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Benzyl adenine is more effective than potassium silicate on decreasing the detrimental effects of heat stress in pepper (*Capsicum annum* cv. *PS301*)

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ABSTRACT- Heat stress causes flower and fruit abscission in pepper. This study was conducted in the greenhouses of Isfahan University of Technology to evaluate the effect of foliar application of Benzyl adenine (BA) and potassium silicate (K_2SiO_3) under heat stress condition on bell pepper. Two factorial experiments based on completely randomized design with four concentrations of BA (0, 0.06, 0.6 and 6 ppm) and the second with two levels of K_2SiO_3 (0 and 5 Mm) both in two temperature treatments (25±2 (optimum) 35±2℃ (high temperature)) with six replicates were conducted. The results of the study indicated that the use of BA (especially 6 ppm) promoted growth parameters and increased proline, phenol and antioxidant content. Also, application of BA 6 ppm improved cell membrane stability assessed via decreasing electrolyte leakage (EL) and also reduced flower abscission in bell pepper. BA at 6 ppm increased plant height, shoot and root dry weight, proline and total phenol, root fresh weight, potassium (K) concentration and decreased flower abscission. Antioxidant content increased with heat stress in all BA levels. Results of the study indicated that fresh and dry weight of root and K concentration increased with 5 mM K_2SiO_3. Moreover, root fresh weight and K concentration and antioxidant content increased in 5 mM K_2SiO_3 under heat stress.

INTRODUCTION

Pepper is mostly cultivated in center and south of Iran, with warm climate. Heat stress detrimentally affects the productivity of many plant species including green pepper. Heat stress affects plant growth, though heat-threshold level differs significantly at different developmental stages (Wahid et al., 2007). Bell pepper needs optimum day/night temperatures of 25/21 °C during flowering. The exposure of flowers to high temperatures leads to flower and fruit abscission and reduces yields. Temperatures above 35 °C decrease fruit set especially via decreasing the pollen viability. Pollen exposed to high temperatures normally becomes non-viable and appears to be deformed (Erickson and Markhart, 2002). Moreover, injuries due to high temperatures produced reactive oxygen species (ROS), increased fluidity of membrane lipids, inactivation of enzymes, inhibition of protein synthesis, protein degradation and loss of membrane integrity (Howarth, 2005). Furthermore, high temperature may have negative effects on photosynthesis, respiration and water relations, and also unbalanced levels of hormones and primary and secondary metabolites (Wahid et al., 2007). In order to contrast with heat stress, plants show various mechanisms, including maintenance of membrane stability, inhibiting ROS, production of antioxidants, and accumulation and adjustment of compatible solutes. All these mechanisms, which are regulated at the molecular level, enable plants to grow under heat stress (Wahid et al., 2007). However, not all plant species and genotypes have similar abilities in coping with the heat shock.

One of the most important plant hormone groups which regulate stress condition are named cytokines (CK) (Chernyad'ev, 2009). Cytokines influence a wide range of parameters during plant growth and development (Vomacka and Pospisilova, 2003). Exogenous cytokines have been used to reduce heat stress injury. Applying CK=10μM reversed heat stress injury in wheat (*Triticum aestivum* L.) (Liu et al., 2002). When the roots of maize seedlings were exposed to heat stress, important activities in maize were inhibited because of reduction in internal CK levels of inhibition of maize. All these mentioned effects were strongly dependent on plant species and concentration of hormon (Vomacka and Pospisilova, 2003).

Pottasium plays a basic role in a variety of plant physiological functions including photosynthesis, enzyme activation, osmoregulation, nutrient flow, and the distribution of primary metabolites (Amtmann et al., 2008). Silicon is known to effectively decrease various abiotic stresses such as heavy metal toxicities, and salinity, drought, temperature, chilling and freezing stresses (Shen et al., 2010). The key mechanisms mediated by K_2SiO_3 are alleviation of abiotic stresses in higher plants including: stimulation of antioxidant systems in plants, uptake processes (Liang et al., 2007) and also improved lipid peroxidation, proline and H_2O_2 accumulation in spinach and tomato in stress (Shen et al., 2010) and Electrolyte Leakage (EL) reduction in heat shock in rice plants (Ma, 2004).

