

## Improving Growth and Yield of Wheat under Drought Stress via Application of SiO<sub>2</sub> Nanoparticles

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### ABSTRACT

Silicon (Si) and its derivatives have beneficial effects on a wide variety of plant species, especially under both biotic and abiotic stresses. Yet, their effects on wheat (*Triticum aestivum* L.) plants under drought stress are not well known. Therefore, in order to evaluate the effects of SiO<sub>2</sub> NanoParticles (NPs) under drought stress, wheat seeds were separately sown in pots. Then, the SiO<sub>2</sub> NPs were added to them through soil and foliar application at three stages of plant growth. Results indicated that drought stress significantly decreased majority of the studied traits compared to the normal irrigation. Soil application of NPs, under drought stress, significantly increased leaf greenness (SPAD) and Relative Water Content (RWC) by 12.54 and 84.04%, respectively, compared to the control (NPs= 0 ppm). Moreover, under drought stress, wheat yield also increased by 25.35 and 17.81%, respectively, by foliar and soil application of NPs. Under the same irrigation regimes, soil application of NPs significantly increased plant height and biomass compared to the foliar application of NPs. Finally, our results highlight that usage of the SiO<sub>2</sub> NPs, especially at rates of 30 and 60 ppm, can mitigate adverse effects of drought stress in wheat plants.

**Keywords:** Biomass, Foliar application, Grain protein, Leaf area, Relative water content, SPAD.

### INTRODUCTION

Nanotechnology employs NPs having at least one dimension between 1 and 100 nm (Auffan *et al.*, 2009). Nanomaterials hold great promise regarding their application in agriculture in terms of plant protection and nutrition due to their size-dependent qualities, high surface-to-volume ratio and unique optical properties (Jatav and Nirmal, 2013). SiO<sub>2</sub> NPs is one of the most popular nanomaterial that has been used in this field (Le *et al.*, 2014).

The Si is the second most abundant element in the earth's crust (Jones and Handreck, 1976). Although this element is not considered an essential nutrient for most

terrestrial plants, it plays an important role in enhancing the quality, quantity, and protection of some plants such as rice and wheat (Epstein, 2009). Also, it can be beneficial in mitigating biotic and abiotic stresses such as insect pest attack, diseases, salinity, drought, wounding, and high temperature (Liang *et al.*, 2007; Van Bockhaven *et al.*, 2013). For instance, silica improves photosynthesis parameters of some plants under drought stress (Ma, 2009; Zhang *et al.*, 2013). It has also been related to affect the antioxidant enzyme activity. Kamangar and Haddad (2016) stated that Si partially offset the negative effects of drought stress by increasing the tolerance of grapevine (*Vitis vinifera* L.) by rising the soluble protein content and antioxidant enzyme activities.

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On the other hand, drought stress, as a major abiotic stress, strongly limits growth, development, and yield of plants especially in arid and semiarid regions (Mahajan and Tuteja 2005; Eneji *et al.*, 2008). This stress can damage plant cell membranes, and cell wall architecture, besides inhibiting photosynthesis and cell division (Taiz and Zeiger, 2006). Monakhova and Chernyadev (2002) stated that drought stress significantly decreased photochemical activities and inhibited activities of enzymes of Calvin cycle. There is little information about SiO<sub>2</sub> NPs effect on growth and yield of wheat under drought stress. Therefore, the purpose of this research was studying the effects of SiO<sub>2</sub> NPs on the wheat under drought stress.

## MATERIALS AND METHODS

### Growth Condition

A pot test was carried out in a factorial experiment in randomized complete block design with three replications. The experimental factors included SiO<sub>2</sub> NPs concentrations (0, 30, 60 and 90 ppm), application methods (foliar and root application), and irrigation regimes (normal irrigation and withholding irrigation for 15 days after pollination). The experiment was conducted during the growing season of 2014-2015 at the College of Agriculture, Tarbiat Modares University (35°43' N; 51°8' E; 1215 m sea level), Tehran, Iran.

### Soil Characteristics

Results of the studied soil analysis are

presented in Table 1.

### Plant Materials

The seeds of wheat (*Triticum aestivum* cv. pishtaz) were purchased from the Seed and Plant Improvement Institute, Karaj, Iran.

### NPS

Specific surface area of SiO<sub>2</sub> NPs was >80 m<sup>2</sup> g<sup>-1</sup> and purity was >99%. The size of the NPs was determined through Field Emission-Scanning Electron Microscope (Figure 1, FE-SEM). Average primary particle size was about 40±9.5 nm.

### SiO<sub>2</sub> NPS Suspension Preparation

The SiO<sub>2</sub> NPs were suspended directly in distilled water and dispersed by ultrasonic vibration (100W, 40 KHz) for 30 minutes. Small magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles (Adhikari *et al.*, 2013). Different doses of the SiO<sub>2</sub> NPs suspensions (0, 30, 60, and 90 ppm) were prepared for the pot experiment.

