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EFFECTS OF ZINC AND SALICYLIC ACID ON WHEAT UNDER DROUGHT STRESS

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ABSTRACT

The present study was conducted to investigate whether exogenous application of salicylic acid (SA) and zinc (Zn) could regulate the activities of antioxidant enzymes and ameliorate the adverse effects of drought stress on wheat. Pot experiment was arranged in a Randomized Complete Block Design (RCBD) with two factors and three replications during the year 2013-2014 in Turkey. The control, drought, drought + SA and drought + Zn were first factor, and the wheat cultivars, Basribey and Ziyabey 98, were second factor. Drought stress at grain filling stage significantly decreased plant height, spike length, number of grains per spike, 1000 grain weight, chlorophyll content, relative water content but activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) under drought stress were increased. Both SA and Zn application had positive influence on all parameters in this experiment. The foliar application of Zn increased water use efficiency and thereby reduced the negative effects of drought stress. Besides the foliar application of salicylic acid regulated physiological processes in plants and alleviated the adverse effects of water stress. Results showed that SA and Zn could be used for improving wheat growth under drought stress.

Key words: Antioxidant enzyme activity, Drought stress, Foliar application, Salicylic acid, Zinc.

INTRODUCTION

Drought is the environmental stress that limit plant growth and development, reduce yield and quality especially in arid and semi arid regions. Drought and heat stress lead to some changes on plants' physiological and biochemical properties and productivity. These factors are associated with global climate change (Akbari *et al*. 2013; Feller and Vaseva, 2014). Wheat (*Triticum aestivum* L.) is one of the most important crops in the world. The growth of wheat has been seriously influenced by drought in many regions. In Turkey, the majority of wheat is cultivated in dry-farming areas. Researchers have stated that, plants are very sensitive to drought stress at pollination and grain-filling stages (Zafar *et al*. 2014). Drought stress leads to overproduction of reactive oxygen species (ROS) which is known as a defense mechanism of plants (Carvalho, 2008). Under drought stress conditions, tolerant cells activate their antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) contributed to ROS accumulation (Farooq *et al*. 2009; Morteza Salekjalali *et al*. 2012).

Salicylic acid is a plant growth regulator that plays a significant role in abiotic stress. It was revealed that SA increased the abscisic acid content, leading to the accumulation of proline (Shakirova *et al*., 2003), and soaking grains in acetyl-salicylic acid imoroved the drought tolerance in wheat (Farooq *et al*. 2009). Many studies report the protective effects of SA on plants against salinity (Daneshmand *et al*. 2009; Azooz *et al*. 2011; Ghafiyehsanj *et al*. 2013), drought (Bideshki and Arvin, 2010; El Tayeb and Ahmed, 2010), osmotic stress (Daneshmand *et al*. 2009; Fateh *et al*. 2012), high temperature (Khan, 2012) and heavy metal stress (Popova *et al*. 2012). SA regulates physiological and biochemical properties of plants under abiotic stress and it is also important in disease resistance (Hashempour *et al*. 2014)

Zinc is essential plant micronutrient which is involved in many physiological functions, protein and carbonhydrate synthesis (Yadavi *et al*. 2014). The decrease of zinc on plant have been associated with the drought stress caused by decreases in soil water and consequently, restriction of root growth (Zafar *et al*. 2014). The application of zinc (Zn) under drought conditions would influence crop yield and quality. It plays a significant role in regulating stomata and ionic balance in crops to reducing the detrimental effects of drought (Moghadam *et al*. 2013; Monjezi *et al*. 2013) and also has protective effects on photo oxidative damage caused by ROS (Akbari *et al*. 2013).

The effects of drought were more severe during reproductive stage than vegetative stage in wheat (Kanani *et al*. 2013). So drought conditions coincide with the grain filling period and limit grain yield. The grain filling stage of wheat Zn and SA application can influence the sensitivity of wheat. Maize is most sensitive to drought stress during the grain filling stage with only the flowering and early reproductive period being more sensitive. Water stress leads to grain abortion if it occurs early enough in the post-anthesis stage but is more often associated with poor grain filling and thus decreased grain weight (Lamm and Aboukheira, 2012). This study investigated the effectiveness of salicylic acid (SA) and zinc (Zn) foliar applications in mitigating the adverse effects of drought stress. For this reason we hypothesized that SA and Zn would alleviate the negative effects of drought on yield components and physiological traits.

