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Effect of silicon application on wheat seedlings growth under water-deficit stress induced by polyethylene glycol

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Osmotic adjustment Silicon Water deficit stress Wheat (*Triticum aestivum* L.). **ABSTRACT-** Silicon (Si) is known to ameliorate the deleterious effects of drought on plant growth. We evaluated growth of wheat (*Triticum aestivum* L. cv. Chamran) under water deficit stress induced by polyethylene golycol (PEG 20% w/v) as effected by Si application.Water deficit stress depressed the growth of shoot, relative water content (RWC) and chlorophyll concentration. Addition of 1.0 mM Si could partially improve the growth of shoot and increase the chlorophyll concentrations of stressed plants. The proline concentration in the leaves was markedly increased under water deficit stress, whereas added Si partially reversed this. Water deficit stress decreased the leaf soluble sugar concentration. There were significant negative regressions between proline concentration and shoot dry weight, supporting the view that proline accumulation is a symptom of stress damage rather than stress tolerance. Addition of Si obviously increased Si accumulation in the shoot. Analyses of K, and Ca showed no accumulation of these ions in the shoot under water stress, and added Si even increased their concentrations under water deficit stress. These results suggest that under PEG-induced water stress conditions, increase soluble sugar and decrease electrolyte leakage, contributed to the improved wheat growth by Si.

INTRODUCTION

Drought is one of the main causes of severe yield reductions. Drought is still a serious agronomic problem and also one of the most important factors contributing to crop yield loss. According to the prediction of current climate change models, the frequency and severity of drought will increase in several regions around the world (Shen et al., 2010). In all, drought is not beneficial for plant growth and development, and the increase in plant resistance to drought is an important way to overcome drought problems.

Silicon (Si) is the second most abundant mineral element in the soil after oxygen and comprises 28% of the earth's crust. It is in plants, especially in grasses, in amounts equivalent to those of macronutrient elements such as calcium, magnesium, and phosphorus (Epstein 1999). Si is never found in a free form and is always combined with other elements, usually forming oxides and it is absorbed by plants in the form of uncharged silicic acid, (Ranganathan et al., 2006). The importance of Si has been recently recognized (Epstein 1999; Ma 2004). It is still hard to prove that Si is an essential element for all higher plants, since it is ubiquitous. Si has been shown to be able to promote the growth and development of plants under abiotic and biotic stresses, including water stress (Epstein 1999; Gong et al., 2005, 2008; Hattori et al., 2005). However, the beneficial effects of silicon have been observed in many plants, especially when they are subjected to environmental stresses (Liang et al., 2007).

Results on the beneficial effects of Si in enhancing the tolerance of plants to biotic and abiotic stresses in several crops, and their relevance to the world of agriculture have been widely described (Epstein 1999; Ma 2004). Si benefits to drought tolerance in wheat (Gong et al., 2005), maize (Li et al., 2007), sorghum (Hattori et al., 2005, 2007) and salt tolerance of barley, tomato and cucumber (Al-Aghabary et al., 2004; Zhu et al., 2005) have been related to its effect on the antioxidant enzyme activity.

Mera and Beveridge (1993) suggested that Si can modify the cation-binding properties of cell walls. Different mechanisms for Si-mediated stress alleviation have been proposed by researchers. Si deposition in leaves was reported to be able to decrease transpiration (Matoh et al., 1986), therefore alleviating salt stress. In rice (*Oryza sativa* L.), Si alleviated salt stress by reducing Na⁺ uptake through partial blockage of the transpirational bypass flow, a major pathway of Na⁺ uptake in this species (Gong et al., 2008). The most widely reported mechanism was that Si might lead to osmotic adjustment and decrease the oxidative damage in plants subjected to environmental stresses (Saqib et al., 2008).

Certain cereal crops especially from the Gramineae and Cyperaceae families accumulate large amounts of Si (Mitani and Ma, 2005), and Si application to these crops ensured better growth. Being a member of Gramineae family, wheat is also considered as Si accumulator. Silicon has a positive effect on plants under drought stress. In maize, the addition of Si increased water use efficiency by reducing leaf transpiration and the water flow rate in the xylem vessel (Gao et al., 2006). Hattori et al. (2005, 2007) suggested that Si could facilitate water uptake and transport in sorghum in drought conditions. In wheat, Si alleviated oxidative stress, enhance membrane stability index and decrease electrolyte leakageunder drought (Gong et al., 2005). Si application is reported to enhance leaf RWC under water stress conditions (Matoh et al., 1986). They suggested that a silica-cuticle double layer formed on leaf epidermal tissue is responsible for this higher water potential. Si accumulated in the transpiration organs can lead to the formation of a double cuticle Si layer, which, through the reduction of transpiration, leads to a decrease in water requirement (Epstein, 1999).

