Improving Growth and Yield of Wheat under Drought Stress via Application of SiO\textsubscript{2} 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 (\textit{Triticum aestivum} L.) plants under drought stress are not well known. Therefore, in order to evaluate the effects of SiO\textsubscript{2} NanoParticles (NPs) under drought stress, wheat seeds were separately sown in pots. Then, the SiO\textsubscript{2} 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\textsubscript{2} 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 \textit{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\textsubscript{2} NPs is one of the most popular nanomaterial that has been used in this field (Le \textit{et al.}, 2014).

The Si is the second most abundant element in the earth's crust (Jones and Handereck, 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 \textit{et al.}, 2007; Van Bockhaven \textit{et al.}, 2013). For instance, silica improves photosynthesis parameters of some plants under drought stress (Ma, 2009; Zhang \textit{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 (\textit{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$_2$ NPs effect on growth and yield of wheat under drought stress. Therefore, the purpose of this research was studying the effects of SiO$_2$ 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$_2$ 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.

<table>
<thead>
<tr>
<th>Sandy loam$^a$</th>
<th>pH</th>
<th>EC</th>
<th>OM$^b$</th>
<th>TN$^c$</th>
<th>P</th>
<th>K</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>%</td>
<td></td>
<td>Ds m$^{-1}$</td>
<td>%</td>
<td>ppm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.55</td>
<td>17.25</td>
<td>72.2</td>
<td>7.7</td>
<td>0.4</td>
<td>0.11</td>
<td>0.11</td>
<td>69.46</td>
</tr>
</tbody>
</table>

$^a, ^b, ^c$: Denotes the soil texture, Organic Matter and Total Nitrogen, respectively.
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Figure 1. Field Emission-Scanning Electron Microscope (FE-SEM) image of SiO$_2$ 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$_2$ 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:

\[
RWC \, (\%) = \frac{(FW-\text{DW})}{(TW-\text{DW})} \times 100
\]

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 7th 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 cm²) and the lowest LA (9.19 cm²), 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 3. Analysis of variance for the effects of SiO$_2$ NPs application and irrigation regimes on some agronomic traits of wheat.

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

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

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$_2$ NPs application and irrigation regimes on some agronomic traits of wheat$^a$

<table>
<thead>
<tr>
<th>NPs (ppm)</th>
<th>Application methods</th>
<th>Irrigation regimes</th>
<th>LA (cm$^2$)</th>
<th>SPAD</th>
<th>RWC (%)</th>
<th>1000-Grain weight (g)</th>
<th>No. grains per spike</th>
<th>Yield (g bush$^1$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Foliar</td>
<td>Normal</td>
<td>16.66$^a$</td>
<td>48.89$^b$</td>
<td>31.49$^c$</td>
<td>24.15$^b$</td>
<td>38.46$^b$</td>
<td>13.90$^d$</td>
</tr>
<tr>
<td>30</td>
<td>Foliar</td>
<td>Normal</td>
<td>18.75$^a$</td>
<td>48.94$^b$</td>
<td>57.12$^b$</td>
<td>25.00$^b$</td>
<td>44.92$^a$</td>
<td>16.49$^a$</td>
</tr>
<tr>
<td>60</td>
<td>Root</td>
<td>Normal</td>
<td>18.50$^{ab}$</td>
<td>49.50$^b$</td>
<td>58.18$^{ab}$</td>
<td>24.92$^{ab}$</td>
<td>48.14$^a$</td>
<td>14.89$^c$</td>
</tr>
<tr>
<td>90</td>
<td>Root</td>
<td>Normal</td>
<td>16.91$^{bc}$</td>
<td>52.85$^a$</td>
<td>59.17$^a$</td>
<td>26.09$^a$</td>
<td>45.48$^a$</td>
<td>15.55$^b$</td>
</tr>
</tbody>
</table>

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).
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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$^{-1}$). In contrast, the maximum grain yield (26.05 g bush$^{-1}$) 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$^{-1}$) were observed in the treatment without foliar application of NPs. In contrast, the highest biomass (70.22 g bush$^{-1}$) 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 SiO₂ NPs application and irrigation regimes on some agronomic traits of wheat.¹

<table>
<thead>
<tr>
<th>NPs (ppm)</th>
<th>Application methods</th>
<th>Irrigation regimes</th>
<th>Protein (g/100 g)</th>
<th>Potassium (g/100 g)</th>
<th>Phosphorus (g/100 g)</th>
<th>Plant height (cm)</th>
<th>Biomass (g bush⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Root</td>
<td>Normal</td>
<td>6.53</td>
<td>0.90</td>
<td>0.35</td>
<td>68.28</td>
<td>50.33</td>
</tr>
<tr>
<td>30</td>
<td>Root</td>
<td>Drought</td>
<td>7.58</td>
<td>1.17</td>
<td>0.40</td>
<td>63.77</td>
<td>53.40</td>
</tr>
<tr>
<td>60</td>
<td>Root</td>
<td>Normal</td>
<td>7.63</td>
<td>0.76</td>
<td>0.29</td>
<td>68.30</td>
<td>72.93</td>
</tr>
<tr>
<td>90</td>
<td>Root</td>
<td>Drought</td>
<td>8.54</td>
<td>1.11</td>
<td>0.35</td>
<td>67.08</td>
<td>55.78</td>
</tr>
<tr>
<td>0</td>
<td>Foliar</td>
<td>Normal</td>
<td>8.17</td>
<td>0.87</td>
<td>0.33</td>
<td>72.78</td>
<td>62.06</td>
</tr>
<tr>
<td>30</td>
<td>Foliar</td>
<td>Normal</td>
<td>7.80</td>
<td>0.88</td>
<td>0.32</td>
<td>69.58</td>
<td>59.73</td>
</tr>
<tr>
<td>60</td>
<td>Foliar</td>
<td>Normal</td>
<td>7.77</td>
<td>1.13</td>
<td>0.37</td>
<td>63.73</td>
<td>54.34</td>
</tr>
<tr>
<td>90</td>
<td>Foliar</td>
<td>Normal</td>
<td>7.08</td>
<td>0.78</td>
<td>0.30</td>
<td>71.19</td>
<td>65.83</td>
</tr>
</tbody>
</table>

¹ 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 (Abasazadeh 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 SiO₂ NPs application in both irrigation regimes, especially in plants under drought stress. The SiO₂ 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$_2$SiO$_3$ 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-Bebahanizadeh et al. (2016) and Sayed et al. (2012). Moreover, the proline content increased in wheat plant leaves under drought stress when SiO$_2$ 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$_2^-$) by forming H$_2$O$_2$, and then, the H$_2$O$_2$ 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$_2$ NPs, in the plants under drought stress, seems to indicate the effectiveness of this compound as an antioxidant system inducer 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$_2$ 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...
CONCLUSIONS

Results of this study showed that drought stress affected the growth and yield of wheat plants. Use of SiO$_2$ 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$_2$ 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$_2$ 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$_2$ NPs initiates these effects.

REFERENCES

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ارزیابی اثر نانویترات دیاکسید سیلیسیوم بر گندم تحت تنش خشکی، بدور گندم به طور جدایگانه در گلدان ها کشف شد. سپس نانویترات از طریق محلول پاشی و مصرف خاکی (در سه مرحله) به آنها اضافه شد. نتایج نشان داد که تنش خشکی، اکثریت صفای اندازه غیری شده و به طور معنی‌داری نسبت به آبیاری نرمال کاهش داد. مصرف خاکی نانویترات در گیاهان تحت تنش خشکی به طور معنی‌داری داری سبزینگی بیشتر از نسبت آب برگ را به ترتیب 13/64/0/84 در مقیاس با کنتراست (نانویترات= 0) افزایش داد. در گیاهان تحت تنش خشکی، محلول پاشی و مصرف خاکی نانویترات عملکرد را به ترتیب 2/0/81 % و 17/1 % در مقیاس با کنتراست به طور معنی‌داری افزایش داد. در رژیم‌های آبیاری مشابه، کاربرد خاکی نانویترات به طور معنی‌داری ارتقاء و بیوماس گیاه را در مقیاس با محلول پاشی نانویترات افزایش داد. در نهایت، نتایج آشکار کرد که مصرف نانویترات دیاکسید سیلیسیوم به واسطه غلظت 60 و 90 پی‌پیام می‌تواند اثرات منفی تنش خشکی را در گندم کاهش دهد.