For this purpose, a thorough understanding of morphological and physiological responses of plants to high temperature, mechanisms of heat tolerance and possible strategies for improving crop thermotolerance is imperative. To this aim, this research intended to investigate the effect of K_2SiO_3 and BA foliar application, in order to increase thermotolerance of sweet pepper.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Two factorial experiments based on completely randomized design at Research Greenhouse of Isfahan University of Technology were designed. For this purpose, seedlings of *Capsicum annum* var. *PS301* were grown in cell trays for 45 days. When seedlings had 3-4 true leaves, they were transplanted to pots with a capacity of 6 liter filled with soil, sand and compost fertilizer with the ratio of 2:2:1. Treatment applications were started after placing the transplants in the pots and with the appearance of the first flower bud. Plants were sprayed with four different concentrations of BA (BA0=0), (BA1=0.06), (BA2=0.6) and (BA3=6) ppm and two concentrations of K_2SiO_3 (Si1=0), (Si2=5) mM on the flower initiation stage. Temperature treatments were applied in controlled environment greenhouse during flower initiation and induction till 50 % of flower was opened about 14 days.

Measurements

Abscission of flowers and young fruits was counted during the experiment. Plant height and fresh and dry weight were measured. K concentration was determined by atomic absorption spectrophotometer after digestion with HCl (Murillo-Amador et al., 2007). EL was used to assess membrane permeability based on Lutts et al. (1996). Total phenolic content was determined using the Folin-Ciocalteu. The absorbance was measured at 725 nm with spectrophotometer. The results were expressed in gallic acid equivalents (mg/100 g fresh weight) using a Gallic acid (0–0.1 mg/mL) standard curve. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve (Singleton et al., 1999). Proline accumulation was determined using Bates et al.'s method (1973). To determine 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of methanolic extracts obtained from sweet pepper, Sanchez-Moreno et al.'s (1998) method was used. bsorption at 515 nm was measured by spectrophotometer. The DPPH free radical scavenging activity was computed as Follows:

$$Radical\ scavenging\ activity = \frac{A0 - As}{A0} \times 100$$

$A0$ is the absorbance of the control.
As is the absorbance of the sample.

Statistical Analysis

Analysis of variance was performed by software Statistix (Ver.8) and comparisons of mean data were analyzed by LSD test at the 5% level (Haghighi et al., 2017).

RESULTS AND DISCUSSION

The Main Effect of Heat Stress, BA and K_2sio_3 on Plant Parameters

It was found that fresh weight of root, plant height, fresh and dry weight of shoot decreased significantly when high temperature was applied and flower abscission increased significantly by raising the temperature from 25 to 35. The main effect of BA showed that plant height, fresh and shoot dry weight, and fresh and root dry weight were increased significantly by increasing concentrations of BA compared with the control so that in 6 ppm BA treatments plant height, dry weights of shoot and dry weights of root increased by 18, 26 and 30%, respectively. Also, it caused a decreased in the flower abscission. There was no significant change with the application of K_2SiO_3 on the plant height, fresh and dry weight of shoot and flower abscission; but, this caused the increase of 20% of fresh and dry weight of root (Table 1).

Heat stress led to a significant increase in the EL, proline, antioxidant and phenol content. On the other hand, it caused a decrease in the concentration of K in relation to the control plant. The application of BA with the concentration of 6 ppm caused the increase of 57% proline, 15% of antioxidant content and 20% of phenolic compounds. The EL decreased with the increase of BA concentration by 11% compared to the control. Proline, antioxidant content, phenol and EL did not show significant differences with the application of K_2SiO_3; but, the application of K_2SiO_3 caused the increase of 12% K concentration (Table 2).

The Interactive Effect of BA and Heat Stress on Growth and Physiological Parameters

Plant height and shoot fresh weight decreased with heat stress when BA was applied (with 0.6 and 6 ppm) and plant height increased in stress condition. The highest shoot fresh weight was in 0.6 and 6 ppm (Fig. 1a and b). Shoot dry weight decreased with heat stress and increased with 6 ppm BA even in stress condition (Fig. 1c).