MATERIALS AND METHODS

Plant materials, experimental design, cultivation practices and competition indices: In order to evaluate the effects of salicylic acid and zinc application on yield components and physiological traits of wheat cultivars under late season drought stress condition, an experiment was conducted in completely randomized design with two factors and three replications during 2013-2014 growing season. The control (normal irrigation), drought stress (deficit irrigation), drought + SA and drought + Zn were used as first factor. Drought susceptible (Basribey 95) (Yavas *et al*. 2011) and drought resistant (Ziyabey 98) (Dennis *et al*., 2002) wheat cultivars were placed in second factor plots. Foliar spraying of SA and Zn were performed at growth stage (GS) 61 and growth stage (GS) 69 on the Zadoks scale by manual sprayer, respectively. Each pots (18x18x15 cm) was filled with soil with volume 3.8 liter. Water deficit was imposed by withholding water at the grain filling stage for 10 days. The soil fertility was improved by using 15-15-15, ammonium nitrate and urea at the rate of 15, 5 and 7 kg da^{-1}, respectively, before planting. The soil texture class is sandy-loam, pH=7.6, total N% =0.10, available P = 2.08 ppm and available K=124 ppm.

Total phosphorus and potash fertilizer were used at planting and the remaining N fertilizer was added at two growing stages of wheat (tillering and stemming). A volume 500 µM of the SA and the concentration of 0.3 % Zn from zinc sulfate have been utilized. Irrigation of control treatments were routinely made until the end of growth period while irrigation of drought stress treatment was interrupted at grain filling stage. At the end of the stress periods, rewatering was carried out. Plant height was obtained from soil surface until spike tip in the main stem, excluding awns. Spike length was measured from the collar to spike tip without considering awns. The number of grains per spike was determined by counting grains on every spike from a subsample of 10 plants. For 1000 grain weight measure, some seeds were selected randomly from five selecting samples and after counting by 100 seeds and then weighted. Leaf relative water content was determined by method developed by Barrs and Weatherley (1962). Second leaf of randomly selected four plants was used for determining relative water content. Fresh weight (FW) immediately recorded, and then leaves were soaked for 4 hours in distilled water. Turgid weight (TW) was recorded followed by drying for 24 hours at 80 ºC for dry weights (DW). Relative water content (RWC) was calculated according to the following formula: $RWC = [(FW - DW) / (TW - DW)] \times 100$. Leaf chlorophyll contents were determined at mid-bloom stage with Apogee Chlorophyll Meter according to Akhkha *et al.* (2011). Superoxide dismutase (SOD) was assayed according to the method of Dhindsa *et al*. (1980). Peroxidase (POD) was measured as described by Polle *et al.* (1994). Catalase (CAT) was determined by the procedure described by Aebi (1984).

Statistical analysis: Variance analysis was carrid out with the SPSS (SPSS Inc. 1999). Differences among means were tested through LSD and α ($P<0.05$) was considered for significance.

RESULTS AND DISCUSSION

The results showed that the interaction between drought treatment and cultivar was significant for all characters except 1000 grain weight. The drought stress had significant effects on yield components such as plant height, spike length and number of grains/spike; leaf characteristics such as relative water content and chlorophyll content in both cultivars. The control treatment had the highest values but drought stress given the lowest values for all characters (Table 1 and 3). Yield of both cultivars were reduced significantly by drought stress. Two plant growth regulators (PGRs) affected plant height. The effects of PGRs were generally more evident in Basribey (a shorter cultivar) than Ziyabey 98. The tallest plants were observed in the control treatment and the shortest under drought conditions. These results are in agreement with those obtained by (Raza *et al*. 2012; Yan and Shi, 2013; Maqbool *et al.* 2015) they reported that drought stress reduced wheat plant height. Anosheh *et al.* (2012) reported that after the exogenous applications of salicylic acid and chlormequat chloride reduced the harmful effects of drought. The positive effects of foliar spray of SA and Zn on plant height of wheat under drought stress were in accord with the results observed by some investigators (Aldesuquy *et al*. 2012; Azimi *et al.* 2013). In one study it was found that drought stress on wheat reduced the plant height, but with Zn application significantly inceased the plant height (Monjezi *et al.* 2013). Maximum grains number mean (43.4) was obtained at full irrigation and decreased by drought conditions (35.0). SA and Zn foliar applications were between control and drought without SA and Zn treatments. SA took place at forefront in terms of number of grains/spike, whereas Zn given the higher spike length. These findings also are in agreement with Farooq *et al.* (2009) and Monjezi *et al.* (2013). A similar protective effect of zinc sulfate was also observed by Malek-Mohammadi *et al.* (2013). At high number of grain per spike leads to higher grain yield in plants Sharafizad *et al.* (2013), but drought stress significantly reduced kernel yield, row no pear ear, kernel no per row, cob diameter and ear length (Zamaninejad *et al.* 2013). According to results, it was founded that drought stress reduced spike length (5.8 cm) and maximum spike weight (8.4 cm) obtained from control conditions. SA and Zn applications increased spike length compared to control. Sharafizad *et al.* (2013) also reported that moderate dosage of salicylic acid (1.2 mM) increased spike length of per spike. 1000 grain weight values were affected by SA and Zn application, but no significant differences were observed between treatments

