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EFFECTS OF SILICON ON THE BIOCHEMICAL CHARACTERISTICS OF WHEAT UNDER DROUGHT STRESS CONDITIONS

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Abstract – In order to determine the effect of drought stress on the biochemical properties of wheat, it was investigated in a factorial experiment in the form of a randomized complete block design with three replications in the research field of the Soil and Water Research Institute in the crop year 2018-2019. and 2019-2020.The treatments included drought stress at three levels without stress, mild stress as the first factor. The second factor of Potassium silicate was investigated at four control levels of 0 and 20 kg/ha as soil application and foliar spraying application with concentrations of 2.5 and 5 kg/ha in Sivand wheat cultivar. The results of analysis of variance showed that the effect of drought stress on Malondialdehyde, Superoxide dismutase, Ascorbate peroxidase, Catalase, Glutathione peroxidase and plant growth was significant ($p<0.01$) and Proline traits and seed yield were significant 5% level ($p< 0.05$). The effect of Silicon on superoxide dismutase traits ($p<0.01$) and malondialdehyde, ascorbate peroxidase, catalase, glutathione peroxidase, breeding and seed yield was significant ($p<0.05$). The results of the treatment interaction showed that catalase and glutathione peroxidase traits were significant ($p<0.01$). As a result, the use of silicon by improving biochemical characteristics can help wheat to overcome drought stress.

Keywords: catalase; grain yield; proline; protein; wheat.

Introduction

Wheat constitutes the major food of most people in the world and accounts for the highest area under cultivation of agricultural lands worldwide. Owing to its unique properties, wheat is the most important crop on the globe (Tester & Langridge, 2010). In Iran, wheat also accounts for the highest area under cultivation, and this issue doubles the importance and the need for planning and optimal management of resources and wheat-producing factors (Emam, 2007). Crops are exposed to multiple environmental stresses, all of which affect their growth, thereby influencing crop production levels. Drought stress is among the most destructive stresses that reduce the productivity of crops more than other stresses (Majidi & Amiri, 2020). Drought is the most important factor limiting the growth and yield of crops and affects 40-60% of agricultural lands worldwide (Sinclair, 2011). High fluctuations are observed in the yields

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of successive years due to shortage and uneven distribution of precipitation from one year to another. Additionally, an increase in the evapotranspiration rate causes the incidence of drought stress during the growth period of plants (Gonzalez et al., 2010). To prevent the destructive effects of stress, plants use a complex defense system including enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase, ascorbate peroxidase (APX), and glutathione reductase (GR), as well as non-enzymatic antioxidants including ascorbic acid, glutathione, carotenoids, and tocopherol (Verma et al., 2015). With the incidence of environmental stresses, increasing both the activity of antioxidant enzymes and the content of osmolytes plays a vital role in tolerance to biotic and abiotic stresses in plants (Epstin, 1999). Gong et al. (2005) concluded that silicon (Si) application would increase the activity of antioxidant enzymes (SOD, CAT, and GR). Their results indicated that drought stress led to an increase in H_2O_2 levels whereas Si reduced H_2O_2 levels, acid phospholipase activity, and damages caused by oxidant stress.

The essential factor related to crop production is good plant nutrition, which plays a remarkable role in increasing yield. Accordingly, the role of some nutrients, such as Si, has been of interest to some plant nutrition researchers (Gong & Chen, 2012). Si is a nonessential but useful nutrient affecting the growth and health of plants. Most plants can absorb Si and the absorption rate is in the range of 0.1-10% of plant biomass depending on the plant type (Cherif & Belanger, 1992).

The effect of Si on plant yield may arise from its precipitation in the leaf width, increasing leaf strength (Adatia & Beasford, 1986), elevating chlorophyll concentration in the leaf unit area (Maghsoudi et al., 2016), and increasing photosystem II efficiency (Popovic et al., 2003). More chlorophyll concentration can improve photosynthesis in plants (Maghsoudi et al., 2016). An increase in tolerance to environmental stresses, including drought, by Si application has been reported in several studies (Maghsoudi & Emam, 2016).

Gong et al. (2005) investigated the effect of Si on the defense of wheat against oxidative stress under drought stress in different stages of growth and development. They reported that the use of Si increased plant water potential under drought stress at the filling stage, but this was not true for the heading stage. The researchers observed SOD inhibition and an increase in peroxidase (POX) activity at the pod filling stage by drought stress. At the grain filling stage, Si application resulted in an increase in SOD activity and a reduction in POX activity under drought stress. CAT activity was slightly elevated under drought stress, and Si application did not change H_2O_2 levels and soluble protein content at the heading stage under drought stress, but it led to a decrease in H_2O_2 concentrations and a rise in soluble protein content.

Ideal soil fertility is a major factor in increasing wheat production. Good plant nutrition is also a solution to reduce the detrimental effects of stresses and plays a marked role in reducing its yield. In this regard, the role of some nutrients, such as Si, has attracted the attention of some plant nutrition researchers (Gong & Chen, 2012). Therefore, this research was conducted to evaluate the effects of Si on agronomic and physiologic traits of the Sivand wheat cultivar in drought stress conditions.

Table 1. The results of mixed ANOVA for the effects of Si on biochemical traits of wheat in drought stress conditions.

SOV	df	Mean of squares				
		MDA	**SOD**	**APX**	**CAT**	**GPX**
Year	1	0.000272ns	3.104ns	0.00012ns	0.0000001ns	1610.00ns
Year repeat	4	0.002544	1.836	0.000311	0.0001488	3274.0*
Drought stress	2	0.912712**	588.844**	0.676901**	0.0083294**	4403403.00**
Year × stress	2	0.005676*	2.066ns	0.002625ns	0.0001059**	1956.00**
Si	3	0.0605*	95.831**	0.02609*	0.0005404*	385861.00*
Year × Si	3	0.00298*	0.328ns	0.000602ns	0.0000435**	412.00ns
Si × stress	6	0.000612ns	4.602*	0.003785*	0.0000361**	69046.00**
Year × stress × Si	6	0.000517ns	0.808ns	0.000579ns	0.0000022ns	159.0ns
Error	44	0.004294	3.91	0.000307	0.0000966	1104
cv (%)	***	9.71	11.7	4.09	22.7	5.35

*, **, and ns: significant difference at 5% and 1% probability levels and no significant difference, respectively.

Table 2. Comparison of average Si and drought stress on the biochemical traits of wheat.

Treatments		MDA (µmol.g)	SOD (µmol.g)	APX (µmol.g)	CAT (Unit.mg[1]Protein)	GPX (µmol.g)
Drought stress	S1	0.481	11.35	0.255	0.027	138.778
	S2	0.871	18.513	0.437	0.039	764.96
	S3	0.672	20.858	0.591	0.064	958.177
	LSD	**0.107**	**3.25**	**0.028**	**0.016**	**54.68**
Si	SI1	0.753	13.643	0.38	0.038	454.946
	SI2	0.679	16.965	0.461	0.05	683.863
	SI3	0.649	18.219	0.415	0.04	554.379
	SI4	0.618	18.802	0.455	0.046	789.365
	LSD	**0.09**	**2.81**	**0.024**	**0.013**	**47.35**

The averages of the treatments with a difference higher than LSD are significantly different at the 5% level.

Materials and methods

This study was carried out as a randomized complete block design with three replications in the research field of the Soil and Water Research Institute in the 2018-2019 and 2019-2020 crop years. The geographic coordinates of the study area include 50° 57' E and 35° 45' N with an altitude of 1280 m from the sea level. In Alborz province, the climate falls into temperate to cold areas with an average precipitation of 250 mm. Experimental treatments were drought stress at three levels without stress, mild stress, and severe stress with 75, 50, and 25% usable moisture in the soil as the first factor. The second factor was potassium silicate at four levels of no Si application (control), 20 kg/ha as soil application at the bolting stage, and spraying at 2.5 and 5 kg/ha at bolting, heading, and seed dough stages, which were examined in Sivand wheat cultivar (drought-sensitive) for 2 years. Wheat plants were cultivated on December 10, and six planting lines (4 m and 20 cm in length and width, respectively) were determined in each plot.

Measurement of traits

Proline content (mg/g leaf fresh weight) accumulated in the leaves at the flowering stage was measured by spectrophotometry at 520 nm wavelength (Bates et al., 1973). CAT activity was determined according to Boominathan & Doran (2002) as described below. First, 900 µl of the reaction solution (containing a 10 mM H_2O_2 solution in PVP-free phosphate buffered saline and 100 µl of enzymatic extract) was poured into a cuvette. Then, H_2O_2 was added to the reaction solution, and the reduction caused by H_2O_2 decomposition by CAT activity was immediately measured with a spectrophotometer (Uvi Light XS 5 SECOMAM) at 240 nm wavelength for 1 min, followed by calculating CAT activity.

APX activity was assessed at the flowering stage according to Boominathan & Doran (2002). Initially, 900 µl of the reaction solution (625 µl of EDTA-containing phosphate buffer, 175 µl of ascorbic acid, 50 µl of H_2O_2, 50 µl of BSA, and 100 µl of enzymatic extract) was poured into a cuvette. Then, the reduction in ascorbic acid caused by the enzyme activity was measured with a spectrophotometer at 290 nm wavelength for 1 min, followed by measuring APX activity. SOD activity was measured through its ability in preventing the photoreduction of nitroblue tetrazolium (NBT) chloride at the flowering stage as described by Dhindsa et al., (1981). To this end, 3 ml of the reaction solution was prepared to contain 50 mM potassium phosphate, 13 mM methionine, 75 µM NBT chloride, ethylene in 0.1 mM tetraacetic acid, 360 µM riboflavin, and 30 µl of crude extract. After stirring the mixture, the spectrophotometer cells were exposed to a 15 W fluorescent light at a distance of 35 cm for 10 min. The reaction was stopped by turning off the light, and the mixture absorbance was read at 560 nm. One unit of SOD activity was defined as the amount of enzyme that could prevent the photoreduction of NBT chloride by 50%. The specific activity of the enzyme was reported as the enzyme units in mg of protein.

The malondialdehyde (MDA) biomarker was determined using a previously reported method (Heath & Packer, 1986). Based on this method, 0.2 g of terminal young leaf fresh tissue was weighed and pulverized in a porcelain mortar containing 5 ml of 1% trichloroacetic acid (TCA). The resulting extract was centrifuged at 10,000 rpm for 5 min. Then, 4 ml of 40% TCA containing 0.5 thiobarbituric acid (TBA) was added to 1 ml of the supernatant. The resulting mixture was heated in a water bath at 95 C for 30 min, cooled immediately on ice, and re-centrifuged at 10,000 rpm for 10 min. The optical density of this solution was read at 532 nm using a spectrophotometer. The MDA-TBA red complex is the substance of interest for absorbance in this wavelength. The absorbance of other non-

Table 3. Comparison of the average effect of year × Si and drought stress on the biochemical traits of wheat.

Treatments			MDA (µmol.g)	SOD (µmol.g)	APX (µmol.g)	CAT (Unit.mg[1]Protein)	GPX (µmol.g)
Year × stress	Y1	S1	0.478	11.523	0.263	0.026	135.085
		S2	0.861	19.03	0.424	0.038	750.729
		S3	0.692	20.791	0.592	0.066	961.913
	Y2	S1	0.484	11.177	0.248	0.029	142.472
		S2	0.882	17.997	0.45	0.04	779.191
		S3	0.653	20.925	0.59	0.061	954.441
	LSD		**0.07**	**2.3**	**0.02**	**0.011**	**38.66**
Year × Si	Y1	SI1	0.769	13.665	0.371	0.038	456.856
		SI2	0.664	17.31	0.466	0.047	678.504
		SI3	0.65	18.437	0.416	0.041	548.393
		SI4	0.624	19.047	0.453	0.047	779.883
	Y2	SI1	0.738	13.62	0.388	0.038	453.037
		SI2	0.694	16.62	0.456	0.052	689.222
		SI3	0.649	18.001	0.413	0.039	560.366
		SI4	0.611	18.557	0.458	0.045	798.847
	LSD		**0.06**	**1.99**	**0.017**	**0.009**	**33.48**

The averages of the treatments with a difference higher than LSD are significantly different at the 5% level.

specific pigments was determined at 600 nm and subtracted from this value. The concentration of this biomarker was calculated using a 1.56×10^5 extinction coefficient, and the measurement results were calculated based on µmolg^{-1} F. Seed protein content was obtained by multiplying seed nitrogen (%) by 6.25 (Jones et al., 1991).

To measure grain yield in individual plots, 0.5 m was eliminated from the initial and end of lines. Then, all spikes in three middle lines were harvested manually along 2 m. Grains were dried in an oven and then separated manually (Alavi Fazel, 2015).

Statistical analysis of data

The uniform variance of experimental errors was verified using Bartlett's test by Minitab software. Statistical calculations were done by MSTATC and Minitab software. Mean values were compared by the least significant method at the 5% probability level.

Results

According to the results in the mixed ANOVA table, MDA concentrations and the effects of Si, year × stress, and year × Si under drought stress were significant at 1% and 5% probability levels, respectively. However, the interaction of drought stress × Si was not statistically significant. As shown in the comparison of means (Table 2), the highest (0.871 µmol.g) and lowest (0.481 µmol.g) MDA concentrations were recorded under mild and no drought stress (control) conditions, respectively. The plants in treatments without Si application and 5 kg of Si spraying contained the highest (0.753 µmol.g) and lowest (0.618 µmol.g) MDA concentrations, respectively (Table 2), suggesting the destruction of the plant cell membrane under drought stress.

SOD activity

Based on the results of mixed ANOVA (Table 1), SOD activity was significantly affected by drought stress and Si treatments at the 1% level, and the interaction of drought stress × Si was statistically significant at the 5% level. According to the comparison of means (Table 2), the highest (20.85 µmol.g) and lowest (11.35 µmol.g) SOD activities belonged to severe stress and no drought stress (control) conditions, respectively. The highest (23.52 µmol.g) and lowest (8.14 µmol.g) SOD activities were obtained in severe stress treatments with 5 kg of Si spraying and no Si application without drought stress, respectively (Table 4).

APX activity

The results of mixed ANOVA (Table 1) revealed that APX activity was significantly influenced by drought stress at the 1% level. Si treatments and the interaction of drought stress × Si were statistically significant at the 5% level. As shown in the comparison of means (Table 2), the highest (0.591 µmol.g) and lowest (0.255 µmol.g) APX activities were measured in severe stress and no drought stress (control) conditions, respectively. Si soil application (20 kg) and no use of Si resulted in the highest (0.461 µmol.g) and lowest (0.38 µmol.g) APX activities. The interaction of treatments led to the highest (0.621 µmol.g) and lowest (0.175 µmol.g) APX activities using Si soil application (20 kg) in severe stress and no use of Si in stress-free conditions (Table 4).

CAT

According to the results of mixed ANOVA (Table 1), CAT concentrations were significantly affected by the effects of drought stress and the interaction of year × stress, year × Si, and drought stress × Si at 1% and 5% probability levels, respectively. The comparison of means (Table 2) showed that the highest (0.064 Unit.mg^{-1} Protein) and lowest (0.027 Unit.mg^{-1} Protein) CAT concentrations were measured under severe and no drought stress conditions, respectively. The plants in treatments with Si soil application (20 kg) and no Si spraying contained the highest (0.05 Unit.mg^{-1} Protein) and lowest (0.038 Unit.mg^{-1} Protein) CAT concentrations, respectively (Table 2). The interaction of drought stress × Si using 20 kg of Si soil application resulted in the utmost CAT concentration (0.069 Unit.mg^{-1} Protein) (Table 4). In this study, drought stress led to an increase in CAT activity. Drought stress increases ROS, thereby elevating antioxidant defense (Apel & Hirt, 2004).

Glutathione peroxidase (GPX)

Based on the results of mixed ANOVA (Table 1), GPX concentrations were significantly influenced by the effects of drought stress and the interaction of drought stress × Si at the 1% probability level, and the effect of Si was significant at the 5% level. The comparison of means (Table 2) indicated that the highest (958,177 µmol.g) and lowest (138.778 µmol.g) GPX concentrations belonged to severe and no drought stress conditions, respectively. The treatment with 20 kg/ha of Si spraying and Si-free treatment resulted in the highest (789.365 µmol.g) and lowest (454.946 µmol.g) GPX concentrations, respectively (Table 2). The interaction of treatments produced the highest (1100.13 µmol.g) and lowest (60.89 µmol.g) GPX activities in 20 kg/ha of Si spraying and Si-free treatments in severe and no drought stress conditions, respectively (Table 4).

Protein

As indicated by the mixed ANOVA table, protein content was significantly influenced by drought stress treatments and Si application at 1% and 5% levels (Table 5). A comparison of means revealed that protein content was affected by

Table 4. Comparison of the average interaction effect of Si and drought stress on the biochemical traits of wheat.

Treatments			MDA (µmol.g)	SOD (µmol.g)	APX (µmol.g)	CAT (Unit.mg^{1}Protein)	GPX (µmol.g)
Si × stress	S1	SI1	0.567	8.146	0.175	0.024	60.893
		SI2	0.492	12.39	0.284	0.032	130.913
		SI3	0.453	12.204	0.245	0.025	125.877
		SI4	0.413	12.66	0.318	0.028	237.43
	S2	SI1	0.957	15.45	0.395	0.032	482.218
		SI2	0.87	18.855	0.479	0.048	939.743
		SI3	0.843	19.525	0.421	0.034	607.345
		SI4	0.815	20.223	0.453	0.043	1030.533
	S3	SI1	0.737	17.332	0.569	0.058	821.727
		SI2	0.677	19.65	0.621	0.069	980.933
		SI3	0.652	22.928	0.578	0.06	929.917
		SI4	0.625	23.522	0.596	0.068	1100.132
	LSD		**0.05**	**1.62**	**0.014**	**0.008**	**27.34**

The averages of the treatments with a difference higher than LSD are significantly different at the 5% level.

severe and no stress treatments at the highest (14.60%) levels. Treatments with 5 kg and no Si application led to the highest (12.64%) and lowest (10.16%) effects, respectively, on protein content (Table 6).

Proline

The results of mixed ANOVA (Table 5) indicated that proline content was significantly affected by drought stress treatments at the 5% level. A comparison of means showed that proline content was affected by severe and no drought stress treatments at the highest (15.56 mg·g) and lowest (7.42 mg·g) levels (Table 6).

Grain yield

According to the mixed ANOVA table, grain yield was significantly affected by drought stress treatments and Si application at the 5% level (Table 5). A comparison of means showed that severe and no drought stress treatments exerted the lowest (4931.33 kg.ha^{-1}) and highest (6872.16 kg.ha^{-1}) effects on grain yield. Treatments with 5 kg and no Si application led to the highest (12.64%) and lowest (10.16%) effects, respectively, on grain yield (Table 5).

Discussion

In this study, considering the water shortage crisis and saving water resources and on the other hand, the important role of silicon in reducing the negative effects of drought stress, we investigated the effect of silicon on biochemical characteristics of wheat by applying drought stress. According to the results obtained from the analysis of variance and comparison tables, it can be stated, insufficient water supply at the vegetative growth stage influences plant establishment and development, stem growth, and reduces substance accumulation in these organs (Aslam et al., 2015).

In drought stress conditions, SOD makes up the first line of defense against active oxygen radicals in the cell and catalyzes the reduction of the superoxide radical to H_2O_2 and molecular oxygen. In the next step, the resulting H_2O_2 is scavenged by APX and CAT enzymes (Amini, 2014). APX and SOD antioxidants can directly react with the superoxide radical and other reactive oxygen species (ROS), which can reduce the damage severity (Israr & Sahi, 2006).

Our results indicate an increase in APX activity in stress conditions as the transcription of some genes related to antioxidant enzymes (e.g., GPX or APX) increases in drought stress conditions to improve plant status, which plays an important role in reducing ROS and the resulting damage (Marivani et al., 2019). Similarly, Kavas et al. (2013) reported that an increase in APX activity in drought stress conditions could inhibit ROS, including H_2O_2, which accumulates during stress.

In this study, drought stress led to an increase in CAT activity. Drought stress increases ROS, thereby elevating antioxidant defense (Apel & Hirt, 2004).

High protein content in drought stress conditions relative to optimum situations can be associated with the reduced duration of growth and development in water-limited treatments, leading to a reduction in the carbohydrate-to-protein ratio and an increase in protein content. Thalooth et al. (2006) reported that deficit irrigation stress resulted in the impaired photosynthesis process, the activity of enzymes, and protein synthesis, affecting the transfer of metabolites to seeds.

Proline plays an essential role in osmoregulation, and the rise of proline content in plant tissues somehow indicates the activation of the osmoregulation mechanism, which provides the ground for more uptake of water and elements from the root environment (Munns, 2002). An increase in leaf proline content in drought stress conditions may result from protein degradation and decomposition of the carbon making up the leaf structure of the plant (Zadehbagheri et al., 2012).

Paknezhad et al. (2017) presented evidence that drought stress at reproductive stages led to reductions in most traits of yield and yield components compared to the control treatment, and the utmost yield reduction was observed in a drought stress treatment applied at the flowering stage. Based on the findings of Shahdi Komele & Kavousi (2004), Si significantly increased the rice plant yield by improving the morphological status and altering the chemical composition of this plant. To explain the mechanism of this result, they deduced that Si could reduce the toxicity of microelements and adjust the uptake of macroelements, thereby affecting the growth, yield, and weight of plant shoots.

Conclusion

Oxidative stress is a consequence of drought stress in plants. Antioxidant enzymatic activities often increase in plant cells exposed to environmental stresses, and thus plants can reduce the damage of created oxygen free radicals. Accordingly, drought stress application reduced grain yield in this research. The application of Si spraying (5 kg/ha) in stress-free conditions increased grain yield by 39% compared to the control.

Table 5. Two-year mixed ANOVA for the effects of Si on the yield and some biochemical traits of wheat under drought stress conditions.

SOV	df	Mean of squares		
		Protein	Proline	Grain yield
Year	1	0.629ns	24.21ns	1423547ns
Year repeat	4	0.377	1.289	35345
Drought stress	2	246.936**	425.905*	25933506*
Year × stress	2	2.311ns	4.379ns	470570ns
Si	3	23.141*	46.14ns	9309636*
Year × Si	3	1.624ns	1.335ns	533459ns
Si × stress	6	1.396ns	0.52ns	290284ns
Year × stress × Si	6	0.809ns	1.366ns	291215ns
Error	44	0.415	1.243	216071
cv(%)	***	5.51	10.26	8.17

*, **, and ns: significant difference at 5% and 1% probability levels and no significant difference, respectively.

Table 6. Comparison of average silicon and drought stress on grain yield and some biochemical traits of wheat.

Treatments		Grain yield (kg.ha^{-1})	Protein (%)	Proline (mg.g)
Drought stress	S1	6872.167	8.255	7.421
	S2	5256.333	12.222	9.619
	S3	4931.333	14.604	15.564
	LSD	**764.9**	**1.06**	**1.83**
Si	SI1	4758.222	10.163	8.901
	SI2	6044.889	12.453	10.714
	SI3	5525.111	11.514	11.049
	SI4	6418.222	12.644	12.808
	LSD	**662.4**	**0.91**	**1.58**

The averages of the treatments with a difference higher than LSD are significantly different at the 5% level.

Table 7. Correlations between yield traits and some biochemical traits of wheat due to silica and drought stress.

Traits	Grain yield	Protein	Proline	MDA	SOD	APX	CAT	GPX
Grain yield	1							
Protein	-0.528	1						
Proline	-0.395	0.900**	1					
MDA	-0.742**	0.455	0.110	1				
SOD	-0.495	0.951**	0.854**	0.478	1			
APX	-0.584*	0.966**	0.922**	0.433	0.903**	1		
CAT	-0.509	0.922**	0.937**	0.242	0.814**	0.947**	1	
GPX	-0.553	0.944**	0.840**	0.552	0.932**	0.928**	0.872**	1

*,**: represent significant differences at 5% and 1% levels, respectively.

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