

Effect of zinc foliar application on auxin and gibberellin hormones and catalase and superoxide dismutase enzyme activity of corn (*Zea mays* L) under water stress

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Abstract

To study the effects of zinc foliar application and water stress on quantitative and qualitative characteristics of corn, an experiment was conducted in Varamin, Iran during the growing season of 2011. The experimental design was randomized complete blocks in factorial arrangement with four replications. Factors included water stress (complete irrigation, irrigation after 90 mm of water evaporation and irrigation after 130 mm of water evaporation from class A pan) and the zinc foliar applications were as follows: 0, zinc sulfate and zinc chelate. Result showed that severe water stress decreased seed yield, oil percentage, chlorophyll content, relative water content, zinc grain content, auxin and gibberellin, and it caused an increase in antioxidant enzyme activity. Zinc foliar application increased all tested attributes under severe water stress. The results of these tests showed that zinc foliar application was effective against the detrimental effects of water stress.

Keywords: antioxidant enzymes activity, hormones, chlorophyll, seed yield, oil percentage

Abbreviations: ROS - reactive oxygen species, RWC - relative water content, CAT - catalase, SOD - Superoxide dismutase, GPX - Glutathione peroxidase, Chl - Chlorophyll, H₂O₂ - hydrogen peroxide, GA3 - Gibberellin, IA - Auxin

Introduction

Water is a scarce resource in Iran because of highly variable amounts of rainfall. The intensity of the effects of water stress on plants is variable according to the timing, duration and magnitude of the deficit (Pandey et al, 2001). In many regions of the world, including Iran, water stress is one of the most important factors responsible for decreasing agricultural crop yields. Effective irrigation relies on identification of critical times for irrigation for application in an accurate and well organized irrigation schedule to make irrigation practices more effective and to conserve water resources thus facilitating sustainable agricultural practice (Ngouajio et al, 2007). Water stress induces oxidative stress in plants (Hajiboland and Joudmand, 2009). However, one of the most important effects of moisture shortage is that mobility of some elements such as zinc will be reduced in the soil solution causing plants to encounter deficiency of the element because of restricted root growth (Kafi and Rostami, 2007). The efficacy of fertilizers is reduced under conditions of water stress, especially if the use of these fertilizers is not consistent with the plants' vegetative growth. Zinc sulfate plays a more important role than other fertilizers in regulating stomata and maintaining the ions balance in plant systems to reduce draught stress. Thus, fertilizer consumption should be balanced and efficient during times of water short-

age and this especially applies to that of zinc sulfate (Baybordi, 2006; Babaeian et al, 2010). In this regard, Krishna (1995) reported a significant positive effect of zinc treatment on dry matter, seed and straw yield of mungbean as well as crude protein percentage in seeds. Kassab (2005) indicated that foliar application of Zn, Mg, Mn and Fe significantly increased growth parameters, yield and its components in mungbean plants.

Given the above mentioned points, it seems that zinc foliar application can reduce the effects of water stress and supply essential plant needs because research has shown that zinc increases crop yield, promotes the quality of produce and consequently promotes the enrichment and improvement in the health of a plant community (Borrell et al, 2008).

Materials and Methods

The experiment was conducted in research field of Azad University, Varamin Branch in Iran during the growing season of 2011. The site of the study was situated at 31°51'E and 20°35'N and 1,050 masl. Site of study was located 900 m above sea level. Soil samples were taken prior to the tests in order to determine its physical and chemical properties. A composite soil sample was collected at a depth of 0 - 30 cm. It was air dried, crushed and then tested to determine its physical and chemical proper-