According to results, it was found that drought stress reduced 1000 grain weight and mean maximum 1000 grain weight (35.9 gr) obtained from control (Table 2). 1000 grain weight values were affected by SA and Zn application, but no significant differences were observed between treatments. However this reduction was alleviated by adding SA and Zn. In the experiment of Kanani *et al.* (2013) on wheat, grain yield and many of yield components significantly affected by drought stress on reproductive growth stage. A drought stress induced decrease in 1000 grain weight was previously reported in Farooq *et al.* (2009) and Beigzadeh *et al.* (2013). It has been proposed that zinc spray alleviates drought stress effect on one thousand grain weight Monjezi *et al.* (2013). Similarly Sharafizad *et al.* (2013) also reported that exogenous application of salicylic acid alleviated the adverse effect of drought stress on wheat plants. Maleki *et al.* (2014) also stated that potassium and zinc sulfate can decrease negative effects of drought stress on maize grain yield and yield components.

Relative water content (RWC) is important characteristic that effect plant water relations. Chlorophyll content and relative water content in leaves significantly decreased with drought stress but SA and Zn application inhibited this effect (Table 3). RWC of wheat was higher initially during leaf development than of matured leaf. Under water stress, wheat and rice plants had lower leaf water potential and thus lower relative water content than well-watered conditions (Farooq *et al.* 2009). There were significant differences between genotypes for relative water content in four conditions. The high amount of RWC were shown in control, SA and Zn foliar application, respectively. The results of this study are in good agreement with the findings of Rao *et al.* (2012) and Alam *et al.* (2013) who observed salicylic acid application induced enhancement of RWC. Shahbazi *et al.* (2012) stated that the cell membrane thermostability, lipid peroxidation, relative water content, catalase, and ascorbate peroxidase activities can be used as yield indicators under drought stress. On the other hand, Sayyari *et al.* (2013) reported that dry weight, leaf area, photosynthetic pigments and RWC of lettuce were reduced in drought stress. The results of experiments by Afshari *et al*. (2013) indicate the exogenous application of 300 µM SA was found to increase the RWC in FWS (50 % flowering stage drought stress) condition, while different SA treatments during PFDS (pod formation drought stress) had no significant effect. Kordi *et al.* (2013) also stated that the chlorophyll content of sweet basil plants was reduced under drought stress. In the research conducted by Anosheh *et al.* (2012), indicated the positive effect of salicylic acid on the contents of chlorophyll a and b, and peroxidase activity. According to another study of cowpea chlorophyll index, Afshari *et al.* (2013) found that 300 µM salicylic acid application increased chlorophyll index under water stress conditions. These observations are similar to the findings of Rao *et al.* (2012) and Alam *et al.* (2013).

Table 1. Effects of drought and foliar application on plant height, spike length and number of grains per spike of wheat

	Plant height (cm)		**Spike length (cm)**		**No.of grains/ spike**	
Treatments	**Ziyabey 98**	**Basribey**	**Ziyabey 98**	**Basribey**	**Ziyabey 98**	**Basribey**
Control	98.9 ^{a}	93.8 ^{a}	8.4 ^{a}	8.8 ^{a}	43.4 ^{a}	36.7 ^{a}
Drought	95.7 ^{c}	84.7 ^{d}	5.8 ^{d}	5.8 ^{c}	35.0 ^{d}	33.9 ^{c}
Drought+SA	97.7 ^{b}	86.8 ^{c}	6.4 ^{c}	6.7 ^{b}	36.9 ^{b}	36.0 ^{a}
Drought+Zn	97.7 ^{b}	87.9 ^{b}	6.6 ^{b}	6.9 ^{b}	36.1 ^{c}	35.0 ^{b}
Treatment (A)	**		**		**	
Cultivar (B)	**		**		**	
Treatment x Cultivar (AxB)	**		*		**	
LSD (AxB)	0.6		0.2		0.7	

The sources of variance were as follows: four treatments, two cultivars and interaction between treatment and cultivar. Different letters indicate the significance of difference by LSD test. Least significant difference (LSD) of the Treatment x Cultivar interaction. ns, * and **: Non significant, significant at 5% and 1% levels of probability, respectively.