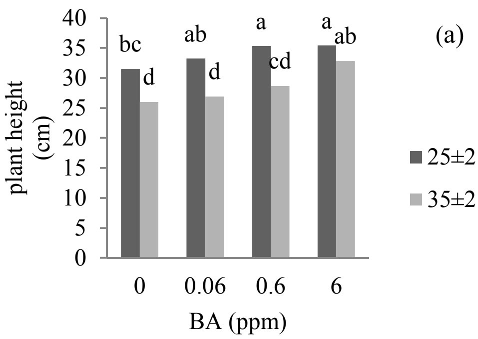
Table 1. Effects of Temperature (°C), BA (ppm), Si (mM) on morphological parameters

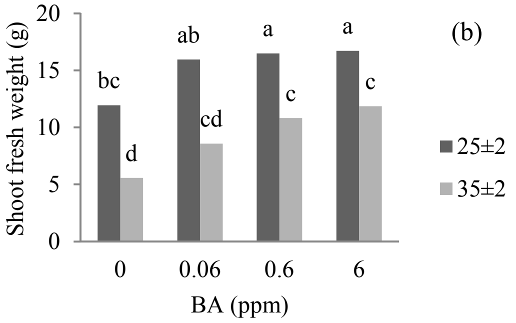
Temperature (° C)	Flower abscission	Root dry weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Shoot fresh weight (g)	Plant height (cm)
25±2	1.96^{b}	0.33^{a}	2.77^{a}	1.66^{a}	14.06^{a}	33.87^{a}
35±2	3.21^{a}	0.33^{a}	2.44^{b}	1.30^{b}	10.42^{b}	28.60^{b}
Benzyl adenine (ppm)						
0	3.33^{a}	0.30^{b}	2.05^{b}	1.43b^{c}	8.76^{b}	28.75^{c}
0.06	2.92^{a}	0.31^{b}	2.66^{a}	1.17^{c}	12.26^{a}	30.08^{bc}
0.6	2.17^{b}	0.34^{ab}	2.79^{a}	1.50^{ab}	13.65^{a}	32.00^{ab}
6	1.92^{b}	0.39^{a}	2.93^{a}	1.81^{a}	14.29^{a}	34.12^{a}
Potassium Silicate (mM)						
0	2.67^{a}	0.30^{b}	2.37^{b}	1.50^{a}	11.87^{a}	32.00^{a}
5	2.50^{a}	0.36^{a}	2.85^{a}	1.46^{a}	12.60^{a}	30.48^{a}

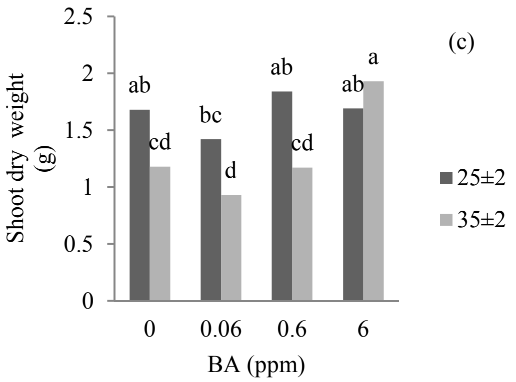
Table 2. Simple effects of Temperature (°C), BA (ppm), Si (mM) on physiological parameters

Temperature (° C)	EL (%)	Total phenol (ppm)	Antioxidant activity (%)	Potassium concentration (ppm)	Proline (µmol/g)
25±2	67.35^{b}	1.79^{b}	50.10^{b}	67.36^{a}	5.76^{b}
35±2	87.24^{a}	2.07^{a}	72.26^{a}	58.91^{b}	9.93^{a}
Benzyl adenine (ppm)					
0	78.94^{a}	1.93^{b}	60.84^{bc}	63.13^{ab}	7.91^{b}
0.06	84.23^{a}	1.82^{b}	51.87^{c}	58.24^{b}	5.70^{c}
0.6	75.89^{ab}	1.65^{b}	61.81^{ab}	66.05^{a}	5.35^{c}
6	70.13^{b}	2.33^{a}	70.20^{a}	65.13^{a}	12.43^{a}
Potassium Silicate (mM)					
0	74.85^{a}	2.05^{a}	60.32^{a}	59.39^{b}	8.10^{a}
5	79.74^{a}	1.81^{a}	62.04^{a}	66.88^{a}	7.59^{a}

