Pak. J. Bot., 45(1): 111-118, 2013.

IMPROVING THE THERMO TOLERANCE OF WHEAT PLANT BY FOLIAR APPLICATION OF ARGININE OR PUTRESCINE

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Abstract

The exposure of wheat plants cv. Giza 168 to high temperature stress (35°C±2) for 4 or 8 hours significantly decreased the growth parameters, the yield components, putrescine (Put), total endogenous polyamines (PAs) contents, total amino acid and total essential amino acid contents, where as, the endogenous spermidine (Spd) and spermine (Spm), ethylene, NH^{+4}, glutamic acid, arginine, proline were increased. Treatment of wheat plants before their exposure to high temperature with arginine or putrescine (1.25 and 2.5 mM) enabled the plants to tolerate the injurious effect induced by high temperature stress via increasing the endogenous Put, Spd , the total PA contents, the content of total amino acids, essential amino acids and the ratio of essential to non-essential amino acids and decreasing the ethylene and NH^{+4} contents as compared with plants exposed to the high temperature stress or the untreated plants. These effects were much more pronounced by applying 2.5 mM of either arginine or putrescine.

Introduction

High temperature is one of the most important environmental factor affecting plant growth and development. It could alter transpiration rate and membrane permeability that leads to growth reduction and poor yield (Morgan, 1990). Application of heat event immediately before anthesis or during anthesis induced a significant reduction in the measured growth parameters (Wollenweber *et al*., 2003) and grain yield (Russel & Wilson, 1994). Macas *et al*., (2000) showed that, grain yield, kernel number and kernel weight of wheat reduced at 35/20°C as compared with 20/20°C. Auld & Paulsen (2003) added that, high temperature stress significantly reduced yield and its components of wheat plants. Miao & Cao (2002) also demonstrated that, putrescine, spermidine and spermine contents decreased when cucumber seeds were germinated under high temperature (35°C**).** In addition free amino acid contents were decreased with increasing stress in maize plant (Willadino *et al*., 1996). Moreover, Kuznetsov *et al*., (2002) stated that, the heat shock treatment of *Mesembryanthermum crystallinum* plant induced intense evolution of ethylene during the first 6 hrs after heating treatment.

Application of polyamines either prior to heat shock or during heat shock period itself enhanced the recovery of growth of both roots and hypocotyls of *Vigna radiata* seedlings, putrescine being the most effective then spermidine and spermine (Basra *et al*., 1997). Moreover, polyamines were effective in improving grain yield of wheat plant (Iqbal *et al*., 2006; El-Basiouny *et al*., 2008). The exogenous application of putrescine and/or spermidine and/or spermine with concentrations ranged between (0.1-1 mM) led to accumulation of endogenous polyamine contents (putrescine, spermidine and spermine) in wheat plants, (Kubis *et al*., 1991) in leaves and thylakoid membrane of cucumber (He–Lixiong *et al*., 2002). However, with respect to arginine, Kakkar *et al*., (2000) reported that, the endogenous polyamine contents were increased by the addition of arginine. Li *et al*., (2003) also recorded that exposing plant to 38°C caused a gradual increase in spermidine and spermine titers in leaves of cucumber plants. On the other hand, (Yang & Yang, 2002) showed that, the prolonged exposure to high temperature (36/23°C for 12 days) increased polyamines in general and putrescine contents within 6 days of treatment but inhibited their synthesis in response to prolonged exposure in *Brassica alboglabra Bailey* seedlings. Mansour *et al*., (2002) found that, polyamine application (2.5 mM putrescine, 5 mM spermidine and 2.5 mM spermine) decreased polyamine/diamine ratio of wheat plant. Bais *et al*., (2001) reported that, under exogenous putrescine application (40mM), ethylene production was lower in both untransformed and transformed *Cichorium intybus* cultures.

The protective role of polyamines (putrescine, spermidine and spermine) on plants was associated with an inhibition of ethylene evolution on maize plants (Todorov *et al*., 1998). Putrescine treatment also decreased ethylene biosynthesis and directly antagonizes several ethylenes–mediated responses in many terrestrial plants (Matto & White, 1991), and delayed senescence of wheat seedling (Mansour *et al*., 2002).