Si also stimulated root H⁺-ATPase in the membranes, suggesting that Si may affect the structure, integrity and functions of plasma membranes by influencing the stress-dependent peroxidation of membrane lipids. The stimulation of root plasma membrane H⁺-ATPase by added Si under salt stress was responsible for the increased uptake and transport of K⁺ and decreased uptake and transport of Na⁺ in plants (Liang, 1999).

The present study was conducted to document the effects of Si application under water limited conditions on the drought tolerance of sorghum and how drought tolerance of wheat may be enhanced by silicon.

MATERIALS AND METHODS

Plant Culture and Treatments

The experiments were conducted in a glasshouse of the College of Agriculture of Ramin Agriculture and Natural Resources University, Khuzestan, Iran at 2012. Seeds of spring wheat (Triticum aestivum L. cv. Chamran) were sown on a net floating in culture solution (pH 5.6) at room temperature after sterilization of the seeds' surface with 1% sodium hypochlorite for 10 min. The nutrient solution was 0.25 Hoagland solution and had the following composition: 1.5 mM KNO₃, 0.25 mM NH₄H₂PO₄, 0.5 mM MgSO₄.7H₂O, 1.25 mMCaCl₂.2H₂O, 23.125 µMH₃BO₃, 4.575 µM MnCl₂.4H₂O, 0.3825 µM ZnSO₄.7H2O, 0.16 µM CuSO₄.5H2O, 0.175 µM (NH₄)6Mo₇O₂₄.4H₂O, and 0.0144 µM FeNaEDTA. Seven-day-old seedlings were transplanted into plastic buckets containing the same culture solution that was aerated continuously with an air pump (electromagnetic air pump, China). The seedlings were grown under a light intensity in the range of about 200- 450 µmol m⁻² s⁻¹. The temperature was in the range of 20-30 °C. The relative humidity was approximately 50%. The solution was renewed every 5 days. From the 30nd day, the solution was changed to 0.5 Hoagland nutrient solution.

Silicon (Si) and polyethylene golycol PEG treatments were imposed simultaneously 2 week after transplant and lasted until harvest. Si was introduced by the addition of K_2SiO_4 (pH was adjusted back to 5.6 with diluted H_2SO_4 , K was supplemented in control groups by the addition of K_2SO_4), and drought conditions were simulated by the addition of PEG- 6000 of 20% (w/v) strength to achieve drought (osmotic) stress levels of approximately -2 MPa. The experimental was performed in a completely randomized design for

four treatments including: control (C), 20% (w/v) PEG-6000 (PEG), 1.0 mM Si (Si), and 20% (w/v) PEG-6000 plus 1.0 mM Si (Si + PEG) with three replicates. Measurements were made on the recent fully expanded leaves, 56 days after sowing.

Fresh and Dry Weight Determination

For each treatment, five seedlings were sampled at (before harvest) for the determination of plant fresh and dry weight and mineral content. The seedlings were separated into shoots and roots, dried at $80 \text{ }^{\circ}\text{C}$ for 72 h, and weighed.

Chlorophyll Determination

Chlorophyll was extracted in 80% acetone and the absorbance was read using a UV/vis spectrophotometer at 663, 645 and 750 nm wavelengths. The values of chlorophyll a (Chl.a) and chlorophyll b (Chl.b) were evaluated using the equation number 1 and 2 proposed by Arnon (1949) to compute chlorophyll content.

where A_{663} and A_{645} represent absorbance values read at 663 and 645 nm wavelengths, respectively.

Relative Water Content

Between four to six samples (replications) are taken from a single treatment. Top-most fully expanded leaves are sampled, unless interested in profiling leaves on the plant. Each sample is placed in a pre-weighed airtight (possibly also oven proof) vial. Samples should reach the lab as soon as possible. This is why leaf sampling should be done quickly and it is important to enlist as much help as possible for the job. In the Lab vials are weighed to obtain fresh leaf sample weight (FW), after which the sample is immediately hydrated to full turgidity for 4 h under normal room light and temperature. Samples 1 and 2 above are rehydrated by floating on deionized water in a closed petri dish. Sample 3 above receives water into the vial to a level of 1-2 cm after which the vial is capped. After 4 h the samples are taken out of water and are well dried of any surface moisture quickly and lightly with filter paper and immediately weighed to obtain fully turgid weight (TW). Samples are then oven dried at 80 °C for 24 h and weighed (after being cooled down in a desiccator) to determine dry weight (DW).