Root fresh and dry weight decreased in the same trend in heat stress when BA did not apply. Root fresh and dry weight was the same as that of the control in 0.06 and 0.6 BA in stress condition. Root growth showed a different trend in 6 ppm BA. Fresh root weight decreased with heat stress but its dry weight increased (Fig. 1d and e). Flower abscission increased with heat stress even with BA application, the lowest abscission was 0.6 and 6 ppm BA. In stress condition, the lowest abscission was seen in 0.6 and 6 ppm BA too (Fig. 1f).







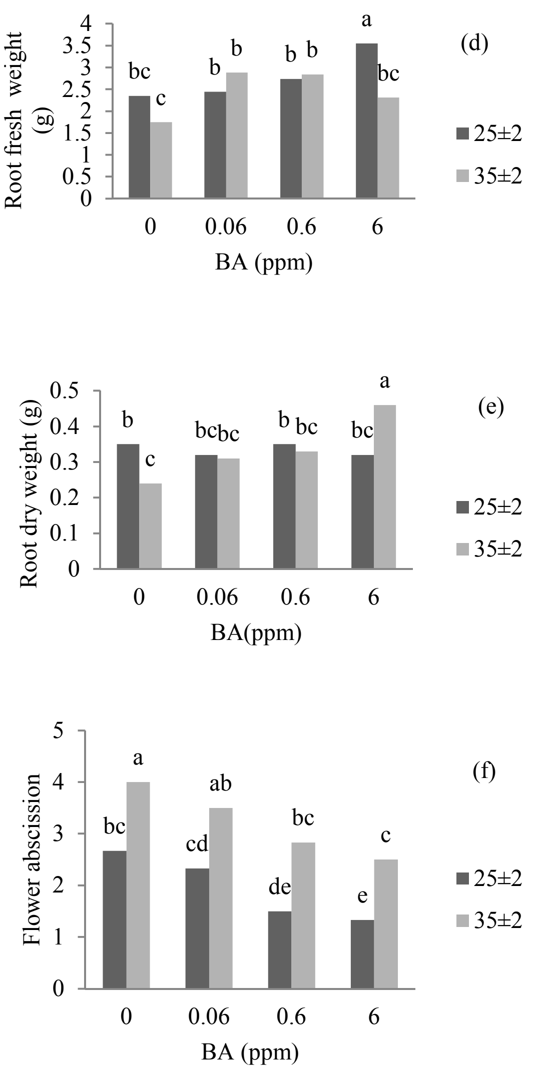


Fig. 1. Interaction between BA (ppm) and temperature on plant height (a), shoot fresh weight (b), shoot dry weight (c), root fresh weight (d), root dry weight (e), flower abscission (f)

Growth characteristics showed that 0.6 and 6 ppm BA was the same in most growth parameters. Height 10.26%, shoot fresh weight 94.07%, root fresh weight 62.28%, root dry weight 37.5% increased and abscission 29.25% decreased in 0.6 ppm BA, respectively. Therefore, regarding growth parameters, it could be concluded that 0.6 ppm BA can decrease the deleterious effect of heat stress. EL and antioxidant content increased with heat stress in all BA levels (Fig 2a and b). Proline content increased with heat stress in all BA levels except in 0.6 ppm which has not changed significantly (Fig 2c). Total phenol increased with heat stress when BA was applied and did not change significantly in 0.6 BA in stress condition (Fig 2d).