In this study we used physiological markers (e.g., growth, endogenous polyamines biosynthesis, ethylene, protein amino acids and yield components) to ascertain that foliar application of arginine or putrescines had improved the thermo tolerance of wheat plant.

Materials and Methods

This investigation is a part of three complementary experiments which were done at the same conditions (El-Bassiouny *et al*., 2008; Khalil *et al*., 2009). The experimental plant used in this investigation was wheat (*Triticum aestivum* cv. Giza 168). Pure strain of grains was obtained from Egyptian Ministry of Agriculture. The chemicals used in the present work are (i) arginine (one of the essential amino acids), (ii) putrescine (a member of polyamine group), they were supplied from Sigma–Aldrich. Green house experiments were conducted in the National Research Centre (Cairo, Egypt) for two successive seasons. A homogenous lots of wheat grains *Triticum aestivum* cv. Giza 168 were sown in pots (50cm in diameter and 50cm in depth) containing equal amounts of clay soil (20kg). Fertilization was done with the recommended dose (5g phosphorus/pot as triple phosphate, 6g nitrogen/pot as urea and 5g potassium/pot as potassium sulphate) during preparation of pots and after sowing. After 15 days after sowing (DAS) thinning was carried out, so as 5 uniform seedlings were left in each pot.

The pots were divided into 5 groups each composed of 30 pots. The plants of the 5 groups were sprayed with H_2O, 1.25 and 2.50 mM arginine or 1.25 and 2.50 putrescine. These treatments were carried out twice (30 and 35 DAS). After 5 days, each group was divided into 3 sets each contain 10 pots, the sprayed plants of the first set were exposed to normal temperature (20^{o}C control), the second set exposed to 35^{o}C ± 2 for 2 times of 4 hours at 2 successive days. The third set exposed to 35^{o}C ± 2 for 4 hours and returned to normal temperature and the exposure to 35^{o}C ± 2 repeated again at the following day on the same set. Three plants from each pot were used for biochemical analysis and the remaining two plants were left to grow for 155 DAS for studying the effect of different treatments on the yield component. The harvest index (HI) and crop index (CI) were determined according to Beadle (1993).

Harvest Index = Economic yield (Grains yield)/Straw yield
Crop Index=Grain yield/Biological yield (Grains yield+Straw yield)

Chemical analysis

Fresh and dry weights of wheat plants were recorded after 48 hrs from exposure to high temperature stress. Endogenous polyamine contents were estimated in fresh leaves. Ethylene content was determined in fresh plants. While, amino acids were determined in fresh tissues of leaves and then calculated as mg/100g dry weight. In addition, total carbohydrate and protein percentages were determined in the powder of dry yield of wheat grains.

Total carbohydrates were determined using the method described by Dubois *et al*., (1956). Total nitrogen was determined by using micro-kjeldahl method described by Peach & Tracy (1956). The protein was calculated by multiplying total nitrogen by 6.25. Putrescine, spermine and spermidine were extracted and determined in all tested samples according to Mietz & Karmas (1977). The method of ethylene determination was essentially similar to that adopted by Luttus *et al*., (1996). Amino acid composition of wheat leaves protein was estimated according to the catalog of amino acid analyzer (1999) LC 3000.

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 & Gomez, 1984).

Results

Growth and yield: The data obtained in this study revealed that exposing wheat plants to the high temperature (heat shock) reduced growth through decreasing fresh and dry weights of shoots below those of the untreated plants (Table 1). It also induced significant reduction of plant height, number of tillers per plant number , weight of spikes per plant, number , weight of grains per plant, weight of 1000 – grains, straw, biological yield per plant and harvest , crop index of plants as compared with those of wheat plants raised at normal temperature. The magnitude of reductions, in most cases was increased with increasing the time of exposure (Table 2). The quality of wheat grains was damaged by high temperature treatments; there were significant decreases in both carbohydrate and protein percentages compared to those of the untreated control plants (Table 2).

Foliar Spraying of wheat shoots with arginine or Put before exposing to high temperature significantly increased fresh and dry weights and water contents of shoots as well as yield components over those of the corresponding plants exposed to high temperature stress alone and in some cases over the untreated plants (Tables 1 & 2). All concentrations used of either arginine or Put induced significant increases in the carbohydrate and protein percentages of stressed wheat grains as compared with those of the corresponding (Table 2). These increments were much more pronounced in response to the application of either substance at 2.5 mM. However, arginine treatments were more effective than Put in this respect.