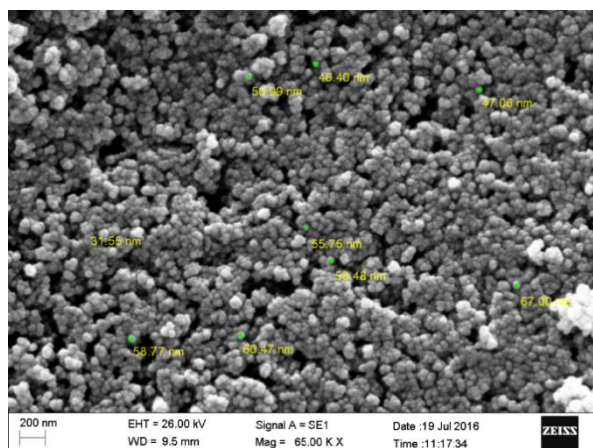
### Treatments

Ten seeds of wheat were surface sterilized and sown in the plastic pot (27 cm in height and 26 cm in diameter) containing 10 kg of soil. Fertilizers were applied to the pots according to the soil analysis. Urea fertilizer was added at rate of 1.03 g N pot<sup>-1</sup> in two equal portions; the first during the seedling

**Table 1.** Physical and chemical properties of soil used in pot experiment.

Sandy loam <sup>a</sup>			pH	EC	OM <sup>b</sup>	TN <sup>c</sup>	P	K	Fe
Clay	Silt	Sand							
	%			Ds m <sup>-1</sup>		%		ppm	
10.55	17.25	72.2	7.7	0.4	0.11	0.11	69.46	616.08	7.76

<sup>a</sup>, <sup>b</sup>, <sup>c</sup>: Denotes the soil texture, Organic Matter and Total Nitrogen, respectively.



**Figure 1.** Field Emission-Scanning Electron Microscope (FE-SEM) image of SiO<sub>2</sub> NPs.

stage and the second at stem elongation stage.

Seedlings were thinned out to allow four plants per pot for data recording. Four concentrations of the SiO<sub>2</sub> NPs (0, 30, 60, and 90 ppm) were applied three times at stage of tillering, stem elongation, and heading in the root and through foliar application. For normal irrigation, soil was frequently irrigated and, in stressed plants, water was withheld for 15 days after pollination. During rainy days, a mobile rain shelter was used in the drought stress treatments to prevent infiltration of the rain.

### Measurements

At the end of one-week stress period, three flag leaves of the four plants in each pot were labeled and some traits were determined *i.e.* SPAD, Leaf Area (LA), content of proline, SuperOxide Dismutase (SOD) activity, and Relative Water Content (RWC). Also, plant height, biomass, yield, and yield components were recorded at the harvest time.

### RWC

The RWC was calculated using the method devised by Mata and Lamattina (2001) using the following equation:

$$RWC (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100 \quad (1)$$

Where, *FW* is Fresh Weight, *DW* is Dry Weight and *TW* is Turgid Weight of leaf samples.

### Leaf Area and SPAD

The Leaf Area (LA) was estimated using portable area meter model Li-3000A LI-COR. Also, the SPAD was measured by chlorophyll meter (SPAD-502, Minolta, Japan).

### Proline and SOD Activity

On the 7<sup>th</sup> day after drought stress, three flag leaves of the four plants in each pot were harvested and frozen in liquid nitrogen immediately for the analysis of SOD activity and proline. Both traits were measured using a spectrophotometer (Specord 200, Analytical Jena, Germany). Proline was determined following Bates *et al.* (1973). The SOD activity was assayed following the method of Giannopolitis and Ries (1977) by monitoring the inhibition of photochemical reduction of Nitro Blue Tetrazolium (NBT) at 560 nm.

### Biomass and Yields

The biomass, yield, and yield components were determined and analyzed when the



grains were mature. The wheat plants were harvested and oven dried at 80°C for 48 hours and then weighed (Gubbins *et al.*, 2011).

### Seed Quality

Contents of phosphorous, potassium, and protein were determined in the dry seeds after harvesting, using Near Infrared Reflectance (NIR).

### Statistical Analysis

Analysis of variance was evaluated by SAS (Version 9.1; SAS Institute Inc., Cary, NC, USA). The significance of differences among treatment means were compared by the LSD test ( $P < 0.05$ ).

## RESULTS

### Analysis of Variance

Analysis of variance showed that NPs concentration, application methods, and irrigation regimes significantly affected all measured traits. The three-way interaction

among NPs concentration, application methods, and irrigation regimes was significant for LA, SPAD, RWC, 1000-grain weight, number of grains per spike, yield, protein, potassium, phosphorus, plant height, and biomass (Tables 2 and 3). There was a significant two-way interaction between NPs concentration and irrigation regimes on SOD activity. Furthermore, there was a significant two-way interaction between NPs concentration and application methods as well as NPs concentration and irrigation regimes interaction on content of proline.

### LA, SPAD, and RWC

Usage of NPs, especially 90 ppm, increased SPAD and RWC compared to the control (NPs= 0 ppm). Application of 30 ppm NPs in plants under non-stressed conditions and no application of NPs in plants under drought stress led to the highest LA (27.00 cm<sup>2</sup>) and the lowest LA (9.19 cm<sup>2</sup>), respectively (Table 4). There was no significant difference between application methods of NPs on LA in the same irrigation regimes. A visible decline in SPAD (44.75) was obtained in the treatment without NPs in plants under drought stress. In contrast, the highest SPAD (55.65) was achieved with

**Table 2.** Analysis of variance for the effects of SiO<sub>2</sub> NPs application and irrigation regimes on some agronomic traits of wheat.

