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The study of humic acid foliar application on physiological and biochemical changes in wheat under irrigation withholding at different growth stages

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ABSTRACT

In order to study effect of humic acid (HA) foliar application and limited irrigation, on physiological and biochemical characteristics of wheat an experiment was conducted in research field of Varamin, Iran during 2012 growing season. The experimental design was laid out in a randomized complete block with a split plots arrangement of treatments in three replications. Main plots included four different levels of irrigation (complete irrigation, irrigation withholding at stem elongation stage, irrigation withholding at flowering stage and irrigation withholding at seed setting stage) and three different concentration of HA foliar application (0, 150 and 300) was allocated to subplots. The results showed that irrigation withholding conditions in different growth stages significantly decreased seed yield and total chlorophyll content but by contrast increased electrolyte leakage, antioxidant enzymes activity and lipid and protein peroxidation. It appears that HA act in plants via a specific form of stress that is detected by anti-stress defense systems in plants. These HA applied to plants can protect against water stress in degraded soils.

Keywords: Humic acid, wheat, irrigation withholding, seed yield, antioxidant enzymes.

INTRODUCTION

Drought stress significantly limits plant growth and crop productivity. The fact that water stress effects on growth and yield are genotype-dependent is well known [1]. Under conditions of water stress and other types of environmental stress, reactive oxygen species (ROS), such as superoxide anion radicals, hydrogen peroxide and hydroxyl radicals, are generated [2]. These free radicals can damage essential membrane lipids as well as proteins and nucleic acids [3]. Plant cells contain an array of protection mechanisms and repair systems that can minimize the occurrence of oxidative damage caused by reactive oxygen species (ROS) [4]. Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymatic (superoxide dismuatase, catalase,ascorbat peroxidase, peroxidase, glutathione reductase [5]. Humic acid (HA) is the active constituent of organic fertilizers and its application may represent an alternative to conventional soil fertilization and a prompt source of N, especially in semi-arid conditions [6,7]. The humic substances, the major component of soil organic matter, have both direct and indirect effects on plant growth [8]. Humic acids can protect plants in water deficient-soils, On the other hand recently, a new mode of action for HA suggests that HA can cluster in roots to affect transpiration and, therefore, the hydraulic conductivity of the roots via colloidal stress [9]. Effects on antioxidative defense mechanisms, reporting the stimulation of catalases (CAT) and the generation of reactive oxygen species (ROS) that act as intermediaries in plant growth [10]. Hence in this field experiment, an attempt was made to investigate the effect of humic acid foliar application on yield and antioxidant enzymes activity of wheat plants under compete irrigation and irrigation withholding at different growth stages.

MATERIALS AND METHODS

In order to study effect of humic acid foliar application and limited irrigation, on physiological and biochemical characteristics of wheat an experiment was conducted in research field of Varamin, Iran during 2012 growing season. Site of study was situated at 35º, 19' N and 51º, 39' E, 900 m above sea level. Before beginning of experiment, soil samples were taken in order to determine the physical and chemical properties. A composite soil sample was collected at a depth of 0-30 cm. It was air dried, crushed, and tested for physical and chemical properties. The research field had a clay loam soil. Details of soil properties are shown in Table 1.

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After plow and disk, plots were prepared. The experimental design was carried out in a randomized complete block with a split plot arrangement of treatments in three replications. Main plots included four different levels of irrigation (complete irrigation, irrigation withholding at stem elongation stage, irrigation withholding at flowering stage and irrigation withholding at seed setting stage) and three different concentration of HA foliar application (0, 150 and 300) was allocated to subplots. Each sub plot consisted 12 rows, 5 m long with 20 cm spaced between rows and 5 cm distance between plants on the rows. For determination of seed yield, the samples consisted of 3 m along the center row of each plot, discarding two rows on the border. The remaining plants were cut at ground level, yield was determined with the experimental combine harvester machine. The humic acid foliar application was applied with a pressurized backpack sprayer (12 l capacity) calibrated to deliver 1000 l ha^{-1} of spray solution. Sprayer was equipped with a spiral solid cone spray nozzle. At the end of growing season crop were harvested and seed yield and biological yield were assayed.

Table 1: Soil properties of the experimental site

Depth	EC (ds m^{-1})	pH	O.C (%)	T.N.V (%)	K (ppm)	P(ppm)	N (%)	Texture
0-30 cm	3.9	7.52	1.12	20.8	395.4	11.9	0.07	Clay loam

Membrane stability assay
Leaf samples (0.5 g) were immersed into 10 ml of -2 bar mannitol solution (14.7 g mannitol per liter) and after 24 h electrical conductivity of the solution was measured.

Chlorophyll assay
Chlorophyll was extracted in 80 % acetone from the leaf samples according to the method of Arnon [11].

Antioxidant enzyme activity assay
Catalase activity was estimated by the method of Cakmak and Horst [12]. Superoxide dismutase activity was determined according to the method of Giannopolitis and Ries [13]. Glutathione peroxidase activity was measured according to method of Paglia and Valentine [14].

Lipid peroxidation assay
The level of membrane damage was determined by measuring MDA as the end product of peroxidation of membrane lipids [15].

Table 2: Analysis of variance on wheat attributes affected by irrigation withholding in different growth stages and humic acid foliar application

S.O.V	df	Seed yield	Total chlorophyll	Membrane stability	Superoxide dismutase	Catalase	Glutathione peroxidase	Malondialdehyde	Dityrosine
Replication	**2**	**407749.71***	**^{ns}0.68**	**^{ns}315.75**	**^{ns}28.13**	**^{ns}512.23**	**^{ns}965.01**	**^{ns}0.01**	**^{ns}0.002**
Irrigation	**3**	**** 20370278.3**	**131.8****	**178652.6****	**278676.52****	**20517.74****	**19760.04****	**67.32****	**** 24.42**
Error (a)	**6**	**77788.51**	**1.01**	**132.74**	**98.29**	**678.52**	**1027.75**	**0.01**	**0.003**
Humic acid foliar application	**2**	**^{ns}39791.64**	**^{ns}4.9**	**8600.99****	**15219.59****	**^{ns}1818.24**	**7511.16****	**0.74****	**1.03****
Interaction	**6**	**^{ns}106786.57**	**^{ns}1.2**	**1595.54***	**^{ns}707.05**	**^{ns}947.71**	**^{ns}1183.41**	**^{ns}0.08**	**0.04***
Error (b)	**16**	**114882.04**	**11.85**	**468.68**	**453.89**	**749.11**	**1165.48**	**0.03**	**0.01**
C.V		**4.76**	**11.84**	**2.33**	**2.69**	**17.06**	**20.04**	**1.57**	**1.84**

*,** and ns significant at 0.05, 0.01 and no significant

Protein peroxidation assay
A standard dityrosine sample was prepared according to Amado et al. [16].

Statistical analysis
All data were subjected to SAS software [17]. Duncan's multiple range tests was used for statistical differences between treatment means and controls. Comparisons with P values 0.05 were considered significantly different.

RESULTS

Analysis of variance showed that the effect of irrigation withholding in different growth stages was significant on all traits experiment. Also the effect of humic acid foliar application was significant on all measured traits experiment except for seed yield, total chlorophyll content and catalase enzyme activity (Table 2). Interaction of experimental factors (irrigation withholding in different growth stages ×

humic acid foliar application) was not significant on all measured traits experiment except for membrane stability and Dityrosine. As can be seen from table 3, seed yield decreased as result of irrigation withholding at stem elongation, flowering and seed setting stages at by 10.56 %, 40.37% and 21.98%, respectively with compared complete irrigation treatment conditions. The stress treatments decreased the number of days required for wheat to reach 50% flowering or maturity, by an average of 4-7 days, if compared with the unstressed control. Similar findings have been reported for faba bean (*Vicia faba* L.), by Mwanamwenge *et al.* [18]. Acceleration of flowering and maturity probably contributed to reduce the impact of drought stress wheat. Furthermore, water deficit decreased seed yield via decrease in photosynthesis and seed number per spike. Similar results are accessible published by other researcher [19]. Our results showed a decrease in the chlorophyll content under water deficit stress (Table 2). It is consistent with the results of Yari *et al.* [20] suggesting that moisture stress reduces leaf chlorophyll content. This decrease of leaf chlorophyll under water deficit is due to the destruction of chlorophyll pigments and the instability of the pigment-protein complex [21].

Table 3: Comparison of main means wheat attributes affected by irrigation withholding in different growth stages and humic acid foliar application

Treatments	Seed yield ($kg.ha^{-1}$)	Total chlorophyll ($mg.lit^{-1}$)	Membrane stability ($\mu s\ cm^{-1}$)	Superoxide dismutase ($\Delta A/mg\ pro.min^{-1}$)	Catalase ($\Delta A/mg\ pro.min^{-1}$)	Glutathione peroxidase ($\Delta A/mg\ pro.min^{-1}$)	Malondialdehyde (nmol g-1 FW)	Dityrosine (nmol g-1 FW)
Irrigation								
Complete Irrigation	8707.8a	a**32.66**	d**763.01**	d**621.13**	c**98.08**	c**105.16**	c**8.25**	d**4.98**
Irrigation withholding at stem elongation	7788b	a**31.66**	b**954.47**	b**813.10**	b**169.18**	ab**188.44**	b**13.48**	b**6.68**
Irrigation withholding at flowering	5192.2d	b**27.62**	a**1100.59**	a**1026.68**	a**214.05**	a**215.14**	a**14.05**	a**8.76**
Irrigation withholding at seed setting stages	6793.7c	b**24.35**	c**884.85**	c**701.53**	b**160.16**	b**172.36**	b**13.56**	c**5.67**
Humic acid foliar application								
Untreated (0 ppm)	a**7183.8**	a**28.38**	a**950.09**	c**758.62**	a**154.92**	b**150.10**	a**12.59**	a**6.80**
Treated (150 ppm)	a**7106.1**	a**29.19**	b**930.01**	b**784.22**	a**151.72**	b**162.46**	b**12.33**	b**6.55**
Treated (300 ppm)	a**7071.4**	a**29.64**	c**897.07**	a**828.98**	a**174.46**	a**198.27**	c**12.09**	c**6.21**

Treatment means followed by the same letter within each common are not significantly different ($P < 0.05$) according to Duncan's Multiple Range test

According to table 3 the highest electrolyte leakage was occurred when wheat plants were treated with irrigation withholding at flowering stage. Electrolyte

leakage decreased as result of humic acid foliar application when these treatments compared with untreated humic acid foliar application (Table 3). Under irrigation withholding at flowering and seed setting stages electrolyte leakage decreased as result of humic acid foliar application with 300 ppm when these treatments compared with untreated humic acid foliar application in this condition (Table 4). Jabari *et al*. [22] indicated that cell walls were destroyed under drought stress because stomata closure under drought conditions decreased carbon dioxide fixation, while photo reactions and compositions like fats, proteins, carbohydrates and nucleic acids [23]. As a result, fatty peroxides destroy cell membrane [24].

Also the result showed that the highest superoxide dismutase, catalase and Glutathione peroxidase enzyme activity were obtained from Irrigation withholding at flowering stage (Table 3). The combined action of SOD and CAT converts the toxic $O_2^{\cdot-}$, H_2O_2 to water and molecular oxygen, averting the cellular damage under unfavorable conditions such as drought stress [25, 26]. It was proved that the drought stress increases the production of reactive

Table 4: Interaction between irrigation withholding in different growth stages and humic acid foliar application on some attributes of wheat

Treatments	Humic acid foliar application	Seed yield ($kg.ha^{-1}$)	Total chlorophyll ($mg.lit^{-1}$)	Membrane stability ($\mu s\ cm^{-1}$)	Superoxide dismutase ($\Delta A/mg\ pro.min^{-1}$)	Catalase ($\Delta A/mg\ pro.min^{-1}$)	Glutathione peroxidase ($\Delta A/mg\ pro.min^{-1}$)	Malon dialdehyde ($nmolg^{-1}$ FW)	Dityrosine ($nmol\ g^{-1}$ FW)
Irrigation	Humic acid foliar application								
Complete Irrigation	Untreated (0 ppm)	a**8707.8**	ab**32.34**	h**771.1**	h**602.96**	e**92.6**	e**98.9**	f**8.36**	j**5.11**
	Treated (150 ppm)	a**8499.7**	ab**32.63**	h**765.32**	h**624.1**	e**97.52**	de**105.55**	f**8.24**	jk**4.95**
	Treated (300 ppm)	a**8915.9**	a**33.01**	h**752.6**	gh**636.32**	ed**104.13**	dce**111.04**	f**8.16**	k**4.88**
Irrigation withholding at stem elongation	Untreated (0 ppm)	b**7727.6**	ab**31.15**	d**964.69**	e**773.11**	cb**189.07**	dce**151.91**	cb**13.94**	d**7.02**
	Treated (150 ppm)	b**7747.8**	ab**31.47**	de**948.73**	d**805.49**	cd**150.2**	dcb**164.99**	d**13.46**	e**6.75**
	Treated (300 ppm)	b**7888.7**	ab**32.38**	de**950**	c**860.69**	cb**168.26**	a**248.41**	e**13.04**	f**6.29**
Irrigation withholding at flowering	Untreated (0 ppm)	d**5290.7**	cb**27.18**	a**1148.59**	b**1000.45**	cb**190.04**	ab**196.54**	a**14.28**	b**9.13**
	Treated (150 ppm)	d**4894.6**	cb**27.21**	b**1110.38**	b**1016.2**	ab**204.53**	ab**209.97**	ab**14.17**	c**8.77**
	Treated (300 ppm)	d**5391.4**	cab**28.47**	c**1042.78**	a**1063.38**	a**247.59**	a**238.91**	cd**13.71**	c**8.38**
Irrigation withholding at seed setting stages	Untreated (0 ppm)	c**6801.2**	c**22.87**	fe**916.01**	g**657.96**	cd**147.99**	dceb **153.03**	cd**13.78**	g**5.95**
	Treated (150 ppm)	c**6785.5**	c**25.46**	f**895.63**	f**691.1**	cb**154.63**	cb**169.33**	d**13.46**	h**5.75**
	Treated (300 ppm)	c**6794.5**	c**24.73**	g**842.9**	e**755.54**	cb**177.86**	ab**194.71**	d**13.45**	i**5.32**

Treatment means followed by the same letter within each common are not significantly different ($P < 0.05$) according to Duncan's Multiple Range test

electron transfer went on in their normal manner. Under such condition, NADP availability will be limited for electron acceptance. Therefore, oxygen can be an alternative electron acceptor which leads to the accumulation of poisonous oxygen species such as superoxide radicals (O_2), peroxide hydrogen (H_2O_2) and hydroxyl radicals (OH^-). The accumulation of active oxygen species, which are produced under stress, damages many cell oxygen species (ROS) [27]. To scavenge these ROS, plants either synthesize different antioxidant compounds or activate antioxidant enzymes. Plants can detoxify ROS by up-regulating antioxidant enzymes, such as SOD, CAT and POX as well as some non-enzymatic antioxidant compounds. It is evident that high levels of antioxidants are related to plant water deficit tolerance [28, 29]. Similar results were reported under drought stress in wheat [30],

Phaseolus acutifolius [31] and tomato plants [32]. Humic acid treatment with high concentration (300 ppm) increased superoxide dismutase, catalase and glutathione peroxidase enzyme activity (Table 3). Under irrigation withholding at different growth stages, humic acid foliar application with 300 ppm increased antioxidant enzyme activity (Table 4). The hypothesis proposed by Asli and Neumann [9] can be justified by these results because colloidal stress may be one explanation for these plant responses to the presence of HA via oxidative stress mechanisms. As such, the theories proposed by other authors regarding the rupture of the HA super-molecule into smaller fragments by rhizosphere acidification, as well as the entrance of HA fragments into plants that exert hormone-like effects, could further support our findings [33, 34, 35].

Also the result of this study showed that the highest malondialdehyde and dityrosine content were observed irrigation withholding at flowering stage (Table 3). It is well known that peroxidation of lipid membranes of higher plants reflects free radical-induced oxidative damage at the cellular level under abiotic stress [36, 37]. Malondialdehyde is often regarded as the product and a reflection of the degree of membrane lipid peroxidation [38]. Therefore, malondialdehyde content in the leaves corn plants was measured under water stress. With the water stress, leaf malondialdehyde content increased. Dityrosine content is often regarded as the product and a reflection of the degree of protein cell plants. The highest dityrosine was observed from stressed plants (Table 3). However, the malondialdehyde and dityrosine content in humic acid foliar application treatments remained lower than that in untreated humic acid foliar application treatment (Table 3). Also, under irrigation withholding at different growth stages, humic acid foliar application with 300 ppm decreased malondialdehyde and dityrosine content (Table 4), which shows that the antioxidant enzymes activity could alleviate the peroxidation of membrane lipids and protein in plant cells.

CONCLUSION

From the study it may be concluded that, in starter feed, the company used antifungal inclusions and less used such things in grower and finisher feeds. As fungal diseases are sensitive for commercial broilers that's why different feed company use high level of fungistat during feed processing. But, this practice is harmful for public health. The use of strong anti-fungals in every level of feeds (Starter, Grower and Finisher) may reduces the fungal contamination of feeds when storage at dealer and farm levels; On the other hand, the feed mill manufacturing process should be maintained properly and post processing contamination should be strictly avoided. Therefore, the occurrence of fungi in commercial broiler feeds may be due to pre and post processing contamination of feed ingredients, bad manufacturing process, contamination of the feed by handlers in the farm, bad feed storage facilities in the farm among others. Due to this fact, regular microbiological and mycotoxicological analysis should be performed for maintaining quality and safety of poultry feed.

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