Table 2. Effects of drought and foliar application on 1000-grain weight of wheat

	1000 grain weight (g)		
Treatments	**Ziyabey 98**	**Basribey**	**Mean**
Control	36.5	35.4	35.9 [a]
Drought	34.9	34.2	34.5 [b]
Drought+SA	36.1	35.3	35.7 [a]
Drought+Zn	35.0	34.7	34.8 [b]
Mean	35.6 [a]	34.9 [b]	
Treatment (A)	**		
Cultivar (B)	**		
Treatment x Cultivar (AxB)	ns		
LSD	LSD (A)=0.2, LSD (B)= 0.4		

The sources of variance were as follows: four treatments, two cultivars and interaction between treatment and cultivar. Different letters indicate the significance of difference by LSD test. Least significant difference (LSD) of the Treatment and Cultivar ns, * and **: Non significant, significant at 5% and 1% levels of probability, respectively.

Table 3. Effects of drought and foliar application on relative water content and chlorophyll content of wheat

	RWC (%)		Chlorophyll content	
Treatments	**Ziyabey 98**	**Basribey**	**Ziyabey 98**	**Basribey**
Control	86.7 [a]	78.3 [a]	51.0 [a]	46.1 [a]
Drought	69.7 [c]	57.7 [d]	49.1 [c]	45.3 [b]
Drought+SA	81.3 [b]	73.7 [b]	49.8 [b]	46.0 [a]
Drought+Zn	70.3 [c]	59.7 [c]	50.0 [b]	45.8 [ab]
Treatment (A)	**		**	
Cultivar (B)	**		**	
Treatment x Cultivar (AxB)	**		*	
LSD (AxB)	1.3		0.6	

RWC: Relative water content. The sources of variance were as follows: four treatments, two cultivars and interaction between treatment and cultivar. Different letters indicate the significance of difference by LSD test. Least significant difference (LSD) of the Treatment x Cultivar interaction. ns, * and **: Non significant, significant at 5% and 1% levels of probability, respectively.

Table 4. Effects of drought and foliar application on SOD, POD and CAT activities.

	SOD ($U\ g^{-1}$)		POD ($U\ mg^{-1}$ protein)		CAT ($U\ mg^{-1}$ protein)	
Treatments	**Ziyabey 98**	**Basribey**	**Ziyabey 98**	**Basribey**	**Ziyabey 98**	**Basribey**
Control	355.0 [d]	339.5 [c]	56.1	60.5 [d]	41.5 [d]	38.9 [d]
Drought	619.3 [c]	341.0 [c]	57.2	61.1 [c]	48.1 [c]	41.0 [c]
Drought+SA	640.0 [a]	353.9 [a]	58.9	91.0 [a]	52.0 [a]	44.1 [a]
Drought+Zn	622.7 [b]	346.3 [b]	58.6	62.5 [b]	50.0 [b]	43.1 [b]
Treatment (A)	**		**		**	
Cultivar (B)	**		**		**	
Treatment x Cultivar (AxB)	**		*		**	
LSD (AxB)	3.2		0.5		0.2	

SOD: superoxide dismutase, POD: peroxidase, CAT: catalase. The sources of variance were as follows: four treatments, two cultivars and interaction between treatment and cultivar. Different letters indicate the significance of difference by LSD test. Least significant difference (LSD) of the Treatment x Cultivar interaction. ns, * and **: Non significant, significant at 5% and 1% levels of probability, respectively.

Antioxidant enzyme activities in both cultivars were increased in drought stressed plants in comparision with that of the control plants. Significant increases were observed for SOD by applications of SA and Zn (Table 4). Simlarly, exogenous application of both PGRs significantly increased the activity of POD. Generally, POD activity was higher in cv. Basribey than Ziyabey 98. CAT activity was also significantly influenced by drought stress. Foliar application of SA and Zn resulted in significant increase CAT activity. In response to drought stress, the activities of CAT, POD and SOD showed an increase in barley and wheat plants (Morteza

Salekjalali *et al.* 2012; Anosheh *et al.* 2012). Other reports have shown that salicylic acid ameliorate the negative effect of drought stress (Fayez and Bazaid, 2014). Previous reports have also shown that salicylic acid application enhanced the activity of POD in barley plants under drought conditions (Habibi, 2012).

Conclusıon: Our results have confirmed the analyzed yield properties, leaf characteristics and antioxidant levels each other. It was observed that leaf physiological properties like yield components, RWC and chlorophyll content were reduced under drought conditions compared to control conditions. Foliar spray with SA mitigated the adverse effects of drought stress on grains per spike. Similar results were observed effect of zinc on spike length. SA mitigated the adverse effects of drought stress. The SA application was significant mitigating effect on RWC and chlorophyll content. It was observed an increase in antioxidant activity in arid conditions and the most significant increase was found in SA application. Ziyabey 98 displayed a significant increase in the activity of SOD and CAT as compared with Basribey. Both decreases in the value of RWC under drought applications and higher SOD and CAT values showed that Ziyabey 98 was more tolerant. As a result, beacuse of the moderating effect of SA application on drought stress, it can be used practially.

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