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Antioxidant Defense System in Heat Shocked Wheat Plants Previously Treated with Arginine or Putrescine.

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Abstract: Two pot experiments were carried out in the screen of National Research Centre during two successive seasons 20/11/2002/2003 - 2003/2004 to alleviate the harmful effects of high temperature stress (35°C ± 2) on wheat cultivar (Giza 168) by the application of arginine or putrescine (0.0, 1.25 and 2.5 mM). Wheat plants are grown normally and sprayed with arginine or putrescine at 30 DAS and left to grow. At 40 DAS (double ridge stage), the plants are exposed to high temperature stress (35°C ± 2) for 4 or 8 hrs while the control plants left at 20°C ± 2. The plant samples were harvested at 48 hrs after the exposure to high temperature. The induced changes in the enzyme activities, lipid peroxidation, nucleic acid contents and protein electrophoretic pattern in the treated wheat plants were determined., The activities of POX, PPO and IAA – oxidase enzymes were increased significantly in wheat plants exposed to high temperature, while, SOD and CAT activities were decreased compared to the values of plants exposed to high temperature stress. Arginine or putrescine treated plants prior to high temperature exposure showed significant reductions in the activities of POX, PPO and IAA – oxidase enzymes and significant increases in SOD and CAT activities than those of the corresponding plants exposed to high temperature stress alone. Exposure of wheat plants to high temperature (4 or 8 hrs) increased significantly the MDA contents in the wheat shoots as a result of lipid peroxidation. Arginine or putrescine treatments induced significant reduction in these contents. DNA contents of wheat plants exposed to high temperature decreased significantly. Such effect was much more pronounced in response to 8 hrs. In the meantime, RNA contents exhibited significant increases over the control. The application of either arginine or Put on wheat plants increased significantly both DNA and RNA contents over the plants exposed to the high temperature stress or the control plants. The changes in the protein electrophoretic pattern of the exposed wheat plants to 4 or 8 h of high temperature exhibited an increase in the number of protein bands as compared to the control. The appearance of new proteins in wheat shoots subjected to the high temperature stress are at molecular weights 111, 90, 70, 45, 32, 24 and 8 KDa. All these proteins are known to be heat shock proteins. Exposing wheat plants to high temperature after arginine or Put treatments exhibited slight increases in the number of bands compared to the plants exposed to the high temperature stress alone. Arginine or Put treatment promotes the synthesis of a new protein bands at M wt 80.9 K Da under normal and high temperature stress. The magnitude of such responses was more pronounced in case of 2.5 mM treatments of either substance.

Key words: Arginine, Putrescine, Wheat, Antioxidant enzymes, Lipid peroxidation, DNA, RNA, Protein electrophoretic pattern, Heat stress.

INTRODUCTION

Heat stress affects the wide spectrum of both biochemical and physiological responses within the plant cells (Roy and Ghosh, 1996). High temperature stress conditions can create a water deficit in plant tissues which in turn lead to injury of cell membranes and to reduction in rates of transpiration, protein synthesis and ion uptake and transport. Also, high temperature can inhibit photosynthetic enzymes as well as loss of permeability of cellular membranes (Levitt, 1980). In nature, plants are subjected to changes of temperature, both during changes in season and over the course of individual days. The temperature of an individual plant cell can change much more rapidly than other factors that cause stress. Thus, like other organisms, plants have evolved strategies for preventing damage caused by rapid changes in temperature and for repairing what damage in unavoidable (Larkindale and Knight, 2002).

