm)</b>						
Control	84.36 e	85.06 e	84.71 D	961.3 e	761.3 f	861.3D
20	98.44 b	93.23 cd	95.83 B	1088 d	1076 d	1082 C
40	93.66 cd	107.9 a	100.8 A	1080 d	1373 b	1227 B
60	95.07 bc	90.71 d	92.89 C	1263 c	1563 a	1413 A
Mean	92.88 A	94.23 A		1098 B	1194 A	
<b>Methionine (ppm)</b>						
Control	84.36 c	85.06 c	84.71 B	961.3 e	761.3 f	861.3D
20	91.46 ab	91.51 ab	91.49 A	1230 b	1073 d	1152 B
40	94.97 a	92.68 ab	93.82 A	1070 d	1146 c	1108 C
60	90.69 b	93.95 ab	92.32 A	1290 a	1230 b	1260 A
Mean	90.37 A	90.80 A		1138 A	1053 B	
<b>Methanol (%)</b>						
Control	84.36 c	85.06 c	84.71 C	961.3 d	761.3 e	861.3C
10	104.5 a	103.0 a	103.8AB	1000 d	1240 b	1120 B
20	104.6 a	105.2 a	104.9 A	1193 c	1430 a	1312 A
30	94.80 b	106.6 a	100.7 B	990 d	1199 c	1094 B
Mean	97.07 B	99.97 A		1036 B	1158 A	
<b>Ethephon (ppm)</b>						
Control	84.36 e	85.06 e	84.71 C	961.3 e	761.3 f	861.3D
200	93.80 bc	98.78 a	96.29 A	1473 bc	1123 d	1298 B
300	95.06 ab	87.25 de	91.15 B	1663 a	1477 b	1570 A
400	92.41 bc	90.16 cd	91.29 B	1420 c	1073 d	1247 C
Mean	91.41 A	90.31 A		1380 A	1109 B	
<b>Salicylic acid (ppm)</b>						
Control	84.36 c	85.06 c	84.71 C	961.3 d	761.3 e	861.3B
25	94.46 ab	93.38 ab	93.92 B	1417 b	1440 b	1428 A
50	95.22 a	90.35 b	92.78 B	1420 b	1420 b	1420 A
100	97.33 a	97.56 a	97.44 A	1620 a	1220 c	1420 A
Mean	92.84 A	91.58 A		1355 A	1210 B	

Values followed by the same letter (s) are not significantly different at  $p < 0.05$ . \*Gem7=Gemmei7

Also, the highest value of grain yield was achieved by Gemmeiza7 when MOH applied at 20%. These results were in complete agreement with Nonomura and Benson (1992)a. The positive effect of MOH on yield may be attributed to stimulation of plant hormone production (Madhaiyan *et al.*, 2006).

Data in Table (8) clearly demonstrated that Eth enhances plant height and grain yield. Sids1 exceeded Gemmeiza7 in grain yield under the same conditions. In this regard, Sids1 gave the highest value of plant height when Eth applied at rate 200 ppm. However, the maximum value of grain yield was

obtained by Sids1 after treatment with Eth at rate 300 ppm. This result goes in line with Saha *et al.* (1995). Results showed that SA enhances plant height and grain yield. There was a significant effect between the two cultivars in grain yield. Sids1 exhibited the higher value than the other cultivar. The maximum value of plant height was achieved by Gemmeiza7 after applied SA at rate 100 ppm. But the highest value of grain yield was obtained by Sids1 with 100 ppm SA. These results were in the same connection with Shakirova *et al.* (2003) and Amin *et al.* (2008).

