

Effect of exogenous salicylic acid under irrigation withholding at different growth stages in wheat (*Triticum aestivum* L.)

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ABSTRACT

Salicylic acid (SA), an endogenous plant growth regulator has been found to generate a wide range of metabolic and physiological responses in plants affecting their growth and development. In order to study effect of salicylic acid foliar application and limited irrigation on quantitative and qualitative characteristics of wheat, this experiment was conducted at Varamin, Iran during 2014 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 filling period stage) and different concentration of foliar application of salicylic acid (0, 150 and 300 µM) were allocated to subplots. The results showed that irrigation withholding conditions in different growth stages significantly decreased seed yield, RWC and total chlorophyll but by contrast increased membrane stability, proline content, SOD and CAT enzymes activity and also lipid and protein peroxidation. Salicylic acid foliar application had positive effect on all attributes in this experiment. In general, the results of the present study indicate that usage of salicylic acid reduces the harmful effects of water deficit stress and increases resistance to drought stress in wheat plant.

Keywords: Salicylic acid, wheat, irrigation withholding, SOD, CAT.

INTRODUCTION

Water is essential for plant growth and agricultural productivity is solely dependent upon water and it is necessary from seed germination to plant maturation [1]. Drought stress is one of the most important abiotic stress factors which are generally accompanied by heat stress in dry season [2]. Plants often suffer from water deficit stress, and the severity of the resulting damage varies depending on the intensity and duration of the stress. Other than the apparent effects of drought stress, the effects of water deficit are not well understood at the biochemical and molecular levels [3,4,5]. Under different conditions particularly environmental stress, reactive oxygen species, such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, are generated [6]. Reactive oxygen species can damage essential membrane lipids as well as proteins and nucleic acids [7]. However, under conditions of environmental stress, production of ROS can increase and endogenous protective activity may then become inadequate. Various associations between water stress and endogenous levels of water-soluble antioxidants have been described [8]. Antioxidant enzymes ascorbate peroxidase (POX), glutathione reductase and non-specific POX also increased significantly under water stress [9]. Various physiological practices are applied to alleviate the adverse effects of water deficit stress on the normal functioning of plants. For example, plant

growth regulators have been applied to plants as a means of improving the growing of those plants. Indeed, there is evidence that the proper application of plant growth regulators can increase plant tolerance to environmental stresses such as drought, heavy metals, salinity, chilling and water-logging [10,11]. Salicylic acid (SA), a plant Phenolic is now considered as a hormone-like endogenous regulator and its role in the defense mechanisms against biotic and a biotic stress has been well documented [12,13]. When applied exogenously at suitable concentrations, SA was found to enhance the efficiency of antioxidant system in plants [14]. SA was found to enhance the activities of antioxidant enzymes, (CAT), peroxidase (POX) and superoxide dismutase (SOD), when sprayed exogenously to the drought stressed plants of *L. esculentum* [10] or to the salinity stressed plants of *B. juncea* [15]. Krantev et al [16] reported the exogenous application of salicylic acid enhanced the activities of antioxidant enzymes ascorbate peroxidase (APX) and SOD in maize plants. The primary objective of the present investigation was to examine the effect of drought stress and salicylic acid on the activities of different antioxidant enzymes and other biochemical exchanges in leaves of wheat. The work was aimed also whether a foliar supply of salicylic acid to plant might be a strategy for increasing the drought stress tolerance.

MATERIALS AND METHODS

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The experiment was conducted at Varamin, Iran during 2014 growing season. Site of study was situated at 31° 51' E and 20° 35' N and 1050 m above sea level. Latitude and longitude of research place were 35°, 19' N and 51°, 39' E, respectively and site of study was located 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. Details of soil properties are shown in Table 1. 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 filling period stage) and different concentration of foliar application of salicylic acid (0, 150 and 300 μM) were 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, 2 m alley was kept to eliminate all influence of lateral water movement. Polyethylene pipeline was performed for control of irrigation as dropping irrigation. After seed sowing, irrigation was applied as required during the growing season. The foliar application of salicylic acid 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 was assayed. One week after irrigation withholding in different growth stages initiation leaf samples was collected and physiological and biochemical changes were assayed.

Relative water content assay

Relative water content was measured, from each plant leaf discs were taken and weighted (fresh weigh, FW). The discs were then placed in distilled water for 5 h at 25°C and then their saturated weights (SW) were measured. The discs were then dried in oven at 70 °C for 24 h to calculate dry weight (DW). RWC were calculated by following formula:

$$RWC = \frac{(FW - DW)}{SW - DW}$$

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 [17].

Proline assay

Proline content of leaves was determined according to a modification of the method of Bates et al [18].

Antioxidant enzyme activity assay

Catalase activity was estimated by the method of Cakmak and Horst [19]. Superoxide dismutase activity was determined according to the method of Giannopolitis and Ries [20].

Lipid peroxidation assay

The level of membrane damage was determined by measuring malondialdehyde (MDA) as the end product of peroxidation of membrane lipids [21].

Protein peroxidation assay

A standard dityrosine sample was prepared according to Amado et al [22].

Statistical analysis

All data were analyzed from analysis of variance (ANOVA) using the GLM procedure in SAS [23]. The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. Duncan's multiple range tests was used to measure statistical differences between treatment methods and controls.

RESULTS AND DISCUSSION

Analysis of variance showed that the effects of irrigation withholding in different growth stages was significant on all measured traits and foliar application of salicylic acid was significant on all measured traits except for RWC and proline content (Table 2). In addition, interaction between these two factors was significant on measured traits experiment such as: catalase enzyme activity and malondialdehyde.

Seed yield

Seed yield decreased as result of irrigation withholding at stem elongation stage, irrigation withholding at flowering stage and irrigation withholding at seed filling period stage at by 6.46 %, 12.00% and 7.29%, respectively with compared complete irrigation treatment conditions. Also, acceleration of flowering and maturity probably contributed to reduce the impact of drought stress in wheat plants. Similar findings have been reported in faba bean (*Vicia faba* L.) [24]. Seed yield increased as result of foliar application of salicylic acid with 300 μM at by 5.86 when this treatment compared with untreated foliar application of salicylic acid (Table 3). Khodary [25] observed a significant increase in growth characteristics, pigment contents and photosynthetic rate in maize, sprayed with SA. Hussein et al [26] in their pot experiment sprayed salicylic acid to the foliage of wheat plants, irrigated with Mediterranean sea water and reported an enhanced productivity due to an improvement in all growth characteristics including plant height, number and area of green leaves, stem diameter and dry weight of stem, leaves and of the plant as a whole.

Relative water content

Table 1: Soil properties of the experimental site

Depth	EC (ds m ⁻¹)	pH	O.C (%)	T.N.V (%)	K (ppm)	P(ppm)	Total N (%)	Texture
0-30 cm	3.1	7.2	0.61	<10	294	10.1	0.069	Clay loam

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

Sources of variation	df	Seed yield	Relative water content	Membrane stability	Total chlorophyll	Proline	Superoxide dismutase	Catalase	Malondialdehyde	Dityrosine
Replication	2	3798.16 ns	0.009 ns	14.82 ns	0.004 ns	0.0000 1 ns	16.04 ns	0.47 ns	0.003 ns	0.000 1 ns
Water stress	3	919071.27**	127.33**	97400.21**	215.9 9**	0.001* *	14805 9.49*	30639 .44**	126.93**	10.51**
Error (a)	6	6982.02	0.04	6.15	0.009	0.0000 04	7.68	2.27	0.0007	0.001
Salicylic acid application	2	402644.40*	0.523 ns	4589.26**	1.87* *	0.0000 4 ns	4489.44**	3280.24**	0.864* *	0.310**
Water stress × Salicylic acid application	6	11307.7 3 ns	0.190 ns	460.6 1 ns	0.078 ns	0.0000 07 ns	367.3 0 ns	270.2 4**	0.131* *	0.024 ns
Error (b)	16	111955.53	1.030	300.9 1	0.103	0.0000 2	453.4 8	62.06	0.033	0.019
C.V (%)		15.52	1.34	2.01	1.13	7.82	2.64	4.21	1.83	3.09

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

Table 3: Comparison of means on some attributes of wheat affected by irrigation withholding in different growth stages and salicylic acid foliar application.

Treatment	Seed yield (kg.ha ⁻¹)	Relative water content (%)	Membrane stability (μs cm ⁻¹)	Total chlorophyll (mg.li t ⁻¹)	Proline (mg.g ⁻¹ FW)	Superoxide dismutase (ΔA/mg pro.min ⁻¹)	Catalase (ΔA/mg pro.min ⁻¹)	Malondialdehyde (nmol g ⁻¹ FW)	Dityrosine (nmol g ⁻¹ FW)
Irrigation	6471.8a	79.62a	731.6 2d	33.12 a	0.011d	652.88d	122.0 5d	5.90d	3.32d
Complete Irrigation	6053.6b	76.60b	844.5 7c	31.58 b	0.022c	771.88c	160.9 9c	7.80c	3.86c
Irrigation withholding at stem elongation stage	5695.1c	74.58c	882.7 7b	26.21 c	0.036b	836.95b	206.7 2b	12.44 b	4.95b
Irrigation withholding at flowering stage	5999.5bc	70.66d	983.4 1a	22.50 d	0.042a	960.22a	257.2 1a	13.83 a	5.72a
Irrigation withholding at seed filling period stage	5864.2b	75.19a	879.9 9a	27.94 c	0.025a	785.00b	170.9 8c	10.24 a	4.62a
Salicylic acid foliar application	6071.4ab	75.30a	860.9 0b	28.39 b	0.029a	808.00a	185.2 9b	10.04 b	4.46b
Untreated (0 μM)	6229.5a	75.60a	840.8 8c	28.73 a	0.029a	823.44a	203.9 5a	9.70c	4.30c
Treated (150 μM)	6471.8a	79.62a	731.6 2d	33.12 a	0.011d	652.88d	122.0 5d	5.90d	3.32d