ties. The soil in the research field was determined as clay/loam. Properties of the soil sample are shown in Table 1. Soil preparation was done with plow and disk, prior to setting out the plots. The experimental design was randomized complete blocks in factorial arrangement with four replications. The factors tested in the experiment were water stress: complete irrigation, irrigation after 90 mm of water evaporation (mild water stress) and irrigation after 130 mm of water evaporation from class A pan (severe water stress); and zinc foliar applications (untreated, zinc sulfate and zinc chelate). Treflan and gallant super were applied to control weed growth. According to soil analysis, phosphorus ($150 \text{ kg ha}^{-1} \text{ P}$) and potassium ($200 \text{ kg ha}^{-1} \text{ K}$) fertilizers were applied to the soil. Nitrogen was supplied from ammonium nitrate source (300 kg ha^{-1}) at three stages: seed sowing, end of the rosette stage, and before the flowering stage. The plots were sown with corn seed (S.C 704) in rows of 75 cm with plant spacing of 20 cm. Corn was planted manually in May 2011. Seeds were sown 6 cm deep. Two seeds were sown in each position and thinning was done in the plots to achieve the desired plant population ($67,000 \text{ plant ha}^{-1}$). After sowing, irrigation was applied as required during the growing season. The plots were 7 m long and consisted of five rows, 0.5 m apart, 2 m alley was maintained between plots to prevent lateral water movement. Zinc foliar application was done at the stage of stem elongation. Foliar applications were applied with a pressurized backpack sprayer (12 l capacity) calibrated to deliver $1,000 \text{ l ha}^{-1}$ of spray solution. The sprayer was equipped with a spiral solid cone spray nozzle.

Seed yield and oil percentage assay

Crops were harvested at the end of the growing season and evaluations were made for seed yield and oil percentage. Oil percentage was calculated using the soxhlet method.

Relative water content assay

Relative water content (RWC) 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). Relative water contents were calculated by following formula (Gupta, 2000):

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

Chlorophyll content assay

The first fully expanded leaf blades were taken to determine chlorophyll (Chl) contents after 30 h of salt stress. For the chlorophyll assay, leaf discs were

ground with 10 ml of 80% acetone (v/v). The amount of chlorophyll a and b was determined spectrophotometrically at 663 and 645 nm, using the method of Arnon (1949).

Concentration of grain zinc assay

Concentration of zinc in air-dried seeds were determined by atomic absorption spectrophotometer (Shimadzu AA-670, Shimadzu, Kyoto, Japan) in clear solution obtained after treating the seed flour with combination of sulfuric acid, salicylic acid, selenium and hydrogen peroxide (H_2O_2).

Gibberellin and auxin assay

The methods for extraction and purification of the four hormones (GA3, IAA) were carried out as previously described with some modifications (Xiao et al, 2001; Haver et al, 2003). Leaf samples were homogenized in cold 80% aqueous methanol extraction medium (1:20, w/v) containing 40 mg l^{-1} butylated hydroxytoluene as an antioxidant. To determine recoveries during extraction and purification, the known amounts of authentic hormones (GA3, IAA) were used as internal standards and were added before homogenization. Then the homogenate was further extracted in the dark at 4°C for 16 h. The extracts were centrifuged at $5,000 \text{ g}$ (4°C) for 10 min, and the supernatant was dried under low pressure and redissolved in 8 ml of 0.1 M ammonium acetate ($\text{pH} = 9$) by using an ultrasonic bath and were subsequently frozen at -20°C overnight. After thawing, the extract was centrifuged at $27,000 \text{ g}$ for 30 min. For purification, the supernatant from the centrifugation step was applied to a preconditioned column combination of PVPP (polyvinylpyrrolidone; Sigma, St Louis, MO, USA), DEAE-Sephadex G-25 (Whatman, Maidstone, Britain) and Chromosep C18 column (C18 Sep-Pak cartridge, Waters, Milford, MA, USA). The column combination was eluted with 0.01 M ammonium acetate, $\text{pH} = 8$. The PVPP column was removed and acidic hormones were eluted from the DEAE column with 1.5M acetic acid and collected in a second Chromosep C18 column attached to the DEAE column. Sep-Paks were washed with distilled water first, and the hormones were eluted from the cartridges with 50% methanol and dried under low pressure. Finally the hormone fraction was redissolved in 50% methanol and subjected to high-performance liquid chromatography (HPLC) analysis.

Antioxidant enzyme activity assay

Catalase (CAT) activity was measured according to Chandlee and Scandalios (1984), with modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer ($\text{pH} = 7$), 0.4 ml of 15

Table 1 - Soil properties of the experimental site.

Depth	EC (ds m^{-1})	pH	OC (%)	TNV (%)	K (ppm)	P (ppm)	Total N (%)	Texture
0-30 cm	4.1	7.4	0.71	<10	368	25.9	0.079	Clay loam

mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm.