Table 1. Effect of foliar treatments of arginine or putrescine at 30 DAS on fresh & dry weights and relative water content of wheat shoots exposed for two periods (4 and 2 times 4 hrs) of high temperature stress (35^{o}C ± 2) at 40 DAS. Means with the same letters are significantly not different.

Treatment		Fresh weight / plant (g)			Dry weight / plant (g)			Relative water content (%)		
		Time of exposure to high temperature (hrs)								
		0	4	8	0	4	8	0	4	8
Control		2.5^{cde}	1.9^{fg}	1.7^{g}	0.65^{bc}	0.58^{c}	0.55^{c}	74	70	67
Arginine (mM)	1.25	3.2^{ab}	2.9^{abc}	2.5^{cde}	0.80^{a}	0.77^{ab}	0.73^{ab}	75	74	71
	2.5	3.4^{a}	3.0^{abc}	2.9^{ad}	0.82^{a}	0.79^{ab}	0.75^{ab}	75	74	74
Putrescine (mM)	1.25	2.9^{ad}	2.4^{cf}	2.2^{efg}	0.78^{ab}	0.76^{ab}	0.73^{ab}	73	69	67
	2.5	3.3^{a}	2.7^{be}	2.3^{def}	0.81^{a}	0.78^{ab}	0.74^{ab}	76	71	67

Table 2. Effect of foliar treatments of arginine or putrescine at 30 DAS on yield components of wheat plants exposed for two periods (4 and 2 times 4 hrs) of high temperature stress ($35^{o}C \pm 2$) at 40 DAS. Means with the same letters are significantly not different.

	Control			Arginine (mM)						Putrescine (mM)					
				1.25			2.5			1.25			2.5		
	Time of exposure to high temperature														
	0	4	8	0	4	8	0	4	8	0	4	8	0	4	8
Plant height (cm)	89^{a}	68^{d}	66^{d}	67^{d}	63^{de}	58^{fg}	67^{d}	60^{ef}	55^{g}	77^{b}	67^{d}	60^{ef}	72^{c}	63^{de}	57^{fg}
Tillers number/plant	3.5^{c}	2.0^{d}	2.0^{d}	5.7^{a}	2.5^{d}	2.1^{d}	4.7^{b}	2.3^{d}	2.1^{d}	6.0^{a}	2.3^{d}	2.0^{d}	6.3^{a}	2.7^{d}	2.0^{d}
Spikes number/plant	3.0^{d}	1.0^{f}	1.0^{f}	4.8^{bc}	2.0^{e}	1.0^{f}	4.5^{c}	2.3^{e}	1.0^{f}	5.1^{ab}	2.3^{e}	1.0^{f}	5.5^{a}	2.3^{e}	1.0^{f}
Weight of grains/plant (g)	4.7^{f}	3.5^{hi}	2.7^{j}	7.8^{b}	5^{ef}	3.4^{hi}	9^{a}	5.3^{d}	4^{g}	6.7^{c}	4.8^{ef}	3.2^{i}	7.7^{b}	5.0^{de}	3.6^{h}
1000 grains weight (g)	38^{e}	27^{k}	22^{l}	42^{c}	36^{g}	27^{k}	44^{a}	39^{d}	30^{j}	42^{c}	37^{f}	31^{i}	44^{b}	38^{e}	35^{h}
Straw yield/plant (g)	7.9^{ef}	7.2^{g}	6.8^{h}	11.3^{a}	9.1^{c}	8.1^{e}	10.9^{b}	8.6^{d}	7.2^{g}	8.1^{e}	7.4^{g}	7.2^{g}	7.7^{f}	6.6^{h}	6.5^{h}
Harvest index (%)	37^{h}	33^{j}	28^{m}	41^{e}	35^{i}	30^{l}	45^{c}	38^{g}	36^{i}	46^{b}	39^{f}	31^{k}	50^{a}	43^{d}	35^{i}
Crop index (%)	58^{h}	49^{k}	39^{n}	69^{e}	54^{j}	42^{m}	82^{c}	62^{g}	56^{i}	85^{f}	65^{f}	45^{l}	99^{a}	76^{d}	55^{i}
Carbo-hydrate content (%)	48^{h}	47^{i}	46^{j}	53^{d}	50^{f}	49^{g}	55^{a}	54^{b}	50^{f}	52^{e}	50^{f}	49^{g}	54^{b}	53^{c}	52^{de}
Protein content (%)	15^{fg}	13^{h}	12^{i}	16^{bc}	16^{de}	13^{h}	17^{a}	16^{cd}	15^{f}	16^{bc}	15^{fg}	13^{h}	16^{ab}	15^{e}	14^{g}