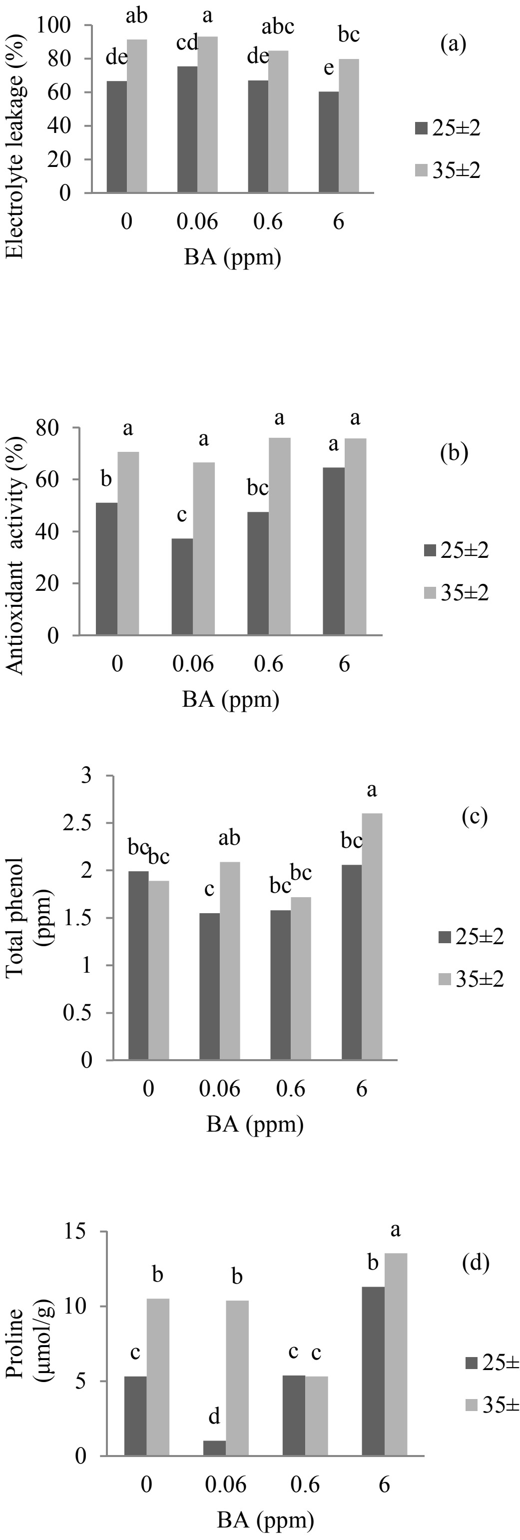


Fig. 2. Interaction between BA (ppm) and temperature on EL (a), antioxidant content (b), proline (c), total phenol (d)

K concentration did not change with 0.6 and 0.06 ppm BA significantly while it decreased in other treatments except in 0.6 ppm (Fig. 3).

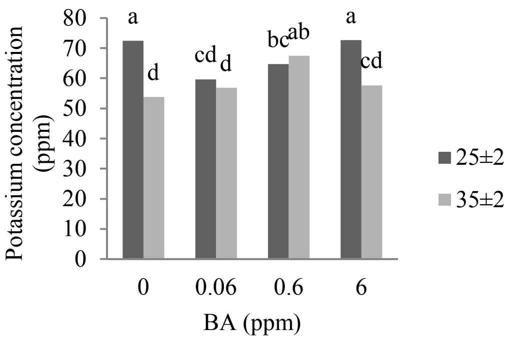
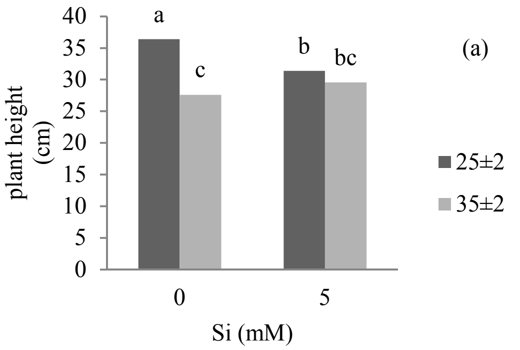
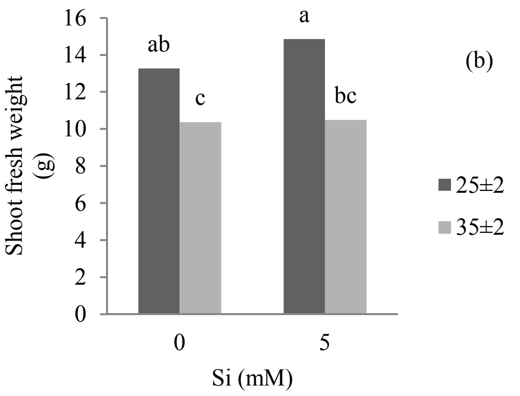