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Heat shock treatment (50oC) for one or two hours increased peroxidase activity of sorghum (Choudhary *et al.,* 1993). Keles and Öncel (2002) indicated that, a catalase (CAT) activity was decreased at supraoptimal temperature of wheat seedlings. Liu and Huang (2002) showed that, the activities of superoxide dismutase and catalase decreased at both high soil temperature (20/35°C) for bentgrass plants. Also, Auld and Paulsen, (2003) recorded an increase in polyphenol oxidase (catechol oxidase) enzyme of wheat plant subjected to high temperature stress (30/25°C).Heat shock treatment increased lipid peroxidation of *Vigna radiate* (Basra *et al.,* 1997). Liu and Huang (2002) illustrated that the content of lipid peroxidation product increased at both high soil temperature (20/35°C) for bentgrass plants. Elevation the temperature to 40°C caused significant increases (greater than 3- fold compared with unheated controls) in lipid peroxidation after 2 days and this increase reached up to 6- fold after 3 days of treatment in *Arabidopsis* plants, (Larkindale and Knight, 2002).High temperature stress induced a reduction in DNA contents of plants. Stress, in general, induces the release of reactive oxygen species (ROS) which accompanied with the induction of DNAase activities, enhanced DNA fragmentation and methylation (Papadakis and Roubelakis – Angelakis, 2005). Lanciloti *et al.* (1996) found that, the exposure of barley to 40°C for 2.5 hrs resulted in the induction of mRNA to form HSP 70, and suggested that, the sub lethally heat shocked cells were returned at 25°C (for 6 hrs) where, mRNA levels recovered to nearly the levels of non heat shocked control. It was shown that, when seedlings exposed to temperature five or more degrees above optimal growing temperatures, synthesis of most normal proteins is repressed and transcription of certain mRNA to be translated to form a small set of "heat shock proteins" (HSPs) is initiated (Nagao *et al.,* 1986). Hendershot *et al.* (1992) indicated that, both low and high molecular weight HSPs are synthesized in wheat as response to heat stress when leaf temperature increase ≈ 10°C above the optimal growth temperature of 18 to 23°C. Accumulation of HSP 101 mRNA was strong under heat shock conditions in maize (Nieto-Sotelo *et al.,* 1999).

Polyamines regulate the enzyme activities of plants. These regulations may be attributed to the potential effect of putrescine which acts as radical scavenger (Todorov *et al.,* 1998). Also, Choudhary *et al.* (1993) revealed that, putrescine treatment decreased peroxidase activity of sorghum. Velikova *et al.* (2000) confirmed that, pretreatment with spermidine and spermine reduced peroxidase and increased catalase activities of bean plant. Also, Bekheta and El-Bassiouny (2005), found that, foliar application of putrescine on wheat plant decreased peroxidase, IAA - Oxidase and polyphenol oxidase and increased catalase and superoxide dismutase activities.Polyamines act as antioxidants by inhibiting lipid peroxidation of plants (Tadoline, 1988). Also, lipid peroxidation decreased as a result of external supply with different concentrations of PAs on wheat, (HuiGuo *et al.,* 2006).Polyamines are able to bind several negatively charged molecules, such as DNA (Pohjanpelto and Holtta, 1996), proteins and membrane phospholipids (Tassoni *et al.,* 1996). They are involved in protein phosphorylation (Chang and Kang, 1999), post transcriptional modifications (Mehta *et al.,* 1994) and conformational transition of DNA (Basu *et al.,* 1990). Several investigators stated that, PAs have several modes of actions where they bind strongly to RNA and DNA, retarding RNase and DNase actions, so it can retard senescence (Papadakis & Roubelakis – Angelakis, 2005). Polyamines are important cellular constituents to specific regulatory proteins. They provide a possible mechanism for the formation of polyamine – protein complex. Proteins and membrane phospholipids (Tassoni *et al.,* 1996).

The present work aims to study the effect of heat shock on antioxidant system of wheat plants previously sprayed with arginine or putrescine.

MATERIALS AND METHODS

The experimental plant used in this investigation was wheat (*Triticum aestivum* var. Giza 168). Pure strain of grains obtained from Egyptian Ministry of Agriculture.

The chemicals used in the present work were (i) arginine (one of the essential amino acids), (ii) putrescine (member of polyamine group). They were supplied from SIGMA – ALDRICH

This experiment carried out to investigate the effect of selective concentrations and date of spraying of either arginine or putrescine on alleviation of heat shock injury of wheat plants. Two pots experiments were carried out in the screen of National Research Centre, Dokki, Giza, Egypt during two successive growth seasons (15 / 11 / 2003 and 2004).A homogenous lots of wheat grains *Triticum aestivum* var. Giza 168 were sown in pots (50 cm in diameter) containing equal amounts of clay soil. Fertilization was done with the recommended dose i.e (5 g phosphorous / pot as triple phosphate, 6 g nitrogen / pot as urea and 5 g potassium / pot as potassium sulphate) during preparation of pots and after sowing. Watering was carried out according to the usual practice. After 15 days from sowing thinning was carried out, so as five uniform seedlings were left in each pot.