Endogenous polyamine contents: The endogenous levels of Put, Spd, Spm and total PA contents were variable in response to wheat shoot exposure to 4 or 8 hrs of high temperature stress. Put and total PA contents were significantly decreased. The opposite trend was observed in response to Spd and Spm contents which exhibited marked increases as compared to the untreated control (Fig. 1).

Ethylene contents: Exposure of wheat shoots to heat stress for 4 or 8 hours increased the ethylene biosynthesis (1.59 fold at 4 hrs and 2.16 fold at 8 hrs) as compared with those of the untreated plants (Fig. 2). All concentrations used of either arginine or Put decreased the ethylene biosynthetic activity of all stressed plants and in some cases of control ones. The magnitude of reduction was much more pronounced by application of 2.5 mM of putrescine followed by arginine (Fig. 2).

Amino acid composition: High temperature stress decreased the total amino acid contents, total essential amino acid contents (therionine, valine, methionine, leucine, isoleucine and phenylalanine) and the ratio of essential to non essential amino acids. However, the same treatments increased markedly glutamic, proline, alanine, tyrosine, histidine, lysine, NH_4^+ (is very toxic) and arginine contents in wheat leaves compared to those of the untreated ones (Table 3). The magnitude of variation was increased with increasing time of exposure to high temperature (4-8 hours). Proline is one of the most important amino acids; which accumulated under high temperature stress in the present work (Table 3). In this trend, simultaneous treatment of *Nicotiana sylvestris* cells with high temperature ($40^{o}C$) resulted in transient proline accumulation which can be correlated with an increase in thermo tolerance (Shevyakova *et al*., 1994**).** In respect to the foliar supply of arginine or put, in most cases increases in the content of total amino acids, essential amino acids and the ratio of essential to non-essential amino acids were observed compared to plants exposed to high temperature alone or untreated plants (Table 3). It is worthy to mention that, application of Put or arginine before exposing wheat plants to the high temperature stress increased the amino acids (arginine, proline and methionine), while decreased the NH_4^+ contents as compared with the untreated plants or plants exposed to high temperature only (Table 3). The obtained data were supported by Kesba (2005) who reported that, L–arginine treatments enhanced the levels of arginine, aspartic, glutamic, proline and methionine in grape roots.

Generally, foliar application of either arginine or Put on wheat plant before exposing to high temperature induced significant increases in both methionine and PA concomitantly with the reduction in ethylene (Table 3; Figs. 1and 2).

Discussion

Growth and yield: The reduction in fresh weight of wheat shoots in response to heat shock treatment concomitantly with the decrease in water content (Table 1) can be ascribed to the effect of high temperature on the membrane permeability and the transpiration rate (Morgan, 1990). The reduction in the yield components of wheat plant in response to high temperature stress might be attributed to the inhibitory effect of high temperature on growth (Table 2) and reduction of total PAs (Fig. 1) which are involved in the regulation of plant growth and development. Also, the high temperature stress decreased antioxidant enzymes activity leading to accumulation of H_2O_2 and consequently increased lipid peroxidation (Khalil *et al*., 2009), and ethylene production. This resulted in reduction of growth and consequently grain weight which associated with a decrease of starch accumulation (carbohydrate content) and the disruption of normal protein synthesis (protein content) under high temperature stress. The decrease in starch synthesis under high temperature might be due to the reduced conversion of sucrose to starch or to the alteration in catalytic activity of a number of enzymes in the pathway of starch synthesis (Wallwork *et al*., 1998). In addition, Stone & Nicolas (1998) stated that, the heat shock proteins which putatively provide protection from stress, could damage wheat quality, since the synthesis of normal protein is largely replaced by heat shock proteins during a heat shock event (Ristic *et al*., 1992; Kasim, 2006).