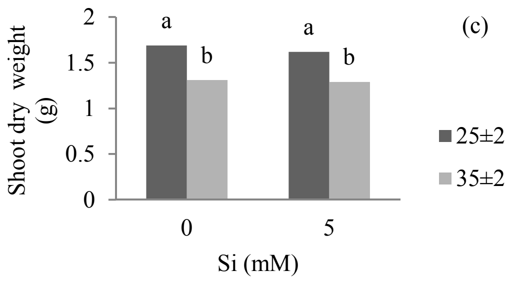
Fig. 3. Interaction between BA (ppm) and temperature on K concentration

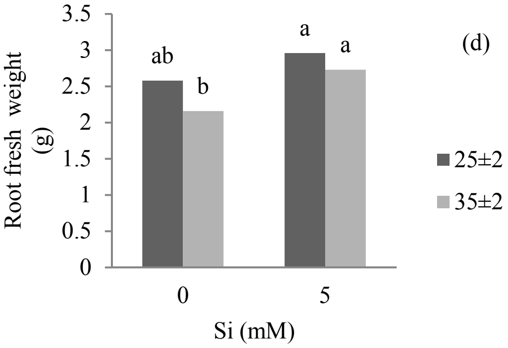
The Interactive Effect of K_2sio_3 and Heat Stress on Growth and Physiological Parameters

Plant height decreased in heat stress and did not change significantly when K_2SiO_3 was applied (fig. 4a). Shoot fresh and dry weight decreased with heat stress in both levels of K_2SiO_3 (Fig. 4b and c). Root fresh weight decreased in heat stress and did not change significantly when K_2SiO_3 was applied (fig 4d). Root dry weight did not change significantly in all treatments (data were not shown).









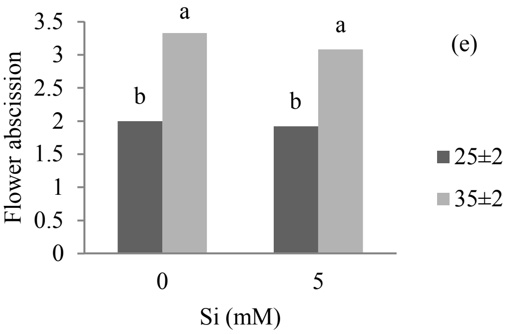
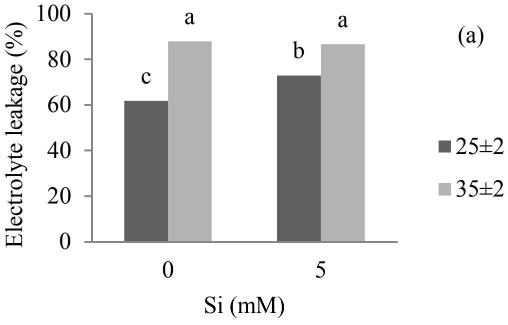


Fig. 4. Interaction between K_2SiO_3 (mM) and temperature on plant height (a), shoot fresh weight (b), shoot dry weight (c), root fresh weight (d), flower abscission (e)

Flower abscission (Fig. 4e), EL, antioxidant content and proline increased in both levels of K_2SiO_3 when heat stress was applied (Fig. 5a, b and c). Total phenol increased with heat stress although it was not significant in either level of K_2SiO_3 (Fig. 5d).