RWC (%) = (FW – DW) / (TW – DW) × 100 (3)

Electrolyte Leakage

Electrolyte leakage was used to assess membrane permeability. This procedure was based on Lutts et al. (1996). Electrolyte leakage was measured using an electrical conductivity meter. Leaf samples of one randomly chosen plant per replicate were taken from the youngest fully expanded leaf and cut into 1 cm segments. Leaf samples were then placed in individual stoppered vials containing 10mL of distilled water after three washes with distilled water to remove surface contamination. These samples were incubated at room temperature (ca. 25 °C) on a shaker (100 rpm) for 24 h. Electrical conductivity (EC) of bathing solution (EC₁) was read after incubation. The same samples were then placed in an autoclave at 120 °C for 20 min and the second reading (EC₂) was determined after cooling solution to room temperature. The electrolyte leakage was calculated as EC₁/EC₂ and expressed as percent.

Determination of Proline and Soluble Sugar

Proline content of leaves was determined according to a modification of the method of Bates et al. (1973). Samples of leaves (0.2 g) were homogenized in a mortar and pestle with 3 mL sulphosalicylic acid (3%, w: v), and then centrifuged at 18 000×g for 15 min. 2 mL of the supernatant was then added to a test tube, to which 2 mL glacial acetic acid and 2 mL freshly prepared acid ninhydrin solution (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL 6 mol L-1 orthophosphoric acid) were added. The test tubes were incubated in a water bath for 1 h at 100°C and then allowed to cool to room temperature. 4 mL of toluene was then added to the tubes and then mixed on a vortex mixer for 20 s. The test tubes were allowed to stand for at least 10 min, to allow separation of the toluene and aqueous phases. The toluene phase was carefully pipetted out into a glass test tube and its absorbance was measured at 520 nm in a spectrophotometer. The content of proline was calculated from a standard curve, and was expressed as mg.g-1 DW. Soluble sugar was measured by the spectrophotometric method described by Zhang et al. (2006). Dry samples of wheat leaves were boiled in distilled water for 30 min. The extract was filtered through two layers of cheesecloth. The filtrate (0.5 ml) was mixed with 1.5 ml distilled water and 1 ml of 9% phenol, and then 5 ml H₂SO₄. Tubes with this mixture were left at room temperature for 30 min. Color change was estimated using a UV-Vis spectrophotometer at 485 nm. The soluble sugar concentration was determined using a standard curve.

Chemical Analysis

Silicon in shoot tissues was determined by the blue silicomolybdate procedure as described by Figen et al. (2008). To the digestion solutions was then added 0.08

M H₂SO₄ and 40% HF. Color development was accomplished by adding 1.5 ml of this solution to 1.5 ml of the reagent mixture of 0.08 M H₂SO₄ and ammonium molybdate (20 g L⁻¹), then 1.5 ml of 0.25 M tartaric acid; finally, 1.5 ml of 0.2 M ascorbic acid was added. After mixing the tubes, absorbance at 811 nm was measured estimated using a UV-Vis spectrophotometer. The silicon concentration was determined using a standard curve.

Ground samples were ashed at 550 °C for 6 h. The white ash was taken up in 2M hot HCl, filtered into a 50mL volumetric flask and made up to 50mL with distilled water. Na, K and Ca were determined in these sample solutions. K in the sample solution was analyzed using a flame photometer and Ca with an atomic absorption spectrophotometry (Chapman and Pratt, 1982).

Statistical Analysis

Statistical analysis was conducted using SAS release 9.1and the analysis of variance (ANOVA) was followed by Fisher's protected LSD test to identify homogeneous groups within the means. Significant differences among treatments were considered at $p \le 0.05$.

RESULTS AND DISCUUSION

Effects of PEG and Si on the growth and chlorophyll concentrations of wheat

Results showed that PEG-induced water stress significantly decreased the growth of wheat seedlings. Compared with the control, in the absence of added silicon the shoot fresh and dry weights under water stress were decreased by 43 and 50%, respectively, whereas these levels were decreased by 29 and 22%, respectively in the presence of added silicon (Table 1). Our results showed no improvement of root growth in stressed plants by adding Si. Added silicon did not change the chlorophyll concentrations of leaves in nonstress conditions. Drought stress significantly decreased the pigment content. Compared to the control, the contents of chlorophyll a, b and total chlorophyll were subsequently decreased to 36, 43 and 38%, respectively, in drought stress conditions, while Si caused an increase in these contents under drought stress and there was no significant difference between control and Si treatments (Table 2).