SOV	DF	Mean square					
		LA	SPAD	RWC	SOD	Proline	1000-Grain weight
Rep	2	1.58	1.34	35.34	178.38	65.32	4.26
NPs (A)	3	13.69 *	42.94 **	2141.11 **	58.50 **	921.70 **	7.59 <sup>ns</sup>
Application methods (B)	1	4.08 <sup>ns</sup>	81.53 **	62.49 <sup>ns</sup>	4.106 <sup>ns</sup>	228.50 **	37.47 **
Irrigation regimes (C)	1	2821.33 **	278.59 **	23818.65 **	252.54 **	22533.76 **	5333.87 **
A×B	3	5.47 <sup>ns</sup>	50.09 **	1262.17 **	2.57 <sup>ns</sup>	71.52 **	40.55 **
A×C	3	5.16 <sup>ns</sup>	39.00 **	906.17 **	54.87 **	589.09 **	14.07 *
B×C	1	5.33 <sup>ns</sup>	560.88 **	376.82 **	16.40 <sup>ns</sup>	3.56 <sup>ns</sup>	235.01 **
A×B×C	3	11.83 *	100.43 **	963.73 **	4.30 <sup>ns</sup>	7.69 <sup>ns</sup>	51.78 **
Error	30	3.91	3.66	4.28	7.03	15.21	4.38
CV (%)	-	11.17	3.82	4.86	11.04	6.94	8.36

\*, \*\*, and ns: Significant at 0.05, 0.01 probability level and not significant, respectively.

**Table 3.** Analysis of variance for the effects of SiO<sub>2</sub> NPs application and irrigation regimes on some agronomic traits of wheat.

SOV	DF	Mean square						
		No grain per spike	Yield	Protein	Potassium	Phosphorus	Plant height	Biomass
Rep	2	8.44	44.97	3.32	0.01810	0.00915	9.30	3.15
NPs (A)	3	202.13 *	14.34 **	0.81 **	0.25515 **	0.00726 **	9.62 <sup>ns</sup>	149.71 **
Application methods (B)	1	97.07 <sup>ns</sup>	56.55 **	1.55 **	0.38880 **	0.00046 **	362.23 **	332.01 **
Irrigation regimes (C)	1	0.71 <sup>ns</sup>	3827.04 **	12.98 **	0.36750 **	0.05266 **	46.41 **	465.50 **
A×B	3	411.84 **	12.86 **	20.98 **	0.17365 **	0.00931 **	95.16 **	244.72 **
A×C	3	818.00 **	13.30 **	2.93 **	0.02615 **	0.00351 **	44.35 **	450.14 **
B×C	1	782.95 **	62.83 **	13.94 **	0.06750 **	0.00226 **	1.54 <sup>ns</sup>	8.56 <sup>ns</sup>
A×B×C	3	222.50 *	8.39 **	4.53 **	0.03135 **	0.00511 **	49.90 **	38.44 *
Error	30	59.68	0.61	0.13	0.00006	0.00001	4.10	11.41
CV (%)	-	17.45	5.16	4.62	0.83	0.99	2.99	5.66

\*, \*\*, and ns: Significant at 0.05, 0.01 probability level and not significant, respectively.

application of 60 ppm NPs in plants under non-stress conditions. Moreover, application of 90 ppm NPs to plants under non-stressed conditions and in the treatment without NPs in plants under drought stress caused the highest RWC (88.27%) and the lowest RWC (21.94%), respectively.

On the other hand, drought stress significantly decreased mean of LA, SPAD and RWC compared to the normal irrigation, while the use of NPs in plants under drought stress significantly increased mean of LA, SPAD and RWC of leaves compared to the control. Also, in plants under drought stress, root application of NPs had better effect on SPAD and RWC of leaves compared to the foliar application of NPs.

### Proline and SOD Activity

Content of proline was affected by NPs concentration and application methods (Figure 2-a), as well as NPs concentration and irrigation regimes (Figure 2-b). The highest content of proline was obtained in the treatment without soil application of SiO<sub>2</sub> NPs (Figure 2-a). In contrast, the lowest content of proline was observed at foliar application of 30 ppm NPs. Also, content of proline was significantly

increased in plants under drought stress compared to the plants under non-stressed conditions (Figure 2-b).

On the other hand, the highest SOD activity was obtained by using 30 and 60 ppm NPs in plants under drought stress. In plants under non-stressed conditions, usage of NPs had no significant effect on SOD activity compared to the control (Figure 3). Drought stress significantly increased SOD activity compared to the normal irrigation.

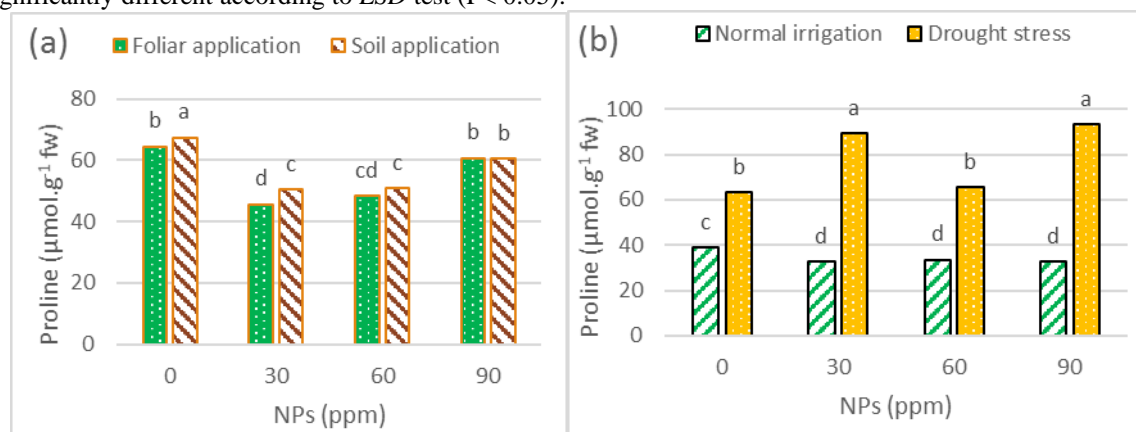
### Grain Characteristics

The results demonstrated that usage of NPs significantly decreased potassium and phosphorus compared to the control. The highest grain protein (10.21%) was found when 30 ppm NPs was used as soil application in plants (Table 5). In contrast, the lowest grain protein (5.90%) was observed in the treatment without NPs soil application. Foliar application of NPs with 0 ppm showed the lowest potassium (0.76%) and phosphorus (0.29%) values in the grains. Also, the highest potassium (1.17%) and phosphorus (0.40%) were obtained in plants under drought stress without application of NPs. Drought stress significantly increased grain protein,

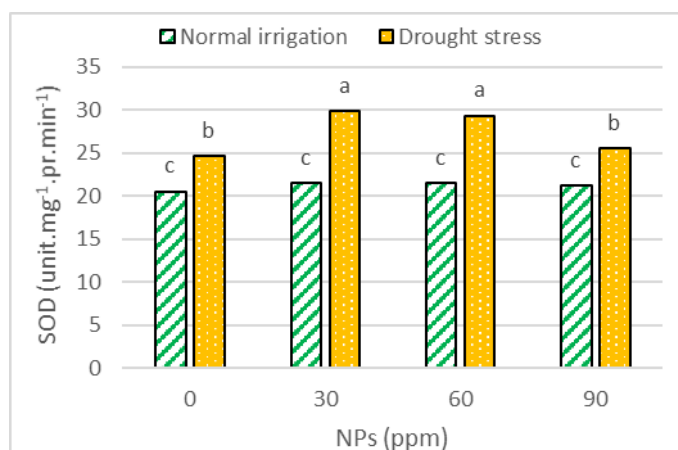
**Table 4.** Effects of SiO<sub>2</sub> NPs application and irrigation regimes on some agronomic traits of wheat.<sup>a</sup>

NPs (ppm)	Application methods	Irrigation regimes	LA (cm <sup>2</sup> )	SPAD	RWC (%)	1000-Grain weight (g)	No. grains per spike	Yield (g bush <sup>-1</sup> )
0	A		16.66 <sup>c</sup>	48.89 <sup>b</sup>	31.49 <sup>c</sup>	24.15 <sup>b</sup>	38.46 <sup>b</sup>	13.90 <sup>d</sup>
30			18.75 <sup>a</sup>	48.94 <sup>b</sup>	57.12 <sup>b</sup>	25.00 <sup>ab</sup>	44.92 <sup>a</sup>	16.49 <sup>a</sup>
60			18.50 <sup>ab</sup>	49.50 <sup>b</sup>	58.18 <sup>ab</sup>	24.92 <sup>ab</sup>	48.14 <sup>a</sup>	14.89 <sup>c</sup>
90			16.91 <sup>bc</sup>	52.85 <sup>a</sup>	59.17 <sup>a</sup>	26.09 <sup>a</sup>	45.48 <sup>a</sup>	15.55 <sup>b</sup>
	Foliar		18.00 <sup>a</sup>	48.74 <sup>b</sup>	50.35 <sup>b</sup>	24.16 <sup>b</sup>	45.67 <sup>a</sup>	14.12 <sup>b</sup>
			17.41 <sup>a</sup>	51.35 <sup>a</sup>	52.63 <sup>a</sup>	25.92 <sup>a</sup>	42.83 <sup>a</sup>	16.29 <sup>a</sup>
	Root		25.37 <sup>a</sup>	52.46 <sup>a</sup>	73.76 <sup>a</sup>	35.58 <sup>a</sup>	44.37 <sup>a</sup>	24.13 <sup>a</sup>
			10.04 <sup>b</sup>	47.64 <sup>b</sup>	29.21 <sup>b</sup>	14.50 <sup>b</sup>	44.13 <sup>a</sup>	8.28 <sup>b</sup>
0	Foliar	Normal	18.50 <sup>ab</sup>	46.20 <sup>e</sup>	44.55 <sup>e</sup>	21.48 <sup>d</sup>	39.74 <sup>cd</sup>	11.70 <sup>e</sup>
	Root		18.33 <sup>ab</sup>	46.42 <sup>e</sup>	43.94 <sup>e</sup>	23.50 <sup>cd</sup>	34.25 <sup>d</sup>	13.98 <sup>d</sup>
30	Foliar		18.50 <sup>ab</sup>	48.18 <sup>de</sup>	49.67 <sup>d</sup>	25.36 <sup>bc</sup>	46.86 <sup>bc</sup>	14.10 <sup>d</sup>
	Root		19.16 <sup>a</sup>	49.69 <sup>cd</sup>	50.88 <sup>cd</sup>	24.81 <sup>bc</sup>	40.10 <sup>cd</sup>	17.11 <sup>a</sup>
60	Foliar	Drought	15.50 <sup>c</sup>	54.17 <sup>a</sup>	52.92 <sup>c</sup>	27.01 <sup>ab</sup>	56.17 <sup>a</sup>	15.67 <sup>c</sup>
	Root		17.83 <sup>abc</sup>	52.81 <sup>ab</sup>	63.36 <sup>b</sup>	24.65 <sup>cd</sup>	42.67 <sup>bcd</sup>	16.09 <sup>bc</sup>
90	Foliar		17.33 <sup>abc</sup>	51.54 <sup>bc</sup>	65.43 <sup>ab</sup>	25.16 <sup>bc</sup>	44.10 <sup>bc</sup>	16.70 <sup>ab</sup>
	Root		16.50 <sup>c</sup>	51.36 <sup>bc</sup>	66.68 <sup>a</sup>	28.36 <sup>a</sup>	50.10 <sup>ab</sup>	16.29 <sup>abc</sup>
0		Normal	25.66 <sup>ab</sup>	50.06 <sup>b</sup>	41.04 <sup>c</sup>	34.01 <sup>b</sup>	39.29 <sup>cd</sup>	22.34 <sup>d</sup>
		Drought	9.16 <sup>d</sup>	44.75 <sup>d</sup>	21.94 <sup>e</sup>	13.73 <sup>c</sup>	30.69 <sup>d</sup>	7.04 <sup>g</sup>
30		Normal	27.00 <sup>a</sup>	50.35 <sup>b</sup>	83.97 <sup>b</sup>	34.50 <sup>b</sup>	39.46 <sup>cd</sup>	26.05 <sup>a</sup>
		Drought	10.00 <sup>cd</sup>	47.44 <sup>c</sup>	30.07 <sup>d</sup>	13.81 <sup>c</sup>	39.48 <sup>cd</sup>	7.45 <sup>g</sup>
60		Normal	24.16 <sup>b</sup>	55.65 <sup>a</sup>	81.77 <sup>b</sup>	36.28 <sup>ab</sup>	51.50 <sup>ab</sup>	23.29 <sup>c</sup>
		Drought	9.20 <sup>d</sup>	48.31 <sup>bc</sup>	32.38 <sup>d</sup>	14.63 <sup>c</sup>	46.24 <sup>bc</sup>	8.48 <sup>f</sup>
90		Normal	24.66 <sup>ab</sup>	54.26 <sup>a</sup>	88.27 <sup>a</sup>	37.55 <sup>a</sup>	56.98 <sup>a</sup>	24.85 <sup>b</sup>
		Drought	11.83 <sup>c</sup>	49.57 <sup>bc</sup>	32.46 <sup>d</sup>	15.83 <sup>c</sup>	50.36 <sup>ab</sup>	10.14 <sup>e</sup>
	Foliar	Normal	25.33 <sup>a</sup>	54.57 <sup>a</sup>	69.82 <sup>b</sup>	34.25 <sup>b</sup>	49.83 <sup>a</sup>	24.19 <sup>a</sup>
		Drought	9.41 <sup>b</sup>	42.92 <sup>d</sup>	27.55 <sup>d</sup>	11.40 <sup>d</sup>	41.51 <sup>bc</sup>	6.05 <sup>c</sup>
	Root	Normal	25.41 <sup>a</sup>	52.36 <sup>b</sup>	77.71 <sup>a</sup>	36.91 <sup>a</sup>	46.74 <sup>ab</sup>	24.08 <sup>a</sup>
		Drought	10.66 <sup>b</sup>	50.34 <sup>c</sup>	30.87 <sup>c</sup>	17.60 <sup>c</sup>	38.91 <sup>c</sup>	10.51 <sup>b</sup>

<sup>a</sup> For a given main effect or two-way interaction, means within each column followed by the same letter are not significantly different according to *LSD* test ( $P < 0.05$ ).



**Figure 2.** Effect of interaction between treatments on proline content. Means with different letters in each column are significantly different according to *LSD* test ( $P < 0.05$ ).



**Figure 3.** Effect of interaction between NPs concentration and irrigation regimes on SOD activity. Means having different letters in each column are significantly different according to LSD test ( $P < 0.05$ ).

potassium, and phosphorus compared to normal irrigation. In the same irrigation regime, protein and potassium were significantly more in foliar application of NPs than soil application of NPs.

### Yield and Yield Components

The highest 1,000-grain weight (37.55 g) was obtained with application of 90 ppm NPs to plants under non-stressed conditions (Table 4). In contrast, the lowest 1,000-grain weight (13.73 g) was observed in the treatment without NPs in plants under drought stress. Foliar application of 60 ppm NPs and soil application of 0 ppm NPs led to the highest (56.17) and the lowest (34.25) number of grains per spike, respectively. Usage of 0 ppm NPs in plants under drought stress led to the lowest grain yield (7.04 g bush<sup>-1</sup>). In contrast, the maximum grain yield (26.05 g bush<sup>-1</sup>) was achieved by application of 30 ppm NPs in plants under non-stressed conditions. In the same irrigation regimes, mean of 1,000-grain weight and grain yield with soil application of NPs were significantly more than foliar application of NPs. In both application methods, drought stress significantly decreased yield and yield components compared to the normal irrigation. Moreover, usage of NPs

significantly increased yield and yield components compared to the control.

### Plant Height and Biomass

The highest plant height was observed with soil application of 90 ppm NPs in plants under non-stressed conditions (Table 5). Root application of NPs had more effect on plants height and biomass. The lowest plant height (60.18 cm) and biomass (49.41 g bush<sup>-1</sup>) were observed in the treatment without foliar application of NPs. In contrast, the highest biomass (70.22 g bush<sup>-1</sup>) was achieved with soil application of 60 ppm NPs. Also, drought stress significantly decreased mean of biomass and plant height compared to the normal irrigation. In both irrigation regimes, usage of NPs, especially 90 ppm, significantly increased plant height and biomass compared to the control.

### DISCUSSION

Drought stress decreased SPAD, LA, and RWC of leaves in plants under drought stress.

There was a close relationship between the SPAD and total chlorophyll concentration for wheat under drought stress (Ommen *et al.*, 1999). Chlorophyll concentration

**Table 5.** Effects of SiO<sub>2</sub> NPs application and irrigation regimes on some agronomic traits of wheat.<sup>a</sup>

NPs (ppm)	Application methods	Irrigation regimes	Protein (%)	Potassium (%)	Phosphorus (%)	Plant height (cm)	Biomass (g bush <sup>-1</sup> )
0			7.76 <sup>b</sup>	1.14 <sup>a</sup>	0.37 <sup>a</sup>	66.48 <sup>b</sup>	55.73 <sup>c</sup>
30			8.29 <sup>a</sup>	0.87 <sup>b</sup>	0.35 <sup>ab</sup>	67.87 <sup>ab</sup>	59.03 <sup>b</sup>
60			7.75 <sup>b</sup>	0.83 <sup>b</sup>	0.32 <sup>b</sup>	67.55 <sup>ab</sup>	64.31 <sup>a</sup>
90			8.06 <sup>ab</sup>	0.84 <sup>b</sup>	0.32 <sup>b</sup>	68.64 <sup>a</sup>	59.60 <sup>b</sup>
	Foliar		8.14 <sup>a</sup>	1.01 <sup>a</sup>	0.34 <sup>a</sup>	64.89 <sup>b</sup>	57.04 <sup>b</sup>
	Root		7.78 <sup>b</sup>	0.83 <sup>b</sup>	0.34 <sup>a</sup>	70.38 <sup>a</sup>	62.30 <sup>a</sup>
		Normal	7.44 <sup>b</sup>	0.83 <sup>b</sup>	0.31 <sup>b</sup>	68.62 <sup>a</sup>	62.78 <sup>a</sup>
		Drought	8.48 <sup>a</sup>	1.01 <sup>a</sup>	0.37 <sup>a</sup>	66.65 <sup>b</sup>	56.55 <sup>b</sup>
0	Foliar		7.65 <sup>d</sup>	1.40 <sup>a</sup>	0.41 <sup>a</sup>	60.18 <sup>e</sup>	49.41 <sup>f</sup>
	Root		5.90 <sup>e</sup>	0.83 <sup>c</sup>	0.32 <sup>bc</sup>	63.84 <sup>d</sup>	56.24 <sup>e</sup>
30	Foliar		7.35 <sup>d</sup>	0.87 <sup>bc</sup>	0.32 <sup>bc</sup>	68.39 <sup>c</sup>	58.40 <sup>cde</sup>
	Root		10.21 <sup>a</sup>	0.86 <sup>bc</sup>	0.35 <sup>b</sup>	69.10 <sup>bc</sup>	57.39 <sup>de</sup>
60	Foliar		8.13 <sup>c</sup>	0.90 <sup>b</sup>	0.35 <sup>b</sup>	67.36 <sup>c</sup>	62.95 <sup>b</sup>
	Root		7.37 <sup>d</sup>	0.86 <sup>bc</sup>	0.34 <sup>b</sup>	71.27 <sup>ab</sup>	70.22 <sup>a</sup>
90	Foliar		8.94 <sup>b</sup>	0.76 <sup>d</sup>	0.29 <sup>c</sup>	68.18 <sup>c</sup>	60.67 <sup>bcd</sup>
	Root		8.17 <sup>c</sup>	0.87 <sup>bc</sup>	0.33 <sup>bc</sup>	72.78 <sup>a</sup>	62.06 <sup>bc</sup>
0		Normal	6.53 <sup>e</sup>	0.90 <sup>c</sup>	0.35 <sup>b</sup>	68.28 <sup>b</sup>	50.33 <sup>e</sup>
		Drought	7.58 <sup>d</sup>	1.17 <sup>a</sup>	0.40 <sup>a</sup>	63.77 <sup>d</sup>	53.40 <sup>de</sup>
30		Normal	7.63 <sup>cd</sup>	0.76 <sup>d</sup>	0.29 <sup>c</sup>	68.30 <sup>b</sup>	72.93 <sup>a</sup>
		Drought	8.99 <sup>a</sup>	0.98 <sup>b</sup>	0.35 <sup>b</sup>	65.88 <sup>cd</sup>	55.68 <sup>d</sup>
60		Normal	7.87 <sup>cd</sup>	0.71 <sup>d</sup>	0.29 <sup>c</sup>	68.98 <sup>ab</sup>	65.80 <sup>b</sup>
		Drought	8.54 <sup>b</sup>	1.11 <sup>a</sup>	0.35 <sup>b</sup>	67.08 <sup>bc</sup>	55.99 <sup>d</sup>
90		Normal	8.04 <sup>c</sup>	0.76 <sup>d</sup>	0.29 <sup>c</sup>	71.34 <sup>a</sup>	62.06 <sup>bc</sup>
		Drought	8.55 <sup>b</sup>	0.98 <sup>b</sup>	0.39 <sup>ab</sup>	67.46 <sup>bc</sup>	61.14 <sup>c</sup>
	Foliar	Normal	7.80 <sup>b</sup>	0.88 <sup>b</sup>	0.32 <sup>b</sup>	69.58 <sup>a</sup>	59.73 <sup>b</sup>
		Drought	7.77 <sup>b</sup>	1.13 <sup>a</sup>	0.37 <sup>a</sup>	63.73 <sup>c</sup>	54.34 <sup>c</sup>
	Root	Normal	7.08 <sup>c</sup>	0.78 <sup>c</sup>	0.30 <sup>b</sup>	71.19 <sup>a</sup>	65.83 <sup>a</sup>
		Drought	9.20 <sup>a</sup>	0.88 <sup>b</sup>	0.38 <sup>a</sup>	66.05 <sup>b</sup>	58.76 <sup>b</sup>

<sup>a</sup> For a given main effect or two-way interaction, means within each column followed by the same letter are not significantly different according to *LSD* test ( $P < 0.05$ ).

decreases under drought stress by chlorophyllase, peroxidase enzymes and phenolic components production (Abaaszadeh *et al.*, 2007). Also, decrease of RWC of leaves in plants under drought stress suggests less relative water absorption or water maintenance in wheat plants, when faced with drought. Moreover, reducing water use efficiency and RWC in plants under drought stress decreased turgor pressure and plant size. Thus, it may be a

reason for decline in LA of wheat plants under drought stress. Similar results were observed by Farooq *et al.* (2009), Zhao *et al.* (2010), and Mamnouie *et al.* (2010).

On the other hand, LA, SPAD, and RWC increased as the result of SiO<sub>2</sub> NPs application in both irrigation regimes, especially in plants under drought stress. The SiO<sub>2</sub> NPs may alleviate the water stress effect on photosynthetic pigments by enhancing endogenous levels of cytokinins,



which stimulate chlorophyll synthesis and improve chloroplast ultrastructure (Liang, 1998). Also, Si is deposited beneath cuticle of leaves, forming a Si-cuticle double layer, consequently, transpiration through cuticle may be decreased by Si deposition (Ma *et al.*, 2001). Therefore, it is suggested that a silica-cuticle double layer formed on leaf epidermal tissue is responsible for higher RWC of leaves. In agreement with our results, Gong *et al.* (2003) found that soil application of Na<sub>2</sub>SiO<sub>3</sub> increased LA, dry mass, RWC, and leaf thickness of wheat plants under water stress. Also, Silica NPs improved water use efficiency, RWC, and chlorophyll content in maize crop (Yuvakkumar *et al.*, 2011; Suriyaprabha *et al.*, 2012).

Amino acid proline has been described as an osmo-protectant and is accumulated along with several abiotic stresses, such as drought stress (Moradshahi *et al.*, 2004), as seen in the present study (Figure 3). Proline accumulation may be due to the increase of proline synthesis or reduction of proline degradation in response to drought stress. It is responsible for the hydration of biopolymers serving as readily utilizable energy and nitrogen source compounds during periods of inhibited growth (Kala and Godara, 2011). Our results are supported by Afshari-Behbahanzadeh *et al.* (2016) and Sayed *et al.* (2012). Moreover, the proline content increased in wheat plant leaves under drought stress when SiO<sub>2</sub> NPs was applied as compared to the control. The Si enhances resistance to various abiotic stresses such as salt, nutrient imbalance, drought, high temperature, freezing by osmoregulation (Ma and Yamaji, 2006). The obtained results are supported by suggestions of Gunes *et al.* (2008) and Crusciol *et al.* (2009), who found silicon increased proline content in drought-stressed plants tissue.

Furthermore, exposure of plants to different biotic or abiotic stresses lead to deregulation or disruption of electric transport chain and, consequently, give rise to the generation of Reactive Oxygen

Species (ROS), which are considered as strong oxidizing and potentially harmful agents for the cells (Kumar *et al.*, 2011). Thus, plants protect cell systems from the cytotoxic effects of drought-accumulated active oxygen species using anti-oxidative enzymes such as SOD, glutathione peroxidase and catalase (Verhagen *et al.*, 2004). The SOD detoxifies superoxide anion free radicals (O<sup>2-</sup>) by forming H<sub>2</sub>O<sub>2</sub>, and then, the H<sub>2</sub>O<sub>2</sub> can be eliminated by catalase and peroxidase (Hasheminasab *et al.*, 2012). These results are in agreement with prior reports that revealed high activities of antioxidant enzymes improved drought tolerance of olive cultivars (Ben Ahmed *et al.*, 2009) and canola (Abedi and Pakniyat, 2010). Also, a higher SOD activity via SiO<sub>2</sub> NPs, in the plants under drought stress, seems to indicate the effectiveness of this compound as an antioxidant system inductor of plants that protect plants from oxidative damage in drought-stressed plants. Silicon partially offsets the negative impact of drought on plants by increasing the activities of SOD, glutathione reductase (Gong *et al.*, 2005), and catalase (Zarafshar *et al.*, 2015). On the other hand, protein accumulation changes the response to drought stress. Drought stress reduces starch deposition in wheat grain, resulting in an increase in grain protein content (Gooding *et al.*, 2003). Some researchers stated that drought stress increased total protein content in rice (Fofana *et al.*, 2010). The role of SiO<sub>2</sub> NPs, especially that of soil application, in increasing ionic content in some of our concentrations, in both irrigation regimes, may be due to their effects on stabilizing cellular membranes through increasing antioxidant substances. This saves cell membranes from oxidative stress and improves plant cell permeability. Also, Si plays an important role in balancing the uptake, transport, and distribution of minerals in drought-stressed plants through water uptake and development of root growth (Hattori *et al.*, 2003; Ahmed *et al.*, 2008). In agreement with our results, Chen *et al.* (2011) stated that silicon improved



