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Foliar Application Effect of Putrescine on Antioxidative Defense of Wheat (*Triticum aestivum* L. var sw _82_9) under Water Deficit Stress

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ABSTRACT: This study was aimed at the role of foliar application of putrescine (Put) on antioxidative defense of wheat (*Triticum aestivum* L. var Sw_82_9) under water deficit Stress. The experiment was done at Islamic Azad University farm, Varamin branch, during the wheat growing season (March to July). Factorial experiment with complete randomized in 12 treatment and 3 replication at three levels application of Put (0, 75 and 150 ppm) was carried out. Significant difference in Put concentration was not seen between withholding irrigation in steam elongation period and foliar application with pure water ($P<0.05$). Significant difference was not seen in super oxide dismutase (SOD) and catalase (CAT) activities between withholding irrigation in grain filling period and foliar application of Put (150 ppm), respectively ($P<0.05$). There was no significant difference in MDA activity between withholding irrigation in flowering, grain filling periods and foliar application of Put with 75 ppm ($P<0.05$) exogenous Put could increase antioxidant enzymes activity under water deficit stress.

Key words: Putrescine, Wheat, Water deficit stress, Polyamins,

INTRODUCTION

Water stress is one of the most important environmental factor which regulate plant condition including growth, production and development. Response to water stress by plants could alter the cellular metabolism, and presenting different defense mechanisms (Hassain *et al.*, 2011).

Plant ability to survival under this stressful condition depends on receiving the kinds of stimuli, generation, transmission of signals and in extremely, promote the different chemical and physiological changes .Various mechanisms are evoked by plants in response to water deficit stress. Plants can resist drought stress by morphological changes e.g. increasing in size of the root system or reducing leaf area via changing biochemical and physiological processes such as antioxidant defense systems and etc (Huang *et al.*, 2001). Polyamines (PAs) including spermine (Spm) and their diamine obligate precursor, putrescine (Put), have frequently described as endogenous plant growth regulations or intracellular messengers mediating physiological responses (Hussain *et al.*, 2012). Put can be directly synthesized from ornithine via ornithine decarboxylase or indirectly from arginine via arginine decarboxylase. PAs occur in cells in the free from, soluble conjugated as well as insoluble _conjugated forms and play the main role in many physiological process like cell division, morphogenesis, metabolism and apoptosis (Duan *et al.,* 2008). Attentions has recently been focused on the role PAs in plant defense against abiotic and biotic stress which various kind of environmental stress like water stress, salinity can change their titer. Increase in PAs can be potently accomplished by inhibition in PAs biosynthetic enzyme activity so, increasing in spm and spermidine of wheat (*Triticum aestivum*) plant are seen under water stress which be associated with a reduction in drought stress (Alcazar, *et al.,* 2010). Classical approaches including exogenous PAs application, enzyme inhibitors use in PAs biosynthesis show a proper model to test different hypotheses, and to answer substantial biological questions derived from pathway manipulation. Current plant response under biotic and abiotic stresses is reactive oxygen species (ROS) production. ROS is characterized by repletion of free radicals and toxic molecules O_2-, H_2O_2, and OH- in tissues and in extremity, damage to plant. Super oxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) enzymes and ascorbate are compound capable of free radical neutralization and inhibition of cell damages (Takahashi and Kukehi 2009). So, this study was aimed at the role of foliar application of putrescine on antioxidative defense of wheat (*Triticum aestivum* L. var Sw_82_9) under water deficit Stress.

MATERIAL & METHODS

Plant material and treatment

The experiment was done on a farm at Islamic Azad University, Varamin branch, Tehran province, Iran during the wheat (*Triticum aestivum* var Sw_82_9) growing season (March_ July. Factorial experiment with complete randomized in 12 treatment and 3 replication at three levels application of Put (0,75 and 150 ppm) was carried out. Wheat seed line were grown at ground with 700m^2 which 18 m^2 were allocated for each treatment. The soil characteristics are summarized in Table 1.

Table 1: Physical and chemical characteristics of experiment soil.

Depth of sampling (Cm)	**Electrical Conduction** (mM.cm^{-1})	**pH**	**Total nitrogen**	**Absorbable phosphorus**	**Sand (%)**	**clay**
0-30	1.45	7.2	0.07	9.4	32	22
30-60	1.65	6.8	0.04	4.7	30.2	16

Malondialdehyde (MDA): MDA measurement was done by Ohkaw *et al.,* (1979). Briefly, 0.2 gr leaves divided to small pieces and homogenized with 2ml trichloroacetic acid 5% in adjacency of ice and centrifuged at 12000 rpm for 15 min.0.5 ml of supernatant mixed with 0.5 ml thiobarbituric acid + trichloroacetic acid 20% and incubated at 96 C for 25 min and again centrifuged at 10.000 rpm for 5 min in cold condition. Absorbance of supernatant was spectrophotometrically read at 532 nm. Thiobarbituric acid and trichloroacetic acid 20% was used as control and MDA was determined using standard curve.

Catalase (CAT): CAT enzyme was measured by method of Paglia (1997). The young leaves were washed by distilled water and homogenated in 0.16 M Tris_phosphate buffer (pH:7.5) and then buffer containing digitonin enzyme was added at the same volume.0.5 ml of homogenized solution was evaluated for protein content and remnant were used for CAT measurement. The elimination rate of H_2O_2 (substrate) was evaluated and buffer containing 0.17 mM phosphate di-sodic, (pH:.7.5) ,0.15 M EDAT and 0.11 mM $MgCl_2$.

Super oxide dismutase (SOD): 3 leaves (subsidiary leaves) were picked up at the morning from the farm and transferred to the laboratory in the icebox. The enzyme was measured by Misra method (Misra and Fridovich 1972). Initially, Tris buffer solution (phosphate, di-sodic, pH: 7.2) was added to 1.3 mM EDTA and 0.1mM monosodic carbonate, then epinephrine (0.25mM) was used as substrate. Enzyme activity was measured by spectrophotometer (absorbance changes) in comparison to standard solution. (Khashuei, 2010).

Dityrosin (Di-Ty): The leaves were washed by distilled water, immediately shattered in Tris-Phosphate buffer (0.16 M, pH:7.5) and homogenated. Buffer containing digitonin enzyme was added at the same volume to homogenated solution. 0.5 ml of homogenated solution was removed for protein evaluation and remnant was used for Di-Ty measurement by method Steven *et al.,* 1978). Enzyme activity was measured by liquid chromatography.

Ascorbate: 1gr of leaves homogenized in 10 ml of 25 mM EPPS buffer (pH : 7.8) and then centrifuged at 15,000 g for 20 min. The supernatant was used for enzyme analysis. Activity of ascorbate peroxidase was measured by Nakano and Asada method (12) at 290 nm. The reaction mixture contained 25 mM phosphate buffer (pH: 7.0), 0.1 mM EDTA, 0.1 mM H_2O_2 ,0.25 ascorbate.

Analysis: The data were analyzed using SAS statistical software. The means were compared by Duncan's multiple range test and ($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Analysis of variance showed that the mean squares of SOD, CAT, Di-Ty and ascorbate concentrations after foliar application of Put were significantly increased with comparison to normal irrigation ($P < 0.05$) (Table 2). The results showed that significant difference was seen between foliar application of Put with foliar application + normal irrigation for MDA ($P < 0.05$).

Table 2: Analysis of variance of the Put effects on antioxidative enzymes.

	df	**Put**	**SOD**	**CAT**	**Ascorbate**	**MDA**	**Di-tyr**
Normal irrigation (a)	3	4646.37	158665.34	31497.9	0.019	127.5	9.36
Foliar application of Put (b)	2	651.97	5607.55	2585.05	0.02	0.33	0.342
(a.b)	6	11.89	757.92	362.76	0.002	0.089	0.085
Error (main agent)	22	2.58	24.55	45.67	0.00003	0.002	0.0008
C.V%	-	2.04	3.14	7.43	3.82	2.73	4.05

ns ,**,* indicate No significance , significancy at 1% and 5% levels, respectively.

Significant decrease was seen for Di_Ty between foliar application of Put and normal irrigation (P < 0.05). The mean range of mutual effect of normal irrigation and foliar application of Put are summarized in Table 3.

Table 3: The mean comparison of normal irrigation and foliar application of Put on antioxidative enzyme activities.

Irrigation	Put (α)	SOD (α)	CAT (β)	AS (γ)	MDA(δ)	Di-Tyr(ε)
Irrigation type a) Normal irrigation	64.28d	62057d	103.97d	0.243d	5.56d	3.18d
b) Withholding irrigation in steam elongation period	81.88c	764.04c	147.1c	0.304c	7.57c	3.68c
c) Withholding irrigation in flowering period	100.72b	838.93	181.73 b	0.325b	12 b	4.89b
d) Withholding irrigation in grain filling period	116.63a	935.31a	244.03a	0.352a	13.58a	5.37a
Foliar application of Put						
a)Foliar application with pure water	83.94c	766.80b	155.99b	0.349a	9.87a	4.46a
b)Foliar application of put (75 ppm)	90.08b	792.6a	166.63b	0.303b	9.7ab	4.26b
c)Foliar application of put (150 ppm)	98.61a	809.75 a	185.00a	0.266c	9.54b	4.12b

α: $(\frac{\Delta A}{mg} por.min-1)$ β : (mg.g^{-1} FT) γ: (n molg^{-1}fw) δ: (n mol g^{-1} fw) ε: (mM)

* Similar letters in each column statistically wasn't significant (*P < 0.05*).

Results showed that significant difference in Put concentration was seen between withholding irrigation in grain filling period and foliar application of Put (150ppm), also significant difference in Put concentration was not seen between stop irrigation in steam elongation period and foliar application with pure water (P<0.05). Significant difference was not seen in SOD and CAT activity between withholding irrigation in grain filling period and foliar application of Put (150 ppm), respectively (P<0.05).

Significant difference was not seen in Di-Ty MDA and SOD enzymes activity in foliar application of Put with 75 ppm and 150 ppm. There was significant difference in CAT activity between normal irrigation and foliar application of Put (150 ppm). There was no significant difference in MDA activity between stop irrigation in flowering, grain filling and foliar application of Put with 75 ppm, also no significant difference was seen between stop irrigation in grain filling and foliar application of pure water for ascorbate activity (P<0.05). The cell membrane stability is important in plants under different stressful conditions such as salinity and water deficit, so elevation of antioxidants levels play the main role in plants. PAs are low molecular weight compounds found in prokaryotes and eukaryotes and their presence are essential for growth and development (Zhao *et al.*, 2007). In this study, the role of foliar application of Put on antioxidative defense of wheat under water deficit stress was investigated. There was significant difference in SOD, CAT, As and Di_Tyr enzymes activities between normal irrigation and foliar application of Put (P < 0.05). Amooaghaie reported the effective role of PAs (Spm, Put) in tolerance of soybean to water deficit stress which in accordance with our study (Amooaghaie, 2011). Rahdari *et al.* was reported the role of PAs on protein, chlorophyll and phenolic compounds in wheat under salinity stress which PAs improved chlorophyll and protein concentrations under salinity stress in root and shot. Shallan *et al.* in 2012 reported that Put caused enhancement of growth, increasing of pigments content, total antioxidant capacity and antioxidant enzyme activities in cotton plant (Shallan *et al.*, 2012). Which in accordance with our study about enzyme activities. Emadi *et al.* reported the effect of foliar application of Put and nutrient elements on grain yield and quality of wheat which Put caused the longest effective grain filling period in wheat, also the highest leaf area duration (Emadi *et al.*, 2013). Effect which in accordance with our study. Verma *et al* (2005) showed that Put increased the glutathions level and antioxygenic enzymes and elevating antioxidant by controlling free radical generation and finally improvement of seedling growth under salinity.

CONCLUSION

It's critical that the exogenous PAs could increase antioxidant enzymes activity and prevention of cell damage specially under water deficit stress, however further studies are recommended.

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