Table 1. Effects of Si and PEG treatments on fresh and dry weight of wheat (per seedling)

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Treatment	Shoot fresh	Shoot dry	Root fresh	Root dry
	weight (g)	weight (g)	weight (g)	weight (g)
Control	4.214 a	0.563 a	1.359 a	0.088 a
Si(1.0 mM)	3.880 a	0.527 a	1.155 a	0.072 a
PEG(20% w/v)	2.388 b	0.280 b	0.642 b	0.062 a
PEG + Si	2.984 b	0.441 a	0.665 b	0.069 a

Within each column, means followed by the same small letters are not significantly different by the LSD test at $p \le 0.05$

	1 5		6
Treatment	Chlorophyll a (mg g ⁻¹ DW)	Chlorophyll b (mg g ⁻¹ DW)	Total chlorophyll (mg g ⁻¹ DW)
Control	8.32 a	2. 98 a	11.28 a
Si(1.0 mM)	7.95 a	2.65 a	10.60 a
PEG(20% w/v)	5.33 b	1.68 b	7.01 b
PEG + Si	7.84 a	2.63 a	10.47 a

Table 2. Effects of Si and PEG treatments on the chlorophyll contents in leaves of wheat seedlings

Within each column, means followed by the same small letters are not significantly different by the LSD test at $p \le 0.05$

Relative water content and electrolyte leakage

Relative water content (RWC) was lower in drought stress compared to control values (16.5%). Si completely restored RWC levels in the PEG treatments (9%). Si did not improve RWC in plants under nonsaline treatments (Table 3). Under the present waterstress conditions, membrane electrolyte leakage increased 4-fold compared with the control in the absence of added silicon. However, in the presence of added silicon, the electrolyte leakage was not changed by water stress (Table 3).

Proline and Soluble Sugar Changes in the Leaves

Si treatment increased the proline level of leaves by 23% under control (Table 3). Water stress increased the proline level 4-fold in the absence of Si, corresponding to 1.5-fold in the presence of added Si compared to the

Si treatment without water stress. Moreover, addition of Si reduced the proline level of water-stressed plants by 30%. Regression analyses showed that there were significantly negative correlations between proline level and both shoot dry weight and leaf chlorophyll concentrations (Fig. 1). The regression relationship between RWC and Shoot dry weight was slightly $(R^2 = 0.87)$ and significantly negative weaker correlations between electrolyte leakage and shoot dry weight ($R^2 = 0.95$; Fig. 2). The soluble sugar concentration in the leaves was significantly increased by added Si in control condition (22%). Water stress reduced the soluble sugar level, with Si-treated plants having higher soluble sugar concentrations compared with the plants under water stress alone (Table 3).

 Table 3. Effects of Si and PEG treatments on Relative water content, Electrolyte leakage, Proline concentration and Soluble sugar concentration of wheat

Treatment	Relative water content (%)	Electrolyte leakage (%)	Proline concentration(mg.g ⁻ ¹ DW)	Soluble sugar (mg g ⁻¹ DW)
Control	85.39 ab	9.93 b	3.56 d	42.83 c
Si(1.0 mM)	89.48 a	13.84 b	5.83 c	55.18 a
PEG(20% w/v)	71.27 c	38.81 a	12.87 a	33.56 d
PEG + Si	78.22 b	16.48 b	9.01 b	45.14 b

Within each column, means followed by the same small letters are not significantly different by the LSD test at $p \le 0.05$



Fig. 1. Regression between shoot dry weight and leaf proline (mg g⁻¹DW) and total chlorophyll



Fig. 2. Regression between shoot dry weight and electrolyte leakage and relative water content (%)

Mineral Nutrients and Si Concentrations in the Shoots

Both Si and water stress decreased K concentrations in the shoots. Under water stress, plants treated with additional Si obviously had lower Ca concentrations in the shoots. The addition of Si also significantly increased the Si concentration in the shoots, whereas water stress decreased its uptake (Table 4). The ameliorative effects of Si on water stress observed in this study are consistent with the results of hydroponic experiments under drought conditions (Gong et al., 2005). Similar results were also obtained in sorghum (Hattori et al., 2005). In field conditions the Si effect on wheat drought tolerance was related to developmental stages and stress intensity (Gong et al., 2008). In maize, Li et al. (2007) observed that the application of Si to soil improved plant growth under different drought conditions. In Brachiaria grasses, application of Si to the soil did not change tolerance to water deficit or affect dry matter yield (De Melo et al., 2003), and it was suggested that the 60% field capacity treatment used in that study was not sufficient to express the role Si plays on soil water deficit tolerance. Therefore, the effects of Si on plant growth may relate to the species used and the stress modes and intensity.