water use efficiency, photosynthesis and mineral nutrient absorption in rice plants, under drought stress. In another study, Si application increased water uptake in the plant under drought stress, thereby stimulated nutrient uptake, especially phosphorus nutrient (Sonobe *et al.*, 2011).

It is well known that drought stress affects plant growth and production by a multitude of molecular, biochemical, and physiological changes (Boutraa, 2010). For instance, closure of stomata and decrease in CO<sub>2</sub> concentration inhibited dry matter production. This was due to limitation of photosynthesis (Reddy *et al.*, 2004) which decreased the yield and its components in plants under drought stress. Moreover, drought stress caused excessive accumulation of intermediate compounds such as reactive oxygen species (Yazdanpanah *et al.*, 2011) which caused oxidative damage to DNA, lipids and proteins and, consequently, decreased plant growth and yield. Reduction in biomass, yield, and grain weight of wheat under drought stress was reported by some researchers (Anjum *et al.*, 2011; Abdoli and Saeidi, 2012).

Moreover, the results showed that using some concentration of SiO<sub>2</sub> NPs increased biomass, plant height, yield, and yield components in both irrigation regimes. Generally, positive effect of Si application in plants is not too obvious under optimum condition, but it is most evident when plant is under suboptimal condition (Henriet *et al.*, 2006). The role of SiO<sub>2</sub> NPs in alleviating the harmful effect of water stress on the growth, yield, and its components may be due to a change in transpiration, improvement in photosynthesis rate and plant water status, changes in ultra-structure of leaf organelles, activation of plant defense systems, maintenance of adequate supply of essential nutrients, and restriction in toxic ions uptake (Sacala, 2009; Parveen and Ashraf, 2010). These findings are in line with Sharifi Rad *et al.* (2014) and Shallan *et al.* (2016).

## CONCLUSIONS

Results of this study showed that drought stress affected the growth and yield of wheat plants. Use of SiO<sub>2</sub> NPs, especially soil application of 30 and 60 ppm, decreased the adverse effects of drought stress. In normal irrigation, there was no significant difference between application methods of NPs. It can be concluded that SiO<sub>2</sub> NPs may produce various metabolites that cause reduction in transpiration, improve photosynthesis rate, affect stomatal conductance, and increase chlorophyll content and photochemical efficiency of leaf. Therefore, the results suggest that application of nano-scale nutrients, SiO<sub>2</sub> NPs, can be helpful to wheat plants either through soil or foliar application in normal irrigation, and soil applications in drought stress. However, further study is required to elucidate how SiO<sub>2</sub> NPs initiates these effects.

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بهبود رشد و عملکرد گندم تحت تنش خشکی از طریق کاربرد نانوذرات دی اکسید-سیلیسیم

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چکیده

سیلیسیم و مشتقاتش اثرات سودمندی بر انواع گونه‌های گیاهی بخصوص در گیاهان تحت تنش خشکی دارد. اثرات آنها روی گندم تحت تنش خشکی به خوبی شناخته نشده است. بنابراین، به منظور



ارزیابی اثر نانوذرات دی اکسید سیلیسیم بر گندم تحت تنش خشکی، بذور گندم به طور جداگانه در گلدان ها کشت شد. سپس نانوذرات از طریق محلول پاشی و مصرف خاکی (در سه مرحله) به آنها اضافه شد. نتایج نشان داد که تنش خشکی، اکثریت صفات اندازه گیری شده را به طور معنی داری نسبت به آبیاری نرمال کاهش داد. مصرف خاکی نانوذرات در گیاهان تحت تنش خشکی به طور معنی داری سبزینگی برگ و محتوای نسبی آب برگ را به ترتیب ۱۲/۵۴٪ و ۸۴/۰۴٪ در مقایسه با کنترل (نانوذرات = ۰) افزایش داد. در گیاهان تحت تنش خشکی، محلول پاشی و مصرف خاکی نانوذرات عملکرد را به ترتیب ۲۵/۳۵٪ و ۱۷/۸۱٪ در مقایسه با کنترل به طور معنی داری افزایش داد. در رژیم های آبیاری مشابه، کاربرد خاکی نانوذرات به طور معنی داری ارتفاع و بیوماس گیاه را در مقایسه با محلول پاشی نانوذرات افزایش داد. در نهایت، نتایج آشکار کرد که مصرف نانوذرات دی اکسید سیلیسیم به ویژه غلظت ۶۰ و ۹۰ پی پی ام می تواند اثرات منفی تنش خشکی را در گندم کاهش دهد.