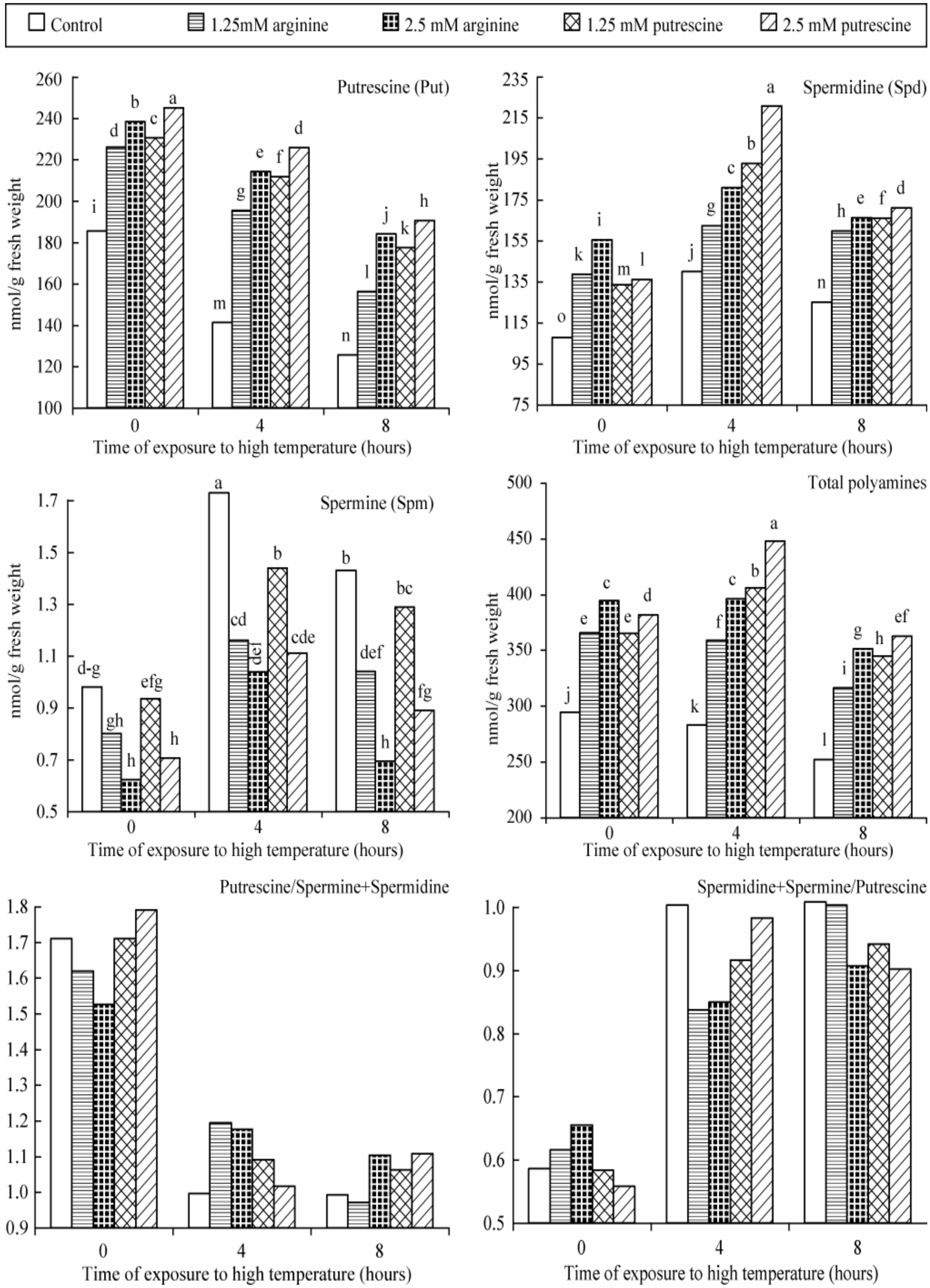

Fig. 1. Effect of foliar treatments of arginine or putrescine at 30 DAS on endogenous polyamine contents (nmol/g fresh weight) of wheat shoots exposed for two periods (4 and 2 times 4 hrs) of high temperature stress (35°C ± 2) at 40 DAS.

Table 3. Effect of foliar treatments of arginine or putrescine at 30 DAS on amino acid compositions (mg/100 g dry weight) of wheat plants exposed for two periods (4 and 2 times 4 hrs) of high temperature stress (35°C ± 2) at 40 DAS. Means with the same letters are significantly not different.

Amino acids	Time of exposure to high temperature (hrs)														
	Control					4 hours stress					8 hours stress				
	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
Aspartic	7	10	12	11	12	6	7	12	10	10	4	5	10	7	8
*Thereonine	4	4	6	5	6	3	3	5	4	5	2	3	3	3	4
Serine	5	8	10	7	9	5	6	9	7	6	4	4	6	5	5
Glutamic	18	24	43	33	56	21	37	50	37	61	23	47	59	42	67
Proline	15	25	39	34	39	28	30	42	40	42	34	33	50	42	45.
Glycine	42	39	35	35	30	33	31	27	29	22	23	19	20	19	16
Cysteine	10	14	-	18	-	-	-	-	-	-	-	-	-		3
Alanine	6	7	8	8	8	4	6	8	6	6	4	4	7	4	4
*Valine	6	8	8	8	9	5	6	7	7	7	4	3	4	4	4
*Methionine	0.4	0.9	1.0	0.7	0.9	0.4	0.6	0.8	0.6	0.8	0.2	0.6	0.8	0.6	0.6
*Leucine	6	9	11	9	10	5	6	9	7	7	4	4	6	5	6
*Isoleucine	14	16	19	17	18	10	12	14	11	12	7	8	11	7	9
*Phenylalanine	1	2	3	3	4	1	1	3	2	2	1	1	2	2	2
Tyrosine	5	6	8	8	10	5	7	8	9	11	6	8	8	9	11
*Histidine	5	8	11	9	10	6	8	12	9	11	7	9	12	9	12
*Lysine	11	14	16	16	19	11	15	18	17	20	12	18	18	19	20
*Arginine	10	15	19	16	19	11	16	19	18	20	12	18	20	18	21
NH_4^+	17	13	8	14	11	18	15	11	19	13	21	17	12	20	15
*Essential	57.5	76.9	94	83.7	95.9	52.4	67.6	87.8	75.6	84.8	49.2	64.5	76.8	67.6	78.6
Non- essential	108	133	155	99	164	102	124	156	138	158	98	120	160	128	159
Total amino acids	165.5	209.9	249	182.7	259.9	154.4	191.6	243.8	213.6	242.8	147.2	184.5	236.8	195.6	237.6
Ess./non-ess	0.53	0.57	0.61	0.85	0.58	0.51	0.55	0.56	0.55	0.54	0.5	0.54	0.48	0.53	0.5

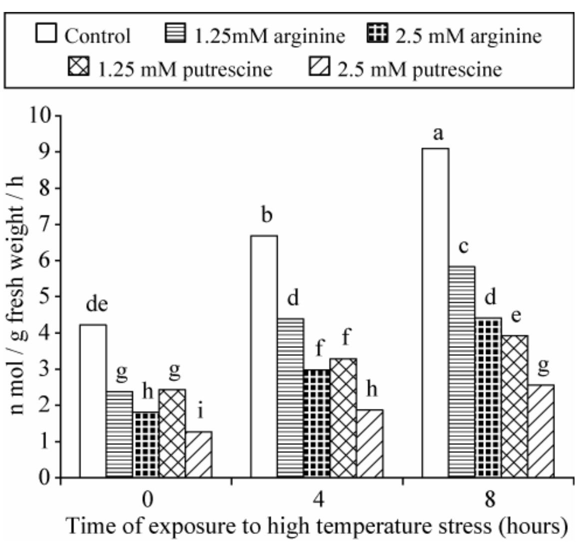

Fig. 2. Effect of foliar treatments of arginine or putrescine at 30 DAS on ethylene contents (nmol/g fresh weight/ h) of wheat shoots exposed for two periods (4 and 2 times 4 hrs) of high temperature stress (35°C ± 2) at 40 DAS.

In contrast to the above result, the quality and quantity of stressed plants were improved in response to Arg or Putt treatment. These results showed the role of PAs in antagonizing the harmful effect of high temperature stress by increasing photo-assimilate and enhancing their translocation to the developing grains in wheat treated plant.