The pots were divided into five groups each composed of 30 pots. The plants of the five groups were sprayed with 0, 1.25 and 2.50 mM arginine or putrescine. Each group was divided into 3 sets each contain 10 pots. These treatments were carried out twice (30 and 35 days after sowing). After five days, the sprayed plants of the first set were exposed to normal temperature (20°C control), the second set exposed to 35°C ± 2 for 4 hours and returned to normal temperature. The third set exposed to 35°C ± 2 for 4 hours and returned to normal temperature and the exposure to 35°C ± 2 repeated again at the following day on the same set.
Activities of some enzymes, Lipid peroxidation, nucleic acid contents and protein electrophorasis were estimated in fresh shoots after 48hrs from exposure to high temperature.

Assay of Enzymes Activities:

The method used for extracting the enzyme is that of MuKherjee and Choudhuri, (1983),.Super oxide dismutase (SOD, EC 1.12.1.1) activity was measured according to the method of Dhindsa *et al.* (1981). Peroxidase (POX, EC 1.11.1.7) activity was assayed using to the method of Bergmeyer, (1974). Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method of Chen *et al.* (2000). Polyphenol oxidase (PPO, EC 1.10.3.1) activity was assayed using to the method of Kar and Mishra(1976).The enzyme activeties were calculated by Kong *et al* (1999). The method used for extraction and the assay of the activities of Indole acetic acid oxidase (IAA oxidase) following the method of Gordon and Weber (1951) as described by Darby Shire (1971). The enzyme activities were assayed by Spekol Spectrocolourimeter VEB Carl Zeiss

***Lipid Peroxidation*:**

The level of lipid peroxidation was measured by determining the malonadialdehyde (MDA) contents. Malonadialdehyde is the product of Lipid peroxidation and that assayed by thiobarbaturic acid reactive substance (TBARS) contents (Hodges *et al.,* 1999).

Quantitative Estimation of Nucleic Acids in Fresh Plant Tissue:

The method used for the extraction of total RNA and DNA is that of Schmidt and Thannhauser, (1945) with some modifications described by Morse and Carter, (1949).Ribonucleic acid (RNA) was estimated colourmetrically by the orcinol reaction as described by Dische, (1953). While, deoxyribonucleic acid (DNA) was estimated by DPA (diphenylamine) colour reaction described by Burton, (1956). The nucleic acids were determination by Spekol Spectrocolourimeter VEB Carl Zeiss.

Determination of Protein Banding Pattern:

Protein extraction was done according to Reuveni *et al.* (1992) with some modifications. Electrophoretic protein profile of wheat shoots were analyzed according to sodium dodocylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique(Sheri, *et al.* 2000), which relates polypeptide maps, molecular protein markers, percentage of band intensity, molecular weight and mobility rate of each polypeptide to standard markers using gel protein analyzer version 3 (MEDIA CYBERNE TICE, USA)

Statistical Analysis:

The results were statistically analyzed using MSTAT- C software. The mean comparisons among treatments were determined by Duncan's multiple range test at 5 % level of probability (Gomez and Gomez, 1984).

RESULTS AND DISSCUSION

Enzyme Activities:

Antioxidant defense system in plants contains enzymatic and non – enzymatic antioxidants. The enzymatic system consists of enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (ARx), monohydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Keles & Öncel, 2002).

The results of the present work showed that, the activities of the antioxidant enzymes (peroxidase (POX) and polyphenol – oxidase (PPO) in wheat shoots exposed to the high temperature stress for 4 or 8 hrs were significantly increased, while, superoxide dismutase (SOD) and catalase (CAT) were decreased as compared with those of the control plants (Fig 1). These results were similar to those obtained by Choudhary *et al.* (1993) who indicated that, heat shock treatment increased peroxidase activities in sorghum plants. In addition,

Auld & Paulsen (2003) reported that, high temperature stress increased the activity of PPO enzyme of wheat. On the other hand, Liu & Huang (2002) found that, the activities of SOD and CAT were decreased in response to high temperature stress in bentgrass plants. Moreover Keles & Öncel (2002) indicated that, CAT activity was decreased at supraoptimal temperature of wheat seedlings.

The antioxidant enzyme system for scavenging the toxic oxygen species acts in various compartments of plant cells. Bowler *et al.* (1992) stated that, antioxidant defense mechanism has been developed for protection against reactive oxygen species (ROS). Edreva *et al.* (1998) revealed that, heat shock induced oxidative damage which influenced by regulating the activities of POX and SOD in *Phaseolus vulgaris* plants.

It has been found in the present investigation that, exogenous application of either arginine or Put decreased significantly the activities of POX and PPO, while enhanced the activities of SOD and CAT of wheat plants as compared with those of the control plant or plants that exposed to high temperature stress without foliar treatment. The magnitude of such response was much more pronounced in response to the application of 2.5 mM of either arginine or Put (Fig 1). These results may be attributed to the potential effect of PAs which act as free radical scavenger(Nayyar and Chander, 2004).. These results are in harmony with those obtained by Bors *et al.* (1989) who found that, PAs could effectively scavenge $O_2^{\cdot}$ generated in both chemical and enzymatic cell – free systems, and added that, PAs inhibit the production of $O_2^{\cdot}$ by the superoxide – dependant conversion of 1 – amino – cyclo – propane – 1 - carboxylic acid to ethylene. Moreover, Papadakis & Roubelakis – Angelakis (2005) confirmed that, exogenous PAs induced significant increases in SOD and CAT in tobacco. Also, Bekheta & El – Bassiouny (2005) reported that, foliar application of Put in general; decreased POX and PPO in wheat plant under salinity stress, however, CAT and SOD activities were increased.

Regarding the effect of high temperature stress on indole acetic acid oxidase (IAA – Oxidase), Fig 1 shows that, exposing wheat plants to 4 or 8 hrs of high temperature induced an increase in IAA – oxidase activity over that of the untreated plant (Fig 1). These results are in agreement with those obtained by Morgan (1990). El – Bassiouny (2004) also, showed a reduction in IAA contents in pea shoots sown at high temperature, and concluded that, high temperature severely inhibited the biosynthesis of auxin and / or increased their oxidation into inactive form. In this connection, Bekheta & El – Bassiouny (2005) indicated that, the increase in stress levels (salinity) is correlated with the increase in the activity of IAA – oxidase in wheat plants.

Arginine or Put treatment led to sharp decrease in IAA – oxidase activity as compared with that of plants subjected to high temperature for 4 or 8 hrs. The magnitude of reduction was increased by increasing the concentration of both substances (Fig 1). These results support by the finding of El – Bassiouny *et al.* (2008) which indicated that, arginine or putrescine treatments increased significantly IAA contents of wheat plants.

Lipid Peroxidation:

The present investigation showed that, exposing wheat shoots to 4 or 8 hrs of high temperature induced a significant increase (1.86 fold or 2.21 fold) in lipid peroxidation, over the control plant (Fig 2). This result was in agreement with those obtained by Larkindale & Knight (2002) using *Arabidopsis thaliana*.High temperature stress is known to damage the cell, and alter most of its components and functions. Some of these damages caused by heat – induced oxidative stress (Karim *et al.,* 1999).

It has been found in the present investigation that, arginine or Put application decreased significantly the malondialdehyde contents under both untreated and plants exposed to high temperature stress. The magnitude of reduction was much more pronounced by applying 2.5 mM of either arginine or putrescine (Fig 2). These results are in a good harmony with those obtained by Tang *et al.* (2004) and Nayyar & Chander (2004) who reported that, exogenous application of PAs decreased the oxidative damage by reducing the malondialdehyde content in response to different stresses in pine and chick pea plants. Several investigators concluded that, the exogenous application of PAs decreased the autooxidation of membrane lipids and stabilize the membrane in wheat (HuiGuo *et al.,* 2006); and maize (Todorov *et al.,* 1998). Similar results were obtained by Basra *et al.* (1997) who found that, PAs maintained membrane integrity during heat shock by decreasing membrane electrolyte leakage and lipid peroxidation, and added that, PAs could ameliorate the effect of heat shock on mung bean plant. Moreover, the recent work of Bekheta & El – Bassiouny (2005) suggested that, PAs stabilize membranes by associating (as organic polycations) with negatively charged phospholipids.

The presented results; in this respect; showed that, lipid peroxidation (Fig 2) was decreased in plant treated with PAs or arginine under high temperature stress compared to plants exposed to high temperature only. This decrease might be attributed to the obvious increase in antioxidant enzymes activity (SOD) and (CAT) (Fig 1) which in turn promoted the scavenging of the harmful free radicals and hydrogen peroxide.

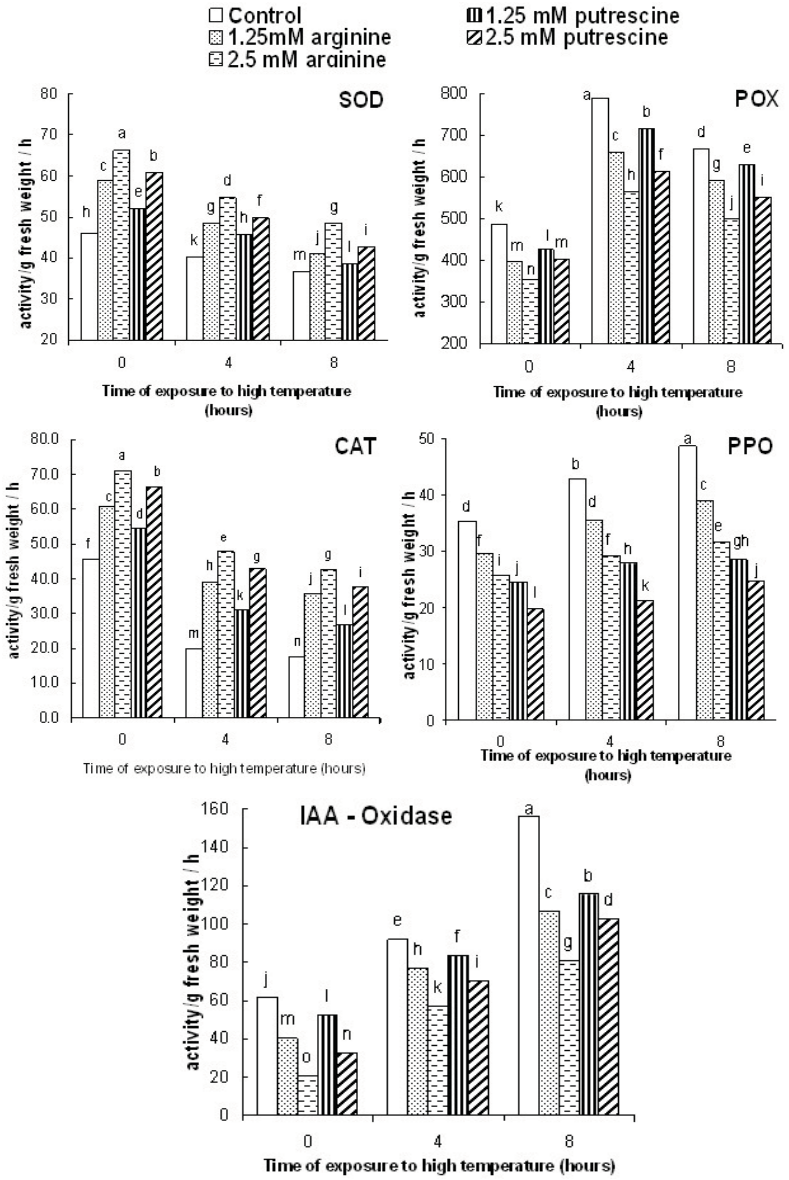

Fig. 1: Effect of foliar treatments of arginine or putrescine at 30 days after sowing on activity of oxidative evzymes (activity / g fresh weight/ h) of wheat shoots exposed for two periods (4 or 8 hre). of high temperature stress (35C±2) at 40 days after sowing.

Nucleic Acid Contents:

DNA content of wheat shoots exposed to high temperature stress; decreased significantly with increasing period of exposure (4 – 8 hrs) (Fig 3). Meanwhile, RNA content exhibited an increase with increasing time of exposure to high temperature compared to that of the untreated plant. The reduction of DNA content in stressed plants may be attributed to the role of ROS which was released at high temperature stress in inducing DNase activities and enhancement of DNA fragmentation. These results are in agreement with those obtained by Papadakis & Roubelakis – Angelakis (2005) who reported that, in tobacco plants, ROS detected at stress was accompanied with induction of DNase activities and enhanced DNA fragmentation and methylation. In this respect, Garg (2002) showed that stress decreased DNA content in soybean plants. Also, Bekheta & El – Bassiouny (2005) revealed that, the reduction in DNA content reached about 36 % in wheat plant exposed to salinity stress.

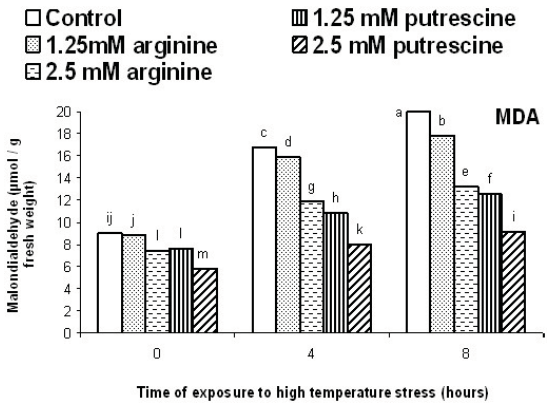

Fig .2: Effect of foliar treatments of arginie or putresine at 30 days after sowing on lipid peroxidation (as malond-ialdehyde contents) of wheat shoots exposed for two periods (4 or 8 hrs) of high temperature stress (35°C±2) at 40 days after sowing.

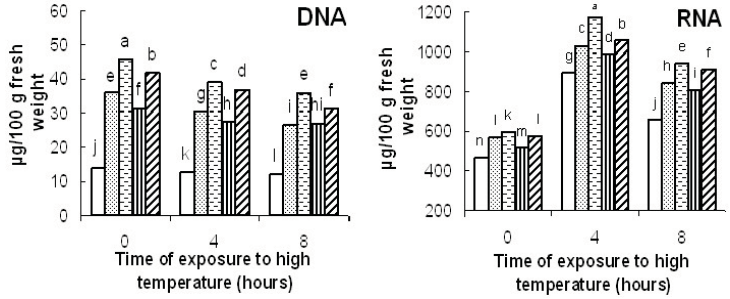

Fig. 3: Effect of foliar treatments of arginie or putresine at 30 days after sowing on nucleic acid contents (µg /100g fresh weight) of wheat shoots exposed for two periods (4 or 8 hrs) of high temperature stress (35°C±2) at 40 days after sowing.

Concerning the effect of high temperature stress on RNA content the results in Fig 3 showed that, RNA content was significantly increased at 4 hrs of high temperature and then decreased at 8 hrs of high temperature but their levels still over the untreated control. These results are in a good harmony with those obtained by Lanciloti *et al.* (1996) who found that, exposure to 40°C for 2.5 hrs resulted in the induction of HSP 70 mRNA.The increase in RNA contents as a result of high temperature stress may be attributed to the mRNA levels of certain enzymes which increased during heat stress. The same conclusion was reported by Lindquist and Craig (1988). Moisyadi and Harrington (1990) suggested that, the increase in the RNA content may be a part of a complex reequilibration of cellular metabolism at elevated temperature in sugarcane. However, several investigators concluded that, HSP mRNA level is strongly induced during heat stress in wheat plants (Helm *et al.,* 1990). Moreover, Nieto – Sotelo *et al.* (1999) indicated that, accumulation of HSP 101 mRNA was high under heat shock conditions in maize plant.

The foliar application of either arginine or Put on wheat plants exposed or without exposure to high temperature increased (significantly) both DNA and RNA contents over plants exposed to high temperature or untreated plants. The magnitude of these increments was much more pronounced in response to 2.5 mM of either arginine or Put (Fig 3). These results are in agreement with those obtained by Chattopadhayay *et al.* (2002). Several investigators stated that, PAs bind strongly to RNA and DNA, so they can protect RNA and DNA from degradation which in turn retarding senescence (Sood & Nagar, 2003). Also, Bekheta & El – Bassiouny (2005) indicated that, PAs induced accumulation of RNA by inhibiting RNase activity. Furthermore, the previous studies of Borrell *et al.* (1996) stated that, the increase in mRNA levels was associated with an enhancement of Put levels in stressed oat leaves. PAs may affect the gene expression via altering the sequence – specific DNA – protein interaction (Panagiotidis *et al.,*1995), activate / modulate protein kinases involved in signal transduction, (Shore *et al.,*1997) or inhibit DNA methylation and fragmentation and / or reduce DNase activities (Papadakis & Roubelakis – Angelakis, 2005).

Protein Electrophoretic Pattern:

The changes in the protein electrophoretic pattern in wheat shoots exposed to 4 or 8 hrs of high temperature exhibit an increase in the number of protein bands to 14 and 12 bands (respectively) as compared to the control (8 bands), Table (1). Also, new proteins appeared in shoots subjected to high temperature at molecular weights 111.2, 90.6, 70.5, 45.6, 32.4, 24.4 and 8.7 K Da. The de - novo synthesis of new set of proteins especially HSPs may be the most important mechanisms involved in the cell protection against heat shock. The same results were obtained by Hendershot *et al.* (1992) detected low (16 – 18 KDa) and high (27 – 94 KDa) molecular weight HSPs synthesized in seedling leaf blades and flag leaves of flowering wheat plants after only 2 hours of heat shock.

The heat shock response and the HSPs along with the correlation of HSP expression with cellular resistance to high temperature led to the hypothesis that HSPs protect cells from the detrimental effects of high temperature and that accumulation of HSPs leads to increased thermotolerance (Landry & Gierasch, 1994). High temperature induced great significant modifications in protein pattern of wheat plant (Table 1). In this connection, Kuznetsov & Shevyakova (1997) reported that, high temperature stress induced the synthesis of polypeptides and accumulated the HSPs at different M wt. (96, 80, 70, 35 and 24 K Da.) in tobacco plants. Also, Pareek *et al.* (1998) found that, when intact rice seedlings were subjected to high temperature the induction of proteins (M wts. 104, 93 and 76 K Da.) were detected. Moreover El-Bassiouny (2004) confirmed this effect where, new set of proteins appeared in pea shoots subjected to high temperatures at M wts. 206, 157, 127, 104, 91, 70, 37, 29 and 23 K Da. Protein at molecular weight (45 K Da) was detected in the wheat shoots exposed to high temperature; in the present work; (Table 1). Several evidences indicated that, the 45 K Da HSPs may play a role in the development of thermotolerance in maize plants. In addition, this HSP was synthesized over a broad temperature range starting from 35°C upwards (Bhadula *et al.,* 2001). High temperature stress induced the disappearance of protein band at M Wt. 34 K Da. in wheat plants (Table 1). This result is in harmony with those obtained by Li *et al.* (2003) who reported that exposing cucumber plants to 40°C caused a loss of 33-K Da polypeptides. On the other hand, Krishnan *at al.* (1989) concluded that, the large increase in the LMW HSP of wheat at 34 K Da was concomitantly with the reduction in normal protein synthesis during heat shock.Synthesis of new protein band at molecular weight 24 K Da in wheat plants subjected to high temperature (Table 1) is supported the previous studies of Annamalai *et al.*(1999) who demonstrated that, three polypeptides of 17 – 24 K Da LMW – HSP were appeared in plants subjected to 35°C.Moreover, the present work showed that, the protein of molecular weight 8.7 K Da appeared in wheat shoot under high temperature stress (Table 1), may be ubiquitin, which is Known as a very small heat shock stress protein consisting of 76 amino acids. Also, Ferguson *et al.,* (1990) stated that ubiquitin could be appear at 8.5 KDa. Ubiquitin appeared to protect protein from degradation by proteases through tagging the protein (Hershko, 1988).

Table 1: Relative area (%) of each protein band of wheat shootr sprayed with different concentrations of arginine or putrescine at 30 dayes after sowing and exposed for two periods (4 or 8hrs) of high temperature stress (35°C±2) at 40 days after sowing.