Glutathione peroxidase (GPX) activity was measured according to [Paglia and Valentine \(1997\)](#) in which 0.56 M (pH = 7) phosphate buffer, 0.5 M EDTA, 1mM NaN_3 , 0.2 mM NADPH was added to the extracted solution. GPX catalyses the oxidation of glutathione by cumene hydroperoxide in the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured with a spectrophotometer.

Superoxide dismutase (SOD) activity was assayed according to [Beauchamp and Fridovich \(1971\)](#). The reaction mixture contained 1.17×10^{-6} M of riboflavin, 0.1 M of methionine, 2×10^{-5} M of potassium cyanide (KCN) and 5.6×10^{-5} M of nitroblue tetrazolium salt (NBT) dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH = 7.8). Three ml of the reaction medium were added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Phillips 40-W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30°C for 1 h. identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank.

Statistical analysis

All data were analyzed from analysis of variance (ANOVA) using the GLM procedure in SAS ([SAS Institute, 2002](#)). It was assumed that the residuals were random, homogenous and with a normal distribution about a mean of zero. Treatment means were compared using LSMEANS ($P < 0.05$).

Results and Discussion

The effects of water stress, zinc foliar application and interaction between the factors were significant on seed yield ([Table 2](#)). It was demonstrated that irrigation after 90 and 130 mm of water evaporation (mild stress and severe stress) decreased seed yields by 14.63% and 33.12%, respectively, when data were compared with the control treatment. This decrease can be attributed to early senescence and a decreased seed filling period. Similar results were obtained in other tests by [Ghorpad et al \(1993\)](#), in which it was reported that water stress significantly de-

creased seed yield. In addition, water stress reduces the transfer of nutrients from leaves to seeds, and given that drought accelerates seed maturation, this process also helps to decrease seed yield by reducing photosynthesis ([Erdem et al, 2006](#)). Furthermore, the highest seed yield was recorded in the treatment of zinc sulfate foliar application ([Table 3](#)). It seems that zinc sulfate has a more important role in regulating stomata and maintaining the ions balance in plant systems that reduce stress induced by drought. The interaction between water stress and zinc foliar application showed that under non-stress conditions, zinc sulfate application increased seed yield ([Table 4](#)). Under stress conditions (both mild stress and severe stress) zinc sulfate application increased seed yield more than zinc chelate application and the control. The treatment of zinc application had superior results in terms of straw and biological yield. The obtained results are in full agreement with the findings of other research by [Basole et al \(2003\)](#), [Gupta et al \(2003\)](#), and [Kassab \(2005\)](#). These results suggest that foliar application of nutrient solutions partially alleviated the adverse effects of water stress on photosynthesis and photosynthesis-related parameters and yield and yield components through mitigating the nutrient demands of water-stressed plants. Related research, [Ved et al \(2002\)](#) stated that foliar applied zinc enhances photosynthesis, stimulates early plant growth and improves nitrogen fixation, grain protein and yield. Water stress during the reproductive stage decreased evaluations for oil percentage. The highest oil percentage was obtained from the control treatment while severe water stress decreased evaluations for oil percentage by 13.20% when data were compared with the control treatment ([Table 3](#)). Decreased oil percentage is attributed to a decrease in seed weight. [Rudra naik et al \(2001\)](#) have reported that water stress decreased seed weight and oil percentage of safflower plants. Oil percentage was affected by zinc foliar application. Zinc improves photosynthesis and assimilates transportation to sinks and finally served to increase evaluations for oil percentage. [Singh and Sinha \(2005\)](#) reported that a decrease in oil concentration might have been caused by oxidation of some polyunsaturated fatty acids. The effect of water stress on chlorophyll content was significant ($P < 0.05$) ([Table 2](#)). Comparison of means showed that in tests for water stress levels, the highest chlorophyll content was obtained under normal

Table 2 - Analysis of variance on attributes of corn affected by water stress and zinc foliar application.

SOV	df	Seed yield	Oil (%)	Chl	RWC	Zn	Gibberellin	Auxin	SOD	Cat
Replication	3	ns	ns	ns	ns	ns	ns	ns	ns	ns
Water stress	2	**	**	*	ns	**	**	**	**	**
Error (a)	6									
Zinc foliar application	2	**	*	*	ns	**	**	**	**	**
Water stress ×										
Zinc foliar application	4	**	ns	*	**	**	**	ns	**	**
Error (b)	18									
CV (%)		14.23	8.48	7.48	3.32	8.60	4.24	3.46	3.75	2.68

*, ** and ns significant at 0.05, 0.01 probability level and no significant, respectively

Table 3 - The comparison of means on attributes of corn affected by water stress and zinc foliar application.