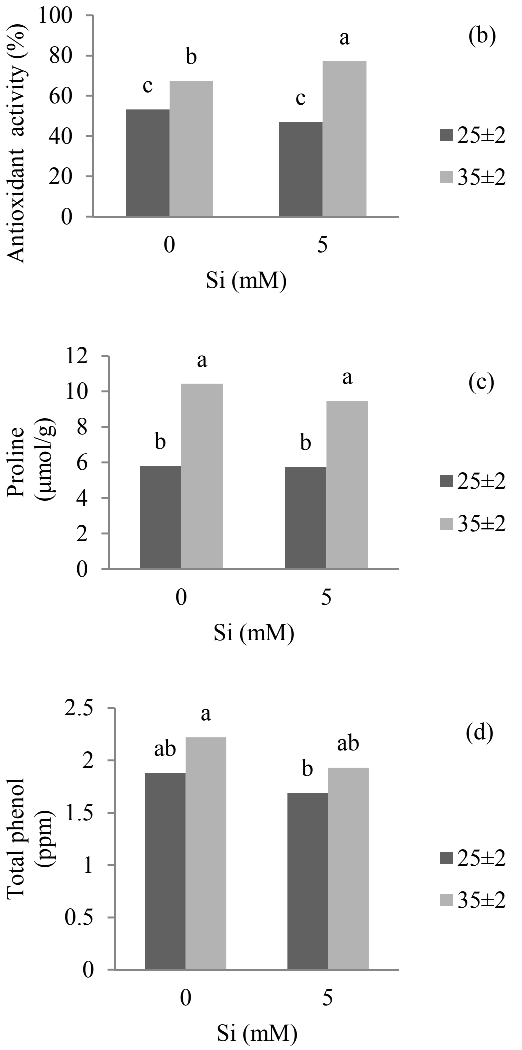


Fig. 5. Interaction between K_2SiO_3 (mM) and temperature on EL (a), antioxidant content (b), proline (c), total phenol (d)

K concentration decreased in stress condition in both K_2SiO_3 levels, but the decline in concentration of 5 mM was less than 0 mM (Fig. 6).

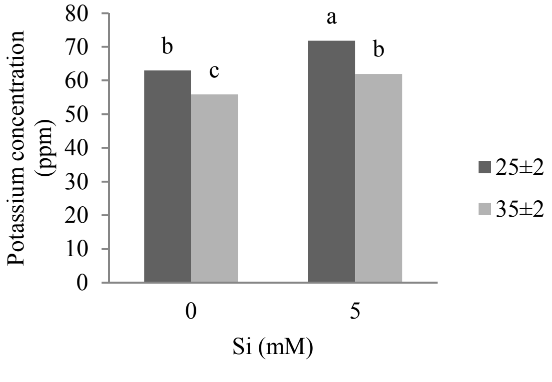


Fig. 6. Interaction between K_2SiO_3 (mM) and temperature on K concentration

The Effect of Heat Stress on Plant Parameters

The effect of heat stress on plant growth depends on intensity and length of stress, plant species and growth stage of the plant. The reduction of photosynthesis and carbohydrate increase in growth inhibitor substances and the reduction of hormones; reduced metabolism and leaf surface reduction are probably the major factors that reduce the growth characteristics (Haroun et al., 2011; Wahid et al., 2007), and therefore the reduction of photosynthesis could be named as limiting factors of the shoots growth during the stress conditions (Bhatt and Srinivasa-Rao, 2005). Inhibition of root growth could be related to the reduction of roots tips/apex expansion mainly because cell walls of plant become hardened. Greenhouse and Growth chamber studies showed that the most harmful effect on the flower abscission happens when the high temperatures are coupled with the appearance of first flower bud. That vary in different plants according to their cultivars and therefore, they respond differently to it (Wahid et al., 2007). It was seen that in pepper, increasing temperature in flower initiation increased abscission too.

In addition to tissue dehydration, heat stress may also induce oxidative stress such as the production of reaction oxygen species that cause cell damage (Liu and Huang, 2005). According to the finding of Xu et al. (2006), reactive oxygen species cause membrane lipid peroxidation and increase membrane fluidity, thus often causes loss of semipermeable membrane and changes in its operation and also increased EL. In this study, it was observed that antioxidant content significantly increased commensurate with temperature (Rodriguez et al., 2016). Probably changes in antioxidant substances can be affected by metabolic changes due to temperature. Also, induction of proline due to heat stress by Goyal and Asthir (2010) has been reported. As a result, due to its role in osmotic adjustment, preserving enzyme, cell membrane stability and, turgