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Putrescine Foliar Application effect on Physiologic and Morphologic Characteristics of Wheat (*Tiriticum aestivum* var sw-82-9) under Water Deficit Stress

Zahra Karimi
Department of Agronomy,
Islamic Azad University, Varamin- Pishva Branch, Varamin, Iran

(Corresponding author: Zahra Karimi)
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ABSTRACT: Water deficit stress is one of the most important environmental stress factors that affect all crop physiological and biochemical processes. In this study, putrescine (Put) foliar application (0, 75 and 150 ppm) was investigated on physiologic and morphologic characteristics of wheat (*Tiriticum aestivum* var sw-82-9) under different levels of water stress as complete randomized experiment in 12 treatment and 3 replication. All experimental traits affected significantly by withholding in irrigation at different growth stages. The grain yield was decreased as result of withholding in irrigation at stem elongation, flowering and seed filling period stages. Withholding in irrigation in different growth stages decreased number of grains per spike, thousand grain weight, total chlorophyll contents and relative water content, whereas increased grain protein, proline content, and electrical conductivity of leaf. Putrescine foliar application specially 150 ppm concentration prevented degradation of leaves protein and chlorophyll and decreased proline by reduction in water deficit stress. In addition, plants were preferred putrescine utilized as a scavenger than the enhancement enzyme activity against stress.

Key words: Putrescine, Wheat, Physiologic characteristics, Water deficit Stress

INTRODUCTION

Various biotic and abiotic stress factors affect plants growth and productivity. The plant normal metabolism initiates responses to environmental changes and makes plants ready to predictable seasonal cycles, thus every deviation of optimized environmental factor is necessarily not stress. The stress term has different meanings which includes unpredictable factors and cause disruption of the equilibrium, injury and aberrant plant physiology. There are different stresses such as salt, drought, heat and oxidative stresses for plants (Zhang. and Yang, 2004). Some countries geographically are at brainless areas, so salt, drought and heat stresses could be inevitable and affect on all plants. Water deficit stress is one of the most important environmental stress factors that affect all crop physiological and biochemical processes and could reduces crop quality and causes plant death (kafi, 2010). Many plants are capable of adaption to water deficit stress by altering their cellular metabolism and engage defense mechanisms such as accumulation of soluble protective proteins and morphological changes like increasing in size of the root and leaves reduction (Amooaghaie, 2011). In recent years, for increasing plant resistance to stress, different methods such as external application of growth regulators are used.

There are different reports in the relationship between polyamines (PAs) and environmental stresses (Bouchereau *et al*., 1999). PAs are small ubiquitous polycations involved in many processes of plant growth and development and found in all living organisms including bacteria, plants and animals (Hussain *et al*., 2011). PAs including spermine (tetramine), spermidine (Triamine) and their obligate precursor of putrescine (a diamine), are involved induction of plant adaptation to stress (Mishra, *et al*., 1994). PAs as the regulators of plant growth involved in a wide range of developmental processes, including cell division, embryogenesis, morphogenesis, flowering, fruit ripening, root development and sustainable membranes collected active radicals (Kaur-Sawhney *et al*., 2003). The role of PAs in reducing the effects of stress in some plants such as pepper, tobacco and pea have studied but there is no study on wheat (Bias, H. P and Ravishankar, 2002). Wheat is one of the most important crops from aspect of cultivation area and production in the world also, it has an important role in food supply of human societies. So in the present study, the effect of different concentrations of putrescine (Put) foliar application was investigated on physiologic and morphologic characteristics of wheat (*Tiriticum aestivum* var sw-82-9) under different levels of water stress.

MATERIALS AND METHODS

The experiment was done at farm of Islamic Azad University, Varamin- Pishva branch, Tehran province, during the wheat (*Triticum aestivum* var Sw-82-9) growing season (March to July). Factorial experiment with complete randomized in 12 treatment and 3 replication at three levels of Put (0, 75 and 150 ppm) was carried out. Wheat seedline 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

A. Evaluation of morphological parameters

Growth is an important drought-sensitive physiological process and serious tool for assessing crop productivity. The wheat length, thousands of grain weight, number of grain per ear length were measured for 10 plants in each group and reported as mean.

B. Grain yield and Harvest Index (HI)

Grain yield was determined at ripening time. HI was calculated by following formulae:

Harvest index (HI%) = 100 × (seed economic yield).(biological yield)$^{-1}$.

C. Evaluation of physiological parameters

Measurement of proline content. Wheat proline content were determined according to the method of Bates *et al*., (1993). The fresh leaves (0.5 gr) were homogenized in 3 ml of sulfosalicylic acid (3%,W/V). The homogenate was filtered through the filter paper.2 ml of filtered solution was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in tubes and incubated in boiling water bath for 1 h. The reaction using ice bath was stopped and contents were extracted with 4 ml of toluene and vigorously mixed for 20 seconds. The chromophore containing toluene was aspirated from aqueous phase and absorbance of solution was measured at 520 nm using spectrophotometer. The proline concentration was measured via standard curve.

Chlorophyll content. Chlorophyll meter was used to measure chlorophyll a, b and ab according to (Sestac and Catasky, 1966) method. The leaves chopped and mixed with calcium carbonate and 10 ml of pure acetone. The resulting mixture was filtered and maintained at dark place in tubes into the ice and water container. The tubes centrifuged for 2 min in 2500 rpm until the pure extract obtained. 1 ml of extract was mixed with 9 ml acetone 80 % and evaluated by spectrophotometer at wavelength of 663 nm and 647nm for chlorophyll a and b, respectively. The Chlorophyll a + b was obtained via following formulae.

Chl.a (mg.L^{-1}) = (12.25 * A663 - 2.79 * A647) * D

Ch.b (mg.L^{-1}) = (21. 5 * A647 - 5.1 * A663) * D

Chl. a + b (mg.L^{-1}) = (7.15 * A663 - 18.71 * A647) * D

Relative Water content (RWC). RWC was measured according to the (Barr, *et al*. 1962) method.

RWC = (FW-DW).(TW-DW)$^{-1}$

TW : sample turgid weight

DW : sample dry weight

D. Electrical conductivity (EC)

EC was measured on four replications of 50-seed samples per lot, weighed on an analytical balance (0.01 g) by EC meter. (Hunson and Hitz 1982).

E. Statistical Analysis

The results were analyzed by an analysis of variance ($P < 0.05$). The means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Water is being economically used for growth process and growth is the main tool for assessing crop productivity in different crops. So, water deficit stress could be accompanied by economic losses for countries. Putrescine foliar application (150 ppm) significantly increased grain yield, protein percent, HI, and E.C of wheat leaf. Withholding irrigation in stem elongation significantly decreased wheat length, thousands of grain weight and biological yield with respect to normal irrigation but putrescine foliar application specially 150 ppm concentration increased considerably mentioned traits (Table 3). Nam *et al*., 2001 have noted drought stress is limiting factor at the initial phase of plant growth specially at stem elongation. Stem length of soybean, potato and rice was decreased under water deficit stress which was in accordance to our study (Kusaga *et al*., 1999). Withholding irrigation in stem elongation increased proline content and decreased chlorophyll content. Rom *et al*., 2005 have reported that the total, a and b chlorophyll contents decreased under drought stress which was in accordance to our study. The maximum proline content obtained in withholding irrigation in grain filling stage with 0.037 mg.g^{-1} FW.

Table 2: Analysis of variance for morphologic characteristics and wheat yield.

		Mean Square							
Sum of Variance (S.O.V)	**d.f**	**Biologic yield**	**Grain yield**	**T. grain weight**	**No. grain per ear**	**No.ear per mm^2**	**Ear length**	**Wheat length**	**Harvest index**
Replication	2	1940.47ns	10569.17ns	0.021ns	1.99ns	260.31ns	0.010ns	0.046ns	0.468ns
Irrigation	3	3008079.75**	1916555.94*	211.98**	338.73**	3116.89ns	2.08**	194.08**	24.03ns
Main error	6	8490.32	38867.75	0.020	0.407	182.77	0.030	1.55	1.97
Put foliar application (P.F.A)	2	835512.93*	472654.81ns	8.84*	75.69ns	4597.90ns	2.54**	24.04ns	4.98ns
Irrigation × P.F.A	6	336466.75*	55577.78*	2.18*	34.56*	2170.21**	0.269*	22.44**	0.210ns
Branch error	16	246466.75	411523.86	2.08	25.67	1951.02	0.163	17.56	28.65
C.V		13.26	13.99	3.45	10.20	9.58	3.99	5.24	13.13

In turn, no significant difference, significant difference at 5 % level and 1 % levels ns, *,**

In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavengering free radicals, and buffering cellular redox potential under stress conditions. It may also act as a protein compatible hydrotrope (Tang, and Newton, 2005), alleviating cytoplasmic acidosis, and maintaining appropriate NADP+ /NADPH ratios compatible with metabolism. Rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages (Hare *et al*., 2002). Furthermore, proline is known to induce expression of salt stress responsive genes, which possess proline responsive elements (e.g. PRE, ACTCAT) in their promoters (Liu, *et al*., 2007: Serano, *et al*., 1999). In response to drought or salinity stress in plants, proline accumulation normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment. For example, in cells of *Distichlis spicata* treated with 200 mM NaCl, the cytosolic proline concentration was estimated to be more than 230 mM (Kuchenbuch and Phillips, 2005). In apical region of maize roots growing at a water potential of 1.6 MPa, proline concentration reached approximately 120 mM and accounted for up to 50% of the osmotic adjustment (Wang, *et al*., 2003). Furthermore, in response to water deficit, concentration of proline increased in maize root. Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. For example, while in salt-tolerant alfalfa plants proline concentration in the root rapidly doubled under salt stress but in salt-sensitive plants the response was slow (Krishnamorthy and Bhagwat 1989).

Table 3: The mean comparison of putrescine foliar application and normal irrigation on some morphologic characteristics.

	Wheat length (cm)	ear length (cm)	No.ear per mm^2	T. grain weight (g)	No. grain per ear	Grain yield ($kg.ha^{-1}$)	Biologic yield ($kg.ha^{-1}$)	Harvest index (%)
Irrigation								
Normal irrigation	82.61 a	10.63 a	478.41 a	47.07 a	54.27 a	6427.0 a	15004.4a	**42.87 a**
Withholding irrigation in stem elongation	73.01 b	10.35 ab	435.27 a	43.50 b	50.65 a	5861.2 ab	14281.7 b	**41.07 a**
Withholding irrigation in flowering stage	81.45 a	9.51 c	460.39 a	41.19 c	40.72 b	5307.1b	13592.2 c	**39.07a**
Withholding irrigation in grain filling	82.64 a	10.03 b	469.59 a	35.50 d	52.93 a	5740.0b	14377.3b	**39.98a**
Putrescine foliar application								
Putrescine foliar application (0 ppm) (Normal irrigation)	78.45a	9.70c	439.63a	40.91b	47.00b	5624.9a	14030.0b	**40.09a**
Putrescine foliar application (75 ppm)	80.08a	10.08b	464.98a	41.92ab	49.92ab	5856.8a	14359.9ab	**40.78a**
Putrescine foliar application (150 ppm)	81.270a	10.616a	478.14a	42.62a	52.00a	6019.8a	14551.7a	**41.37a**

* No significant difference at 5 % level Duncan for means which have the same letters in each column

Similarly, salt-tolerant ecotypes of *Agrostis stolonifera* accumulated more proline in response to salinity than salt-sensitive ecotypes (Liu, *et al*., 2006). Besides positive effects of proline on improving salt tolerant plant at the organism level, considerable improvement in salt tolerance has also been observed at the cellular level. For example, in vitro studies with brown mustard (*Brassica juncea*) indicated that salt-adapted calli had higher accumulation of free proline compared with non-stressed calli (Liu, *et al*. 2008). The available information from different studies suggest that optimal concentrations of proline may be species or genotype dependent, which need to be determined a priori before commercial application of exogenous proline to improve crop stress tolerance. Furthermore, because in most crop species stress tolerance may vary with developmental stages. Drought levels were such that the mean yield of all cultivars under drought stress ranged from 37 to 86% of control yield, corresponding to irrigation cut-off varying from 69 days. In each experiment the grain yield under drought showed highly significant cultivar differences, which appeared consistent between years. Yields were adjusted for drought escape by using a correction factor which ranged from g/m^2 per day advance in flowering, being greater in experiments with less severe drought. The demonstration of linear relationships between cultivar yield and drought intensity, indicated by the mean yield of some or all cultivars, prompted the consideration of cultivar yield under drought as the function of yield potential.

Table 4: The mean comparison reciprocal effect of normal irrigation and putrescine foliar application on morphologic characteristics and grain yield of wheat.

Irrigation	Putrescine foliar application **(P.F.A)**	Biologic yield (kg.ha^{-1})	Grain yield (kg.ha^{-1})	T. grain weight(g)	No. grain per ear	No.ear per mm^2	ear length (cm)	Wheat length (cm)	Harvest index (%)
Normal irrigation	Normal irrigation	14754.8abc	6278.2ab	46.20ab	52.93a	459.45ab	10.26bcd	81.21ab	42.58a
Normal irrigation	P.F.A (75 ppm)	15023.7ab	6420.4ab	47.16a	54.63a	487.78a	10.56abc	82.58a	42.79a
Normal irrigation	P.F.A (150 ppm)	15234.6a	6582.3a	47.85a	55.26a	487.98a	11.06a	84.04a	43.24a
Withholding irrigation in stem elongation	Normal irrigation	13987.5cdef	5662.8abc	42.26de	48.43abc	407.97b	10.06cde	70.29d	40.53a
Withholding irrigation in stem elongation	P.F.A (75 ppm)	14325.6bcde	5875.4abc	43.71cd	50.40ab	435.43ab	10.23bcd	73.61cd	41.04a
Withholding irrigation in stem elongation	P.F.A (150 ppm)	14532.1abcd	6045.5abc	44.54bc	53.13a	462.42ab	10.76ab	75.14bcd	41.66a
Withholding irrigation in flowering	Normal irrigation	13245.4f	5045.7c	40.63e	36.36d	435.79ab	8.86f	79.98abc	38.16a
Withholding irrigation in flowering	P.F.A (75 ppm)	13654.6ef	5342.8bc	41.09e	41.30cd	464.85ab	9.46ef	81.45ab	39.16a
Withholding irrigation in flowering	P.F.A (150 ppm)	13876.5def	5532.7abc	41.85de	44.50bc	480.52ab	10.20bcd	82.94a	39.90a
Withholding irrigation in grain filling	Normal irrigation	14132.4cde	5512.7abc	34.56f	50.30ab	455.28ab	9.60de	82.31a	39.08a
Withholding irrigation in grain filling	P.F.A (75 ppm)	14435.8bcde	5788.6abc	35.71f	53.36a	471.86ab	10.06cde	82.68a	40.14a
Withholding irrigation in grain filling	P.F.A (150 ppm)	14563.6abcd	5918.6abc	36.24f	55.13a	481.62ab	10.43abc	82.94a	40.71a

* No significant difference at 5 % level Duncan for means which have the same letters in each column

Table 5: Analysis of variance some physiologic characteristics of wheat.

	df	Electrical Conductivity (E.C)	Prolin	Relative Water content (RWC)	Chlorophyll (a+b)	Chlorophyll b	Chlorophyll a	Protein yield (Pr.Y)	Protein (%)
		(The Mean Squares)							
Replication	2	3.09ns[a]	0.000002ns	0.184ns	0.0001ns	0.000008ns	0.002ns	162.96ns	0.004 ns
Irrigation	3	103110.64**	0.001**	104.39**	0.664**	0.021**	116.43**	7782.45*	4.93 **
The main error	6	10.005	0.00001	0.085	0.0001	0.00001	0.005	172.05	0.001
Putrescine foliar application	2	5628.14**	0.0001**	1.56ns	0.022**	0.001**	0.532**	7247.28*	0.323 **
Putrescine foliar application × Irrigation	6	647.88*	0.00003*	1.26*	0.100*	0.010**	0.045ns	3132.81**	0.048*
Branch error	16	197.81	0.000008	1.24	0.001	0.0001	0.062	2102.90	0.031
C.V		1.79	12.52	1.49	2.07	2.23	2.13	10.50	2.34

a: In turn, no significant difference , significant difference at 5 % and 1 % levels ns, *,**

Table 6: The mean comparison effect of normal irrigation and putrescine foliar application on some characteristics of wheat.

	E.C ($\mu s.cm^{-1}$)	Proline ($mg.g^{-1}FW$)	RWC (%)	Chlo a+b ($mg.L^{-1}$)	Chlo.b ($mg.L^{-1}$)	Chlo.a ($mg.L^{-1}$)	Pr.Y ($kg.ha^{-1}$)	Pr (%)
Irrigation								
Normal irrigation	681.12d	0.010d	78.54a	2.41a	0.614a	1.79a	424.18 b	6.60 d
Withholding irrigation in stem elongation	718.82c	0.018c	75.68b	2.22b	0.596b	1.63b	429.20 b	7.32 c
Withholding irrigation in flowering	819.01b	0.025b	72.72c	2.04c	0.561c	1.48c	413.20 b	7.78 b
Withholding irrigation in grain filling	919.33a	0.037a	70.76d	1.77d	0.504d	1.26d	479.47 a	8.35 a
Putrescine foliar application								
Water foliar application	806.66a	0.025a	74.03a	2.068b	0.556b	1.511b	411.15b	7.35c
Putrescine foliar application (75 ppm)	783.68b	0.025a	74.51a	2.118a	0.570a	1.547a	455.61ab	7.51b
Putrescine foliar application (150 ppm)	763.37c	0.019b	74.74a	2.154a	0.580a	1.575a	480.23a	7.67a

* No significant difference at 5 % level Duncan for means which have the same letters in each column

Table 7: The mean comparison reciprocal effect of normal irrigation and putrescine foliar application on some characteristics of wheat.

		E.C ($\mu s.cm^{-1}$)	Proline ($mg.g^{-1}FW$)	RWC (%)	Chlo a+b ($mg.L^{-1}$)	Chlo.b ($mg.L^{-1}$)	Chlo.a ($mg.L^{-1}$)	Pr.Y ($kg.ha^{-1}$)	Pr (%)
Irrigation	**Foliar application**								
Normal irrigation	**Normal irrigation**	681.95I	0.010D	78.51A*	2.386A	0.610A	1.780A	408.80BC	6.51H
	P.F.A (75 PPM)	681.27I	0.010D	78.71A	2.410A	0.613A	1.793A	422.53BC	6.58H
	P.F.A (150 PPM)	680.15I	0.010D	78.40A	2.433A	0.620A	1.810A	441.20ABC	6.70H
Withholding irrigation in stem elongation	**Normal irrigation**	744.70G	0.020C	75.08B	2.163C	0.580B	1.586C	402.10C	7.10G
	P.F.A (75 PPM)	720.80H	0.020C	75.79B	2.240B	0.600A	1.640B	431.40BC	7.34FG
	P.F.A (150 PPM)	690.96I	0.016C	76.18B	2.280B	0.610A	1.673B	454.10ABC	7.51EF
Withholding irrigation in flowering	**Normal irrigation**	855.53D	0.030B	72.13CDE	1.986E	0.546D	1.440E	387.03C	7.67DE
	P.F.A (75 PPM)	811.24E	0.026B	72.79CD	2.046DE	0.560CD	1.486DE	416.80BC	7.80D
	P.F.A (150 PPM)	790.26F	0.020C	73.25C	2.096D	0.576BC	1.523D	435.77ABC	7.88CD
Withholding irrigation in grain filling	**Normal irrigation**	944.45A	0.040A	70.41E	1.736G	0.490F	1.240G	446.67ABC	8.11BC
	P.F.A (75 PPM)	921.41B	0.043A	70.75E	1.776FG	0.506EF	1.270FG	481.93AB	8.33B
	P.F.A (150 PPM)	892.12C	0.030B	71.13DE	1.806F	0.516E	1.293F	509.80A	8.61A

* No significant difference at 5 % level Duncan test for means which have the same letters in each column.

CONCLUSION

Putrescine foliar application significantly increased grain yield, protein percent, HI, and E.C of wheat leaf under water deficit stress which could be applicable in prevention of economic losses and refinement of crop productivity.

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