Treatments	Seed yield (t ha ⁻¹)	Oil percentage (%)	Chlorophyll Content (SPAD)	RWC (%)	Zn (mg 100g ⁻¹ grain)	Gibberellin (μ m mg ⁻¹ tissue DW)	Auxin (μ m mg ⁻¹ tissue DW)	SOD (u mg protein ⁻¹)	Cat (u mg protein ⁻¹)
Water stress									
Complete irrigation	8.50c	5.3a	41a	79.56a	89a	164a	447a	585c	114c
Irrigation after 90 mm	11.87a	5.3a	39b	76.15b	81a	125b	394b	778b	141b
Irrigation after 130mm	10.85b	4.6b	37b	73.23b	49b	98c	333c	1028a	180a
Zinc									
Untreated (0 ppm)	8.98c	4.96b	38b	75.72a	48c	98c	335c	586c	113c
Zinc sulfate foliar	12.71a	5.06a	40a	77.02a	99a	162a	444a	1018a	182a
Zinc chelate foliar	11.20b	5.22a	39a	76.20a	72b	126b	396b	787b	140b

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

irrigation treatment and the lowest chlorophyll content was obtained after 130 mm of water evaporation (Table 3). This result is consistent with the results of Yari et al (2005), in which it was suggested that moisture stress reduces leaf chlorophyll content. Also, the effect of zinc foliar application and the interaction between water stress and zinc foliar application was significant (Table 2). Also, the result showed that the highest chlorophyll content was record from the treatment of zinc sulfate foliar application (Table 3). The interaction between water stress and zinc foliar application showed that under stress conditions (both mild stress and severe stress) zinc foliar application increased evaluations for chlorophyll content (Table 4). In this regard, zinc sulfate foliar application increased chlorophyll content more than zinc chelate application and the control in this condition. The physiological characteristic of chlorophyll content is a good indicator of stress resistance because it affects the continuation of photosynthesis under water stress conditions (Jiang and Huang, 2002; Zhang et al, 2006; Zahedi et al, 2012). The results showed that different irrigation and zinc foliar application had a non-significant effect on relative water content RWC (Tables 2 and 3). However, RWC decreased under irrigation treatments of 90 and 130 mm of water evaporation (mild stress and severe stress). RWC is the appropriate measure of plant water status in terms of the physiological consequence of cellular water stress, while water potential is an estimate of a plant's water status and is a useful evaluation to assess water transportation in the soil-plant-atmosphere continuum (Kramer, 1988). A reduction in leaf RWC indicates the decrease of swelling pressure in plant cells and reduces growth. Therefore, plant water potential will be reduced in order to maintain the rate of transpiration (Karam et al, 2006). Also, the interaction between water stress and zinc foliar application was statistically significant (Table 2). Under stress conditions (both mild stress and severe stress) zinc foliar application increased evaluations for RWC (Table 4). Tariq et al (2004) reported that foliar application of micro-nutrients improves crop quality and increases resistance in plants against biotic and abiotic stresses. Analysis of variance showed there was significant difference for each factor and in the interactions between them (Table 2). Irrigation treatments