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***Biochemical counteraction of heat stress injury in wheat .....***

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## المقاومة البيوكيميائية لأضرار الإجهاد الحرارى فى القمح تحت ظروف صحراء الوادى الجديد

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### الملخص العربي

أقيمت تجربتين حقليتين خلال موسمي ٢٠٠٧/٢٠٠٨ ، ٢٠٠٨/٢٠٠٩ بمحطة البحوث الزراعية التابعة لمركز بحوث الصحراء بمحافظة الوادى الجديد (مزرعة الخارجة)، وذلك لدراسة الدلائل البيوكيميائية المرتبطة بمقاومة أضرار الاجهاد الحرارى لصنفين من القمح سدس ١ (مقاوم للحرارة) ، جميزة ٧ (حساس للحرارة) وذلك بتفعيل دور المقاومة المستحثة من خلال استخدام بعض المركبات الكيميائية مثل كلوريد الكالسيوم ، الفينيل الانين ، الميثيونين ، الميثانول ، الإيثيفون ، حامض الساليسيليك مع استخدام الماء العادى كمقارنة. وقد أوضحت النتائج أن كل معاملات الرش الورقى أدت الى تحسين النمو ومحصول الحبوب. وقد لوحظ تفوق صنف سدس ١ عن جميزة ٧ فى صفات النمو والمحصول تحت معظم المعاملات. كما وجد ان معاملة الكالسيوم كانت أفضل المعاملات (٠.٨%) ويليها معاملة الإيثيفون (٣٠٠ جزء فى المليون) ثم معاملة الساليسيليك (٢٥ جزء فى المليون) وكان هذا مرتبطا بمقاومة الاجهاد الحرارى فى القمح. وقد جاءت باقى المعاملات فى المرتبة الثانية. كما أظهرت النتائج ارتباط ذلك بزيادة بعض المكونات البيوكيميائية مثل صبغات التمثيل الضوئى والانزيمات المضادة للاكسدة (الكاتاليز، البيروكسيديز)، ونقص فى بعض المكونات الأخرى مثل الـ malondialdehyde.

أظهر التفريد الكهربى لمشابهات انزيم Superoxide dismutase عن وجود ٥ حزم bands لصنفى القمح. وقد وجد أن الخامسة منها ظهرت فى كل المعاملات بما فيها معاملة المقارنة. كما أظهرت النتائج أن الحزمتين رقم ٣ ، ٤ كانت موجودة فقط فى معاملة الايثيفون لصنفى القمح. حيث أظهرت النتائج وجود حزم جديدة عند معاملة صنفى القمح بالايثيفون بتركيز ٢٠٠ ، ٣٠٠

## Biochemical counteraction of heat stress injury in wheat .....

جزء في المليون. كما أوضحت النتائج حدوث تغيرات في كثافة الحزم تحت معاملات الرش الورقي وكان هذا مرتبطا بمقاومة الاجهاد الحرارى للقمح. كما أوضحت النتائج وجود ١٦ حامض أميني تشمل أحماض أمينية حلقية وغير حلقة cyclic and acyclic amino acids. وقد وجد أن الاحماض الامينية غير الحلقية acyclic تحتوى على أحماض أمينية اليقاتية unsubstituted مثل ( الجليسين، الالانين، الفالين، الليوسين، الايزوليوسين ) ، واحماض أمينية اليقاتية substituted مثل الاحماض التى تحتوى على مجموعة هيدروكسيل ( سيرين ، ثيونين )، ومجموعة ثيو (الميثيونين)، ومجموعة كربوكسيل (أسبارتك ، جلوتاميك)، مجموعة داي أمينو (الليسين)، ومجموعة الجوانيدينو (الارجنين). كما أظهرت النتائج أن الاحماض الامينية الحلقية cyclic تشمل الاروماتية ( الفينيل الانين ، التيروسين ) ، الحلقية غير المتجانسة (الهستيدين) ، وأحماض الإمينو (البرولين). كما اظهرت النتائج زيادة واضحة في محتوى النباتات من الاحماض الامينية كنتيجة لمعاملات الرش الورقي، وقد أعتمد هذا على نوعية مادة الرش والجرعة والصنف النباتي. وقد أظهرت النتائج أن الاحماض الامينية الكربوكسيلية سجلت أعلى القيم مع كل معاملات الرش الورقي المستخدم مقارنة بباقي الاحماض الامينية، وهذا راجع لأن هذه الاحماض تستخدم كبدئات لتخليق معظم الاحماض الامينية الاخرى، والمرتبطة بمقاومة الاجهاد الحرارى في القمح. ويمكن الاستفادة من هذا البحث كمايلي:

- فى دراسة وتقييم أسس التحمل الحرارى للقمح بمنطقة الوادى الجديد والمعروفة بإجهادها الحرارى ، مع التوصية باستخدام التراكيب الوراثية الاكثر تحملا لظروف الاجهاد الحرارى مثل سدس ١ وإرتباط ذلك بالمحصول ومحتوى المكونات البيوكيميائية.
- الإستفادة من الدلائل البيوكيميائية المرتبطة بمقاومة الاجهاد الحرارى، وذلك بدفع الأصناف الحساسة مثل جميزة ٧ على مقاومة الإجهاد الحرارى (تفعيل دور المقاومة المستحثة).

**Table (3): Photosynthetic pigments in leaves as affected by foliar application, wheat cultivars and their interaction at 75 days after sowing.**

Foliar application	Photosynthetic pigments (mg / 100 g fresh wt.)														
	Chlorophyll a			Chlorophyll b			Carotenoids			Chlorophyll a + b			Total pigments		
	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean
Calcium chloride ( % )															
Control	106.0d	115.0d	110.5C	44.00c	25.67d	34.83 C	43.67d	33.33e	38.50C	150.0d	140.7d	145.3C	193.7d	174.0e	183.8C
0.2	161.0a	126.7c	143.8B	56.67b	42.00c	49.33 B	59.00b	49.67cd	54.33B	217.7a	167.0c	192.3B	276.7a	218.3c	247.5B
0.4	147.0b	133.0c	140.0B	77.00a	39.67c	58.33 A	54.67bc	51.67c	53.17B	224.0a	172.7c	198.3AB	278.7a	224.3c	251.5B
0.8	151.0b	163.0a	157.3A	44.00c	51.67b	47.83 B	52.00c	66.00a	59.00A	195.7b	214.7a	205.2A	247.7b	280.7a	264.2A
Mean	141.4A	134.4A		55.42 A	39.75 B		52.33A	50.17A		196.8A	173.8B		249.2A	224.3B	
Phenylalanine (ppm)															
Control	106.0d	115.0d	110.5C	44.00f	25.67h	34.83 D	43.67d	33.33e	38.50C	150.0e	140.7e	145.3C	193.7e	174.0f	183.8C
20	173.0b	171.0b	172.0A	34.00g	53.00d	43.50 C	46.00d	61.67a-c	53.83B	207.0cd	224.0b	215.5B	253.0d	286.0bc	269.5B
40	146.7c	189.0a	167.8A	49.67e	86.00a	67.83 A	57.00bc	67.67a	62.33A	196.3d	275.0a	235.7A	253.3d	342.7a	298.0A
60	157.7bc	152.0c	154.8B	57.00c	72.00b	64.50 B	55.67c	64.67ab	60.17A	214.7bc	224.0b	219.3B	270.3cd	288.7b	279.5B
Mean	145.8A	156.8A		46.17 B	59.17A		50.58B	56.83A		192.0B	215.9A		242.6B	272.8A	
Methionine (ppm)															
Control	106.0e	115.0e	110.5D	44.00d	25.67f	34.83 C	43.67c	33.33d	38.50C	150.0d	140.7d	145.3D	193.7e	174.0f	183.8D
20	160.7b	189.7a	175.2A	85.00a	63.67b	74.33 A	57.33b	71.00a	64.17A	245.7a	253.3a	249.5A	303.0b	324.3a	313.7A
40	133.7d	108.7e	121.2C	32.67e	35.00e	33.83 C	46.67c	46.00c	46.33B	166.3c	143.7d	155.0C	213.0d	189.7e	201.3C
60	149.0bc	139.7cd	144.3B	54.67c	53.67c	54.17 B	47.33c	50.67c	49.00B	203.7b	193.3b	198.5B	251.3c	244.0c	247.7B
Mean	137.3A	138.3A		54.08A	44.50B		48.75A	50.25A		191.4A	182.8A		240.3A	233.0A	