Endogenous polyamine contents and amino acids composition: Amino acids and PAs under stress conditions are directly related in their metabolic pathways and are affected by alteration in enzymatic levels caused by feedback and/or repressive mechanism Slocum & Weinstein (1990). The reduction in Put and PA contents in stressed wheat concomitantly with the intense evolution of ethylene (Fig. 2) might indicate the utilization of S-adenosyl methionine (SAM) into ethylene biosynthesis, in this connection Bouchereau *et al*., (1999) suggested that, the fluxes of SAM forward either ethylene or PAs are extremely responsive to environmental challenges. PAs in general and Put in particular could be reduced via the stimulation of diamine oxidase (DAO) and / or polyamine oxidase (PAO). The decrease in polyamines could be achieved due to the inhibition of certain enzymes which responsible for their synthesis from their precursors; arginine and / or ornithine (Bouchereau *et al*., 1999).

Rabe (1990) and Kasim (2006) reported that, a number of nitrogen–containing compounds accumulate in plants subjected to environmental stress as glutamine, asparagines, proline and ornithine in numerous crop species as barley, oat and peas. Santa Cruze *et al*., (1999) revealed that, glutamic could be converted directly to proline. Several investigators suggested that, proline act as a storage compound of carbon and nitrogen for rapid recovery from stress (Jager & Meyer, 1977) as a free radical scavenger (Smirnoff & Gumbes 1989) and as protective agent of enzyme and membrane (Solomon *et al*., 1994). Proline improves stability of some cytoplasmic and mitochondrial enzymes (Nash *et al*., 1982)). Moreover, Venekamp (1989) suggested that, proline overproduction in stressed conditions is an attempt to regulate cytosolic pH or acidity. In this connection, exogenous application of proline is known to induce abiotic stress tolerance in plants (Ali *et al*., 2007; Kamran *et al*., 2009).

The significant increases in endogenous Put, Spd and in turn total PAs contents (Fig. 1) as result of foliar application of either arginine or Put could occur through the reduction of ethylene biosynthesis since polyamines and ethylene are linked through the common precursor S–adenosylmethionine (SAM), so PA and ethylene could inhibit each other in biosynthesis and/or action (Tari & Csiszar, 2003). In addition, Kesba (2005) reported that, L–arginine treatment enhanced the levels of arginine, aspartic, glutamic, proline and methionine in grape roots. This could be confirmed by the results obtained in the present work which indicated an increase in aspartic, glutamic and arginine and decrease in NH^{+4} content in wheat leaves of the Put and arginine treated plants. Glutamic could be converted directly to proline, (Santa–Cruze *et al*., 1999) or indirectly through the metabolic flux from glutamate under stress conditions which known to be highly in favor of proline synthesis through the glutamate $\Delta 1$ – pyrroline–5 carboxylate (P5C) pathway (Delauney & Verma, 1993). Slocum & Weinstein (1990) reported that arginine and proline accumulation as a result of PA application are considered to be detoxification mechanism to NH^{+4} produced in plants subjected to stress.

Ethylene content: Tari & Csiszar (2003) reported that, PAs and ethylene synthesis were linked through the common precursor (SAM). So, PA and ethylene could inhibit each other in biosynthesis and / or action. Mehionine contains aminopropyl group of the simple diamine Put which contributes to the biosynthesis of PAs through SAM which is also the precursor of the plant hormone ethylene. Ethylene is mostly considered as a stress inducer and PAs as stress inhibitor. So, the role of SAM would seem to be crucial. Feedback controls exist that cause PAs to inhibit ethylene formation and ethylene to inhibit PAs formation (Matto & White, 1991). Thus, each pathway once initiated tends to shut-off the other.

Conclusion

The increases of Spd and Spm in wheat plant after exposure to high temperature stress might be a key factor in cellular protection against heat stress. The predominant amino acids (glutamic, proline and arginine) in the wheat plants exposed to high temperature stress were elevated by prolonging the period of exposure. Among accumulated amino acids, and NH^{+4} which is considered as a very toxic product in plants subjected to high temperature stress.

Foliar application of arginine or Put on wheat plants exposed to high temperature stress, in most cases increased the content of untreated plants. The amino acids particularly (arginine, proline and methionine) while, decreased the NH^{+4} contents as compared with the untreated plants or plants exposed to high temperature. Also, foliar application of either arginine or PUT on wheat plant before exposing to high temperature induced significant increases in both methionine and PAs concomitantly with the reduction in ethylene.

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(Received for publication 28 October 2011)