M WT. K Da.	Time of exposure to high temperature (hous)														
	Control					4					8				
	0	Arginine (mM)		Putrescine (mM)		0	Arginine (mM)		Putrescine (mM)		0	Arginine (mM)		Putrescine (mM)	
		1.25	2.5	1.25	2.5		1.25	2.5	1.25	2.5		1.25	2.5	1.25	2.5
306.6	-	-	6.37*	10.25*	12.26*	-	-	-	-	-	-	-	-	-	-
229.7	52.83	10.1	-	-	7.48	12.23	2.63	0.77	5.44	1.55	8.63	8.93	6.31	8.98	4.32
111.2	-	5.23*	16.52*	13.75*	8.29*	4.83**	7.35**	6.46**	6.57**	5.36**	5.32**	5.98**	4.31**	5.98**	6.32**
90.62	-	-	-	-	-	10.52**	13.35**	10.91**	12.15**	10.37**	7.48**	8.56**	7.47**	8.26**	8.12**
80.9	-	8.76*	9.35*	10.12*	8.87*	-	6.53**	6.75**	4.75**	8.85**	-	2.16**	2.39**	3.86**	3.92**
70.51	-	-	-	-	-	3.42**	3.98**	2.11**	3.13**	2.35**	5.42**	6.11**	5.65**	6.73**	5.32**
63.3	11.37	13.68	7.62	11.03	9.58	7.68	2.6	2.6	2.7	3.1	7.11	7.75	5.33	6.66	4.42
45.6	-	-	-	-	-	3.77**	-	-	-	-	-	-	-	3.36**	1.46**
39.9	7.63	7.53	8.73	12.15	9.98	9.36	7.68	8.37	8.53	9.22	9.99	10.22	10.88	9.81	11.27
37.8	8.6	12.75	13.42	8.34	6.12	10.05	6.58	8.24	7.36	9.28	11.06	12.89	13.96	10.31	15.66
34	5.25	7.23	16.34	10.1	9.16	-	0.73*	6.03*	0.22*	4.17*	-	-	2.12*	-	5.38
32.4	-	8.65*	7.89*	-	4.27*	2.26**	4.36**	3.75**	3.02**	2.57**	2.97**	5.06**	4.21**	2.63**	5.57**
30.6	5.27	11.52	-	12.15	10.92	3.14	6.13	4.11	5.15	4.81	6.07	-	-	7.82	-
24.38	-	-	-	-	-	17.11**	10.62**	10.87**	11.37**	8.48**	20.33**	14.00**	14.86**	11.03**	13.66**
16.5	5.47	8.86	8.07	5.99	2.13	4.3	2.6	4.08	1.62	-	-	-	-	-	-
12.7	3.58	5.69	5.69	6.12	5.99	3.25	7.04	8.39	7.39	8.93	4.67	6.12	8.23	5.03	6.15
8.7	-	-	-	-	-	10.30**	16.12**	18.04**	18.14**	19.34**	11.13**	12.22**	14.28**	9.54**	8.43**
Total number of bands	8	11	10	10	12	14	15	15	15	14	12	12	13	14	14

The combined effect of arginine or putrescine and high temperature stress induced the appearance of 7 new protein bands their molecular weights are 111.2, 90.6, 70.5, 45.6, 32.4, 24.4 and 8.7 KDa as compared with the untreated control. This indicates that both arginine or putrescine and high temperature stress share a

common mechanism. It is also indicated from Table 1 that the protein band of molecular weight 80.9 KDa is specific for arginine or putrescine treatment. The promotive effect of PAs in ameliorating the high temperature injury via inducing the synthesis of heat shock proteins may be attributed to the role of PAs in improving the stability of enzyme or protein molecules which were affected by heat stress. In this respect, El-Bassiouny (2004) indicated that, improving heat tolerance due to putrescine application might be attributed to the induction of new proteins biosynthesis in shoots of pea plants their molecular weights were 94 and 41. In addition, the same treatment increased the level of small HSPs at molecular weights 29 & 23 K Da under high temperature. Bekheta & El – Bassiouny (2005) observed that, foliar application of Put to wheat cultivars induced the synthesis of new set of protein bands at molecular weights 309, 91,70, 36, 21, 17 and 15 K Da which may play a key role in increasing the tolerance of plant to salinity stress.

ABBREVIATIONS

Days after sowing; DAS, Superoxide dismutase; SOD, Catalase; CAT, Peroxidase; POX, Polyphenol oxidase; PPO, Indole acetic acid oxidase; IAA – Oxidase, Malondialdehyde; MDA, Putrescine; Put,

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