Values followed by the same letter (s) are not significantly different at  $p < 0.05$ . \*Gem7=Gemmeiza7



**Table (3). Cont.**

Foliar application	Photosynthetic pigments (mg / 100 g fresh wt.)														
	Chlorophyll a			Chlorophyll b			Carotenoids			Chlorophyll a + b			Total pigments		
	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean	Sids 1	*Gem7	Mean
Methanol (%)															
Control	106.0e	115.0de	110.5C	44.00c	25.67d	34.83 C	43.67d	33.33d	38.50D	150.0c	140.7c	145.3C	193.7d	174.0d	183.8C
10	164.7b	293.0a	228.8A	60.00b	95.00a	77.50 A	57.00c	93.67a	75.33A	224.7b	388.0a	306.3A	281.7bc	481.7a	381.7A
20	134.3cd	165.0b	149.7B	97.00a	54.00bc	75.50 A	75.00b	55.67c	65.33B	231.3b	219.0b	225.2B	306.3b	274.7bc	290.5B
30	150.7bc	166.0b	158.3B	54.67bc	60.33b	57.50 B	55.00c	57.67c	56.33C	205.3b	224.3b	214.8B	260.3c	282.0bc	271.2B
Mean	138.9B	184.8A		63.92A	58.75 A		57.67A	60.08A		202.8B	243.0A		260.5B	303.1A	
Ethephon (ppm)															
Control	106.0bc	115.0b	110.5A	44.00a	25.67d	34.83 C	43.67b	33.33cd	38.50B	150.0a	140.7c	145.3A	193.7b	174.0e	183.8A
200	87.67d	113.7bc	100.7B	33.67c	41.00b	37.33 B	39.67bc	51.67a	45.67A	121.3d	151.0a	136.2B	161.0f	202.7a	181.8A
300	69.00e	132.0a	100.5B	19.67e	17.00e	18.33 D	29.33d	32.00d	30.67C	88.67e	149.0ab	118.8C	118.0g	181.0de	149.5B
400	108.0bc	104.0c	106.0AB	41.00b	41.00b	41.00 A	40.67bc	41.00b	40.83AB	149.0ab	142.0bc	145.5A	189.7bc	183.0cd	186.3A
Mean	92.67B	116.2A		34.58 A	31.17 B		38.33A	39.50A		127.3B	145.7A		165.6B	185.2A	
Salicylic acid (ppm)															
Control	106.0c	115.0c	110.5B	44.00c	25.67d	34.83 C	43.67b	33.33c	38.50B	150.0bc	140.7cd	145.3B	193.7bc	174.0cd	183.8B
25	75.67d	116.7c	96.17C	31.67d	42.00c	36.83 C	35.33c	53.00a	44.17A	107.3e	144.3c	125.8C	142.7e	197.3bc	170.0BC
50	89.00d	77.67d	83.33D	30.00d	60.67b	45.33 B	38.00bc	36.67bc	37.33B	119.0de	138.3cd	128.7C	157.0de	175.0cd	166.0C
100	130.7b	178.7a	154.7A	40.00c	77.67a	58.83 A	41.00bc	56.00a	48.50A	170.7b	156.3a	213.5A	211.7b	312.3a	262.0A
Mean	100.3B	122.0A		36.42B	51.50A		39.50A	44.75A		136.8B	169.9A		176.3B	214.7A	

Values followed by the same letter (s) are not significantly different at  $p < 0.05$ . \*Gem7=Gemmeiza7

**Table (6): Protein amino acids composition in shoot of Sids1 as affected by foliar application at 75 days from sowing.**