Other studies showed the significant Si-induced enhancement of photosynthesis and chlorophyll concentrations in drought-stressed maize plants (Li et al., 2007), sorghum (Hattori et al., 2005), salt-stressed barley (Liang, 1999) and tomato (Al-Aghabary et al., 2004). That Si increases the dry weight wheat seedling under stress might be associated with the increases in chlorophyll content under stress conditions. Adatia and Besford (1986) reported that the addition of Si could increase the chlorophyll content in cucumber plants grown in recirculating nutrient solution.

Table 4. Effects of Si and PEG treatments on K, Ca and Si concentration of wheat

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Treatment	K (mg g ⁻¹ DW)	Ca (mg g ⁻¹ DW)	Si (mg g ⁻¹ DW)	
Control	58.26 a	11.04 a	0.68 c	
Si(1.0 mM)	52.48 b	10.89 a	1.74 a	
PEG(20% w/v)	47.34 c	9.46 b	0.53 c	
PEG + Si	40.84 d	8.18 c	1.31 b	

Within each column, means followed by the same small letters are not significantly different by the LSD test at $p \le 0.05$

In this study, application of Si improved relative water content of PEG-stressed plants, which was consistent with that observed in potted plants (Gong et al., 2005), indicating that added Si could improve the water status of water-stressed wheat. The beneficial effects of Si on plant growth have been linked to decreased transpiration. In an earlier study of rice (Oryza sativa L.), Si-caused physical blocking of cuticular transpiration was suggested to be the cause of Si-induced reduction in transpiration (Liang, 1999). Recent studies of maize (Zea mays L.) showed that Siinduced reduction in transpiration was associated mainly with decreased stomata aperture/conductance (Gao et al., 2006), suggesting the involvement of Si in stomata movement. However, in a previous study we observed that application of Si increased relative water content of drought-stressed wheat in hydroponic. Similar phenomena were also observed in sorghum under drought stress (Hattori et al., 2005). It seems,

therefore, that the increase in RWC by added Si was not mainly through these mechanisms. In sorghum, Si facilitated water uptake and transportation under drought conditions (Hattori et al., 2005, 2007). Therefore, the increase of RWC in the leaves of waterstressed wheat by added Si might have been due to stimulated water uptake and transport. However, this requires further investigation.

A common response of plants to environmental stresses is overproduction of different types of compatible organic solutes, which are of low molecular weight, highly soluble, and usually nontoxic at high cellular concentrations (Ashraf and Foolad, 2007). Proline and soluble sugars are such organic solutes. They can protect plants from stress through different mechanisms, including cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of proteins/enzymes (Ashraf and Foolad, 2007). In this study, an increased proline concentration in wheat leaves was observed under water stress (Table 3). The accumulation of proline in the leaves might be involved in one or more of the above processes and contribute to drought tolerance. However, the actual role of proline in osmotolerance remains controversial. In some studies, accumulation of proline under stress has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants (Nayyar and Walia, 2003). However, other researchers suggested that the accumulation of proline was a symptom of stress injury rather than an indication of stress tolerance (Lutts et al., 1996). In this study, addition of Si decreased proline accumulation in the leaves under water stress (Table 3). There are significantly negative linear correlations between the proline level and both shoot dry weight and chlorophyll. A negative linear correlation is seen between the Electrolyte leakage and shoot dry weight (Fig. 2). Our results seem to support the view that proline accumulation under stress is an injury symptom. A Si-induced decrease in proline accumulation was a sign of stress injury alleviation.

The concentration of soluble sugar, one of the osmosis-regulated molecules in plant tissues, water stress decreased the soluble sugar concentration in the leaves, irrespective of Si addition (Table 3). Compared with the stressed plants without additional Si, plants with added Si had a significantly higher soluble sugar concentration. This suggests that in the water stress conditions used in this study, the catabolism of soluble sugar was enhanced and that adding Si decreased the catabolism under water stress. This observation was contrary to a previous study in which increased soluble sugar levels were found in the field (Zhu at al., 2005). This may be related to the difference in stress modes and durations. Further studies using different stress modes and duration in wheat cultivars need to be done to confirm this.