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

different growth stage (Table 3). RWC is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit, while water potential is an estimate of plant water status and it is useful in dealing with water transport in the soil-plant-atmosphere continuum [27].

Total chlorophyll

The highest total chlorophyll content was related to those plots which were treated with complete irrigation. However irrigation withholding at different growth stages decreased total chlorophyll content. Changes in leaf chlorophyll content with drought and heat injury may involve a severe

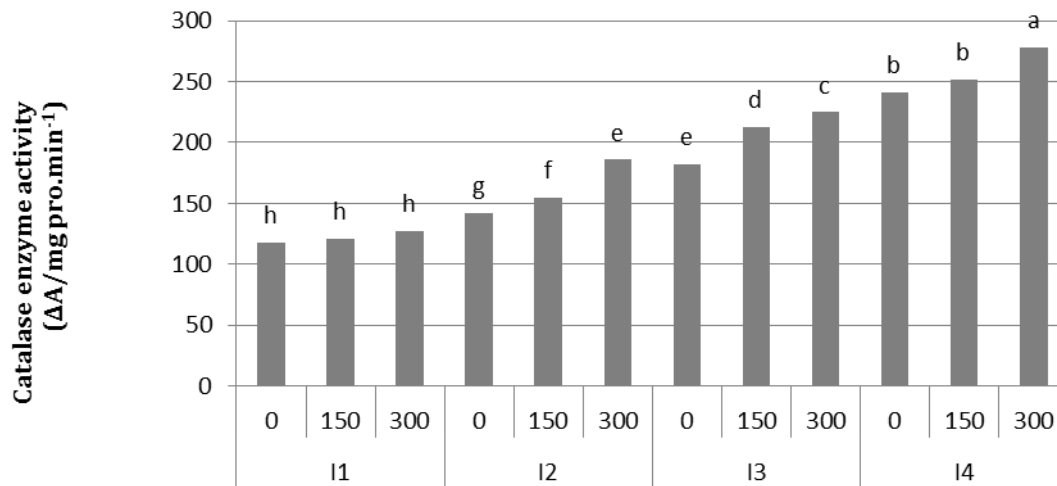


Figure 1: Interaction between irrigation withholding in different growth stages and foliar application of salicylic acid on catalase enzyme activity (I1 =complete irrigation, I2= withholding at stem elongation stage, I3= withholding at flowering stage and I4= withholding at seed filling period stages and 0, 150 and 300 salicylic acid concentrations). All the values followed by the same letter in each column are not statistically different at the $P < 0.05$ probability level.

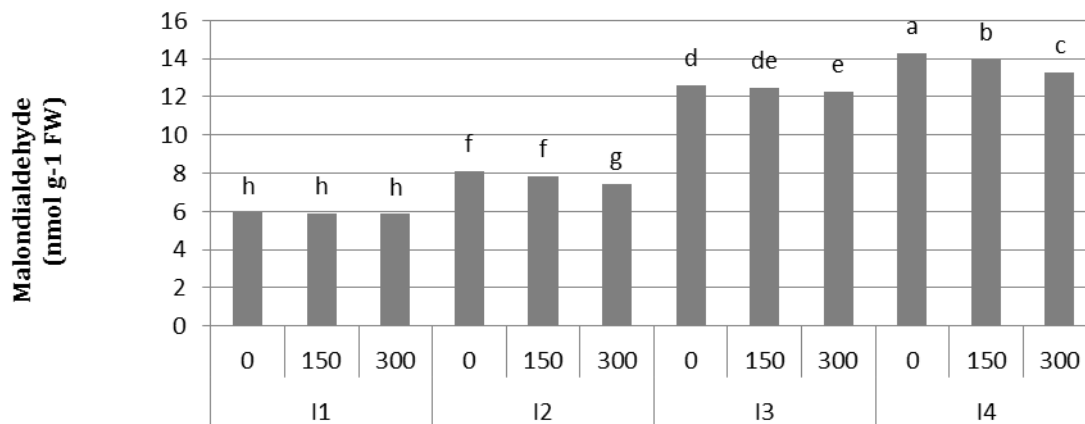


Figure 2: Interaction between irrigation withholding in different growth stages and foliar application of salicylic acid on malondialdehyde. (I1 =complete irrigation, I2= withholding at stem elongation stage, I3= withholding at flowering stage and I4= withholding at seed filling period stages and 0, 150 and 300 salicylic acid concentrations). All the values followed by the same letter in each column are not statistically different at the $P < 0.05$ probability level.

Membrane stability

According to table 3 the highest electrolyte leakage was occurred when wheat plants were treated with irrigation withholding at different growth stages. Electrolyte leakage decreased as result of foliar application of salicylic acid when these treatments compared with untreated foliar application of salicylic acid (Table 3). All this shows that foliar application of salicylic acid can lower the plant's membrane permeability and alleviate the damage on the cell membrane caused by water stress.

chlorophyll photooxidation mediated by oxyradicals [28]. Water deficit stress leads to an increase in free radicals in chloroplasts and destruction of Chl molecules by ROS, which results in reduction of photosynthesis and growth. Singlet oxygen atoms and O_2^- radicals predominantly attack double bond-containing compounds (unsaturated fatty acids and Chl), thus damaging the chloroplast membrane system and photosynthetic reaction centres [29]. It is worth mentioning that total chlorophyll content increased as result of foliar application of salicylic acid with 300μM when these treatments compared

with untreated foliar application of salicylic acid in this condition (Table 3). Salicylic acid is a detoxifier and neutralizer of superoxide radicals and other singlet oxygen species; by prevention of the activity of free radicals it can enhance the Chl content. Moreover, salicylic acid significantly increased chlorophyll a & b and carotenoids [30,31].

Proline

The result showed that the highest proline content in the leaves of plants belongs to irrigation withholding at seed filling period stage (Table 3). PRO protects membranes against the adverse effect of high concentrations of ions and may also function as a PROT compatible hydrotrope and as a hydroxyl radical scavenger [32]. In the current study, irrigation withholding at different growth stages increased the leaf PRO content. The concentration of this metabolite usually increases in response to water deficit stress [33].

Antioxidant enzymes

Plants under irrigation withholding at different growth stages showed a significant increase in SOD and CAT activity in the leaves compared with control plants (Table 3). Application of salicylic acid was associated with a significant difference in SOD and CAT activity in stressed plants; however, application of salicylic acid increased SOD and CAT activity in plants (Table 3 and Figure 1). However as can be seen from figure 1 the highest catalase enzyme activity was obtained from foliar application of salicylic acid with 300 μ M concentration in irrigation withholding at different growth stage condition. The enzymes assayed are scavengers of free radical species. SOD converts one form of ROS (O_2^-) to another equally toxic one (H_2O_2). Hydrogen peroxide is converted to oxygen and water by CAT and POX [34]. The increase in SOD activity was reported in tolerance basmati rice variety [35]. In our study, salicylic acid increased the activity of these enzymes maybe by elimination of free radicals. This increased antioxidant enzyme activity might be due to SA's regulatory role at transcriptional and translational levels. Also, these increases might be due to their direct scavenging function against the toxic free radicals and their promotional effects on synthesis of internal protective antioxidants. SA was found to enhance the activities of antioxidant enzymes, CAT, peroxidase (POX) and superoxide dismutase (SOD), when sprayed exogenously to the drought stressed plants of *L. esculentum* [10] or to the salinity stressed plants of *B. juncea* [15].

Lipid and protein peroxidation

The level of lipid and protein peroxidation of leaves, measured as MDA and dityrosine (DT) content, are shown in Table 3. The levels of MDA and DT were increased in the leaves of plants by irrigation withholding levels. The highest MDA and DT content belong to irrigation withholding at seed filling period stage. One of the best known toxic effects of ROS is damage to cellular membranes,

lipids and PROTs. Plasma membranes are oxidized by ROS to generate MDA. In this study, water deficit stress induced a progressive accumulation of MDA and DT in the leaves of plants. We observed a significant increase in the MDA and DT content in water deficit stressed plants. It was observed that application of salicylic acid decreased the MDA and DT content in the leaves of plants (Table 3 and figure 1). Exogenous salicylic acid partially inhibits these increases because salicylic acid is a scavenger of ROS. SA also affects the lipid peroxidation, which plays a key role in initiating defense response [36].

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