Foliar application		Amino acids (mg/g dry wt.)															
		Acyclic amino acids											Cyclic amino acids				
		Aliphatic unsubstituted					Aliphatic substituted						Aromatic	Heterocyclic	Imino		
		Glycine	Alanine	Valine	Leucine	Isoleucine	Hydroxy		Thio	Carboxy		Diamino				Guanidino	
Serine	Threonine						Methionine	Aspartic	Glutamic	Lysine	Arginine	Phenyl alanine	Tyrosine	Histidine	Proline		
Control	Tap water	1.61	0.92	9.18	2.07	1.32	1.32	1.38	0.03	2.36	3.40	2.19	2.39	1.41	0.21	1.84	2.13
Calcium chloride (%)	0.2	1.76	2.70	2.21	2.35	1.49	0.86	1.42	0.02	4.34	4.76	1.58	1.24	1.28	0.68	1.10	5.67
	0.4	4.00	5.13	4.11	4.29	3.09	3.33	3.19	0.33	7.66	11.45	3.56	3.02	3.50	1.35	2.82	9.45
	0.8	7.25	8.63	6.74	8.79	5.54	6.06	5.43	0.09	14.08	16.92	8.00	4.74	5.97	1.67	4.74	13.93
Phenyl-Alanine (ppm)	20	2.93	3.86	3.82	4.00	2.43	2.31	2.34	0.25	5.77	6.61	2.71	2.02	2.39	0.81	1.81	6.24
	40	3.26	4.68	4.05	4.57	2.90	2.61	2.66	0.05	6.37	7.54	3.15	2.55	2.89	1.40	2.15	6.62
	60	6.12	7.93	7.43	8.08	6.25	5.60	5.08	0.97	11.14	14.49	6.19	4.04	6.07	2.42	4.69	13.60
Methionine (ppm)	20	2.71	4.08	3.48	3.57	2.42	2.26	2.08	0.07	6.42	6.90	2.43	2.13	2.29	0.80	1.78	5.64
	40	3.26	4.68	4.05	4.57	2.90	1.78	1.73	0.53	4.78	5.53	2.28	2.02	2.84	0.92	1.32	5.09
	60	6.12	7.93	7.43	8.08	6.25	1.88	1.66	0.03	4.38	5.64	1.67	1.40	1.88	1.88	1.35	4.43
Methanol (%)	10	0.77	1.66	1.39	1.48	0.92	0.65	0.76	0.08	1.66	2.28	0.92	0.64	0.63	1.42	0.45	3.48
	20	4.51	5.89	4.79	6.16	3.85	4.00	4.08	0.40	9.29	11.29	4.58	4.03	6.57	3.44	4.97	10.30
	30	7.29	8.88	9.63	11.16	8.22	6.27	6.27	1.34	13.41	16.08	9.69	13.48	12.15	2.17	7.44	7.30
Ethephon (ppm)	200	3.66	4.91	4.59	4.35	3.16	3.11	2.73	0.50	7.21	9.24	5.29	6.44	5.53	0.93	3.89	3.48
	300	2.81	3.49	3.63	3.85	2.31	2.04	2.07	0.56	4.47	5.90	3.40	3.46	0.77	1.62	3.78	4.60
	400	4.52	5.40	5.88	6.21	3.97	3.22	3.39	0.52	8.30	9.82	4.77	7.45	4.99	1.42	4.21	5.03
Salicylic acid (ppm)	25	1.93	2.74	2.43	2.44	1.62	1.52	1.58	0.04	3.51	4.36	1.55	1.34	1.50	1.05	1.21	3.98
	50	2.40	3.31	3.51	3.27	1.91	1.95	5.01	0.08	4.39	5.47	2.07	2.11	1.90	1.16	1.40	5.32
	100	5.49	7.35	5.37	7.49	4.95	5.01	4.97	0.49	9.95	13.36	6.45	4.75	5.75	2.50	4.89	12.83

**Biochemical counteraction of heat stress injury in wheat .....**

**Table (7): Protein amino acids composition in shoot of Gemmeiza7 as affected by foliar application at 75 days from sowing.**