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The addition of Si to the nutrient solution increased the Si concentration in wheat shoots (Table 4), which was expected and consistent with other studies in wheat plant (Tuna et al., 2008). Accumulation of inorganic ions is another way for plants to cope with environmental stresses, as observed in previous studies (Zhu et al., 2005). However, in the experimental conditions of our study, no obvious accumulation of inorganic ions was observed in the shoots under PEG stress (Table 4). The results potentially suggest that these ions did not contribute to osmotic adjustment under the water stress used in this study. Although compared to the plants without added Si, stressed plants with added Si had lower K, and Ca concentrations in the shoots, yet, considering the improvement of shoot dry matter by Si, their total contents in the shoots were actually increased. This indicates higher uptake of these ions in the roots. One possible explanation for the increased K uptake could be the stimulating effect of Si on the plasma membrane H⁺-ATPase in the roots, as found in barley under salt stress (Liang, 1999). Calcium is transported via the xylem and its transport is dependent on transpiration (Arndt et al., 2000). Therefore, increased Ca transport in Si-treated plants under water stress was possibly due to increased transpiration by Si under stress, as observed in previous studies (Gong et al., 2005; Hattori et al., 2005). However, the mechanism for the increased uptake of these ions by added Si under water stress remains to be further investigated.

CONCLUSIONS

In conclusion, addition of Si could alleviate PEGinduced water stress in wheat. The alleviative effect was attributed to an enhanced relative water content and growth. According to the organic solutes and inorganic ions investigated, osmotic adjustment seemed to show little contribution to the Si-induced tolerance to the PEG-induced water stress of this study.

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تأثیر کاربرد سیلیسیوم بر رشد گیاهچهای گندم در شرایط تنش رطوبتی ناشی از یلی اتیلن گلیکول عزيز كرملاچعب*، محمد حسين قرينه

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واژه های کلیدی:

تنظیم اسمزی سیلیسیم تنش کمبود رطوبت گندم

چکیده- سیلیسیم بهعنوان اصلاح کننده اثرات تنش خشکی بر رشد گیاه معرف است. در این پژوهش رشد گیاه گندم (... Triticum aestivum L) رقم چمران، تحت شرایط کمبود رطوبتی ناشی از پلی اتیلن گلیکول و کاربرد سیلیسیم مورد ارزیابی قرار گرفته است. تنش کمبود رطوبت، رشد اندام هوایی گیاه، محتوای نسبی آب و غلظت کلروفیل را کاهش داد. اضافه کردن یک میلیمولار سیلیسیم، به صورت جزئی باعث بهبود رشد اندام هوایی و افزایش غلظت کلروفیل گیاهان تنش دیده شده است. غلظت پرولین برگها در شرایط تنش کمبود رطوبت افزایش قابل توجهی داشته و سیلیسیم باعث کاهش آن گردید. تنش کمبود رطوبت، غلظت قندهای محلول برگ را کاهش داد. همبستگی منفی معنیداری بین غلظت پرولین و وزن خشک اندام هوایی مشاهده شد که نشان میدهد تجمع پرولین، به عنوان یک نشانه ای از آسیب تنش تا تحمل آن می باشد. اضافه نمودن سیلیسیم، بهطور واضح غلظت سیلیسیم اندام هوایی را افزایش داد. آنالیزهای پتاسیم و کلسیم نشان داد تجمع این یونهای در شرایط تنش کمبود رطوبت، و زن خشک اندام هوایی مشاهده شد که نشان میدهد تجمع پرولین، در شرایط تنش کمبود رطوبت، نازیان می باشد. اضافه نمودن سیلیسیم، بهطور واضح معنی داری بین نشانه ای از آسیب تنش تا تحمل آن می باشد. اضافه نمودن سیلیسیم، نوزهای در شرایط تنش کمبود رطوبتی و نائی داد. آنالیزهای پتاسیم و کلسیم نشان داد تجمع این یونهای در شرایط تنش کمبود رطوبتی ناشی از پلی تیلن گلیکول، رشد گیاه از طریق افزایش قندهای محلول و کاهش نشت الکتولیتی به وسیله سیلیسیم بهبود یافته است.