of 90 and 130 mm of water evaporation (mild stress and severe stress) decreased contents of Zn in seeds (Table 3). In fact, water stress caused a decrease in transmission of photosynthetic substances to grains and decreased uptake minerals and subsequently reduced the nutrient content in grains. In this regard, zinc sulfate foliar application increased contents of Zn in seeds more than zinc chelate application and the control (Table 3). Other research by Modaihsh (1997) determined that foliar application of micronutrients (Fe, Cu, Zn, and Mn) in the form of sulfate rather than as chelate (either EDTA or EDDHA) generally resulted in higher concentrations of these elements in wheat grain (*Triticum aestivum* L) and both Zn and Mn concentrations were considered to be higher than others. However zinc sulfate foliar application increased contents of Zn in seeds under mild and severe stress conditions (Table 4). The effects of water stress and zinc foliar application on gibberellin and auxin contents were significant (Table 2). The interaction between water stress and zinc foliar application was significant only for gibberellin (Table 2). Results also showed that the highest gibberellin and auxin contents were obtained when corn plants were exposed to complete irrigation (Table 3). By contrast, the lowest evaluations for gibberellin and auxin were obtained in corn plants that were exposed to severe stress conditions (Table 3). Plants respond to water stress by making various adaptations through various physiological and biochemical changes (Monneveux and Belhassen, 1996). These adaptations include changes in endogenous phytohormone levels, especially that of abscisic acid. Determinations of variation of auxin contents under water stress are very contradictory. It has been reported that water stress resulted in a decrease of auxin content in the leaves of wheat (Xie et al, 2003). But other evidence has shown that adaptation to water stress was accompanied with an increase in auxin content (Sakurai et al, 1985; Pustovoitova et al, 2004). This study has demonstrated that water stress had significant and differential effects on gibberellin and auxin contents in corn plants. Gibberellin is able to increase plant growth under osmotic stress (Kaur et al, 1998); however, a decline in levels of gibberellin and auxin were observed in corn plants in response to water stress in this study. It was reported that water stress resulted

Table 4 - The interaction effect between water stress and zinc foliar application on attributes of corn.

Water stress	Foliar application of Zinc	Seed yield [£]	Oil percentage	Chl Content [§]	RWC (%)	Zn [†]	Gibberellin [‡]	Auxin [‡]	SOD [§]	Cat [§]
Complete Irrigation	Untreated(0 ppm)	11.42c	5.05c	37cd	78.6cde	75c	168b	450b	440e	98d
	Zinc sulfate	14.22a	5.48a	45a	80.4a	126a	198a	493a	574d	100d
	Zinc chelate	12.47b	5.46a	42ab	79.7bc	95b	167b	456b	573d	100d
Irrigation after 90 mm	Untreated(0 ppm)	8.95e	5.10a	34cd	74.9e	44d	85d	338d	742c	142c
	Zinc sulfate	12.72b	5.58a	39bc	80.0a	96b	125c	393d	1018b	183b
	Zinc chelate	10.87cd	5.32a	38cd	75.5de	75c	121c	392c	743c	141c
Irrigation after 130mm	Untreated(0 ppm)	6.57f	4.51c	37cd	72.0bcd	26e	84d	274e	741c	142c
	Zinc sulfate	10.42d	4.62bc	40ab	74.4cde	75c	124c	392c	1296a	220a
	Zinc chelate	8.50e	4.60bc	39cd	73.3de	45d	86d	389c	1045b	179b

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test. Units of measurement are as follows: £: (t ha⁻¹); §: (SPAD); †: mg 100g⁻¹ grain; ‡: (µm mg⁻¹ tissue DW); §: (u mg protein⁻¹);

in a decrease in auxin content (Yang et al, 2001; Xie et al, 2003). Results also showed that zinc sulfate application increased gibberellin and auxin contents more than zinc chelate application and the control (Table 3). The interaction between water stress and zinc foliar application showed that under stress conditions (both mild stress and severe stress) zinc foliar application increased gibberellin and auxin contents (Table 4). Zn is a precursor of the auxin (IAA) (Oosterhuis et al, 1991). Thus, zinc foliar application served to increase hormones involved in biosynthesis such as auxin in the treated corn plants. Analysis of variance showed that there was significant difference for each factor and interactions between them (Table 2). In the present study, those plants exposed to water stress showed a significant increase in SOD and CAT activity in leaves (Tables 3 and 4). The enzymes assayed are scavengers of free radical species. SOD converts one form of ROS (O₂[•]) to another equally toxic one (H₂O₂). Hydrogen peroxide is converted to oxygen and water by CAT and POX, which use ascorbate as the hydrogen donor (Hegedus et al, 2001). Water stress may also lead to stomatal closure, which reduces CO₂ availability in the leaves and inhibits carbon fixation (Gossett et al, 1994a,b). An increase in SOD activity was reported in a water stress tolerant basmati rice variety (Singh et al, 2007). In water stress conditions, zinc foliar application increased the activity of these enzymes (Table 4). Zinc sulfate application increased antioxidant enzyme activity more than zinc chelate application and the control (Tables 3 and 4). Zinc is an essential mineral nutrient and a cofactor of over 300 enzymes and proteins involved in cells (Marschner, 1986). Thus zinc foliar application served to increase antioxidant enzyme activity in water stress condition.

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