Foliar application		Amino acids (mg/g dry wt.)															
		Acyclic amino acids											Cyclic amino acids				
		Aliphatic unsubstituted					Aliphatic substituted						Aromatic		Heterocyclic	Imino	
							Hydroxy		Thio	Carboxy		Diamino					Guanidino
Glycine	Alanine	Valine	Leucine	Isoleucine	Serine	Threonine	Methionine	Aspartic	Glutamic	Lysine	Arginine	Phenyl alanine	Tyrosine	Histidine	Proline		
Control	Tap water	2.88	4.05	3.78	3.08	2.38	2.93	2.43	0.08	6.91	9.48	2.64	2.58	2.72	1.06	2.52	4.36
Calcium chloride (%)	0.2	4.05	5.18	4.26	4.64	3.12	3.69	3.76	0.13	7.10	9.33	3.76	3.03	4.24	1.64	3.47	11.51
	0.4	4.51	5.89	4.66	5.23	3.75	4.28	4.15	0.39	8.68	11.76	4.30	3.95	4.83	1.86	4.03	11.09
	0.8	3.68	4.88	4.20	4.01	3.10	3.36	3.36	0.42	4.79	10.01	3.45	2.45	4.12	1.20	2.74	7.33
Phenyl-Alanine (ppm)	20	5.35	6.95	5.41	6.21	4.41	5.42	5.03	0.39	7.66	14.04	5.62	3.61	4.59	1.48	4.27	12.09
	40	3.39	4.59	3.94	4.21	2.79	3.11	3.15	0.39	5.85	8.28	3.21	2.42	3.69	1.09	3.16	7.69
	60	3.66	5.26	4.02	4.40	3.24	3.71	3.33	0.63	7.72	10.44	3.45	2.46	4.00	1.04	3.15	9.81
Methionine (ppm)	20	4.66	6.33	5.03	5.08	3.81	4.74	4.20	0.28	9.02	14.18	4.15	3.45	4.62	1.99	5.41	8.58
	40	3.21	4.28	3.61	3.75	2.69	2.74	2.70	0.51	5.32	7.83	2.91	2.12	3.43	1.09	3.73	7.18
	60	2.44	3.29	2.71	2.78	1.96	2.07	2.13	0.48	4.16	5.99	2.04	1.49	2.22	1.34	2.16	5.28
Methanol (%)	10	3.05	4.10	3.60	3.37	2.36	2.83	2.73	0.08	5.33	7.45	2.71	1.88	3.02	1.58	2.33	7.11
	20	2.83	3.24	2.74	3.06	1.92	2.18	1.83	0.49	4.78	6.04	2.35	1.83	3.15	0.62	2.74	5.72
	30	3.41	4.59	4.07	4.36	2.88	3.12	3.06	0.40	6.96	8.32	3.23	2.93	3.93	0.68	2.96	9.40
Ethephon (ppm)	200	3.81	4.76	4.23	4.24	3.00	3.28	3.65	0.59	8.77	9.94	3.05	2.22	3.66	1.43	2.73	6.52
	300	2.81	3.72	3.29	3.37	2.18	1.99	1.99	0.41	4.63	6.91	2.45	1.99	2.65	1.33	2.15	5.73
	400	4.05	5.39	4.62	5.81	3.65	3.55	3.22	0.55	7.42	10.22	4.32	3.16	5.69	0.76	3.99	7.13
Salicylic acid (ppm)	25	3.54	4.71	4.74	5.71	4.26	3.11	3.24	0.76	5.77	8.05	4.43	2.30	3.16	1.19	3.96	7.72
	50	2.23	3.06	2.38	2.40	1.68	1.78	1.86	0.45	3.54	4.95	1.96	1.29	2.06	1.13	1.72	4.92
	100	3.53	5.03	3.94	4.02	2.93	3.57	3.23	0.15	7.02	9.92	3.25	2.43	3.59	1.39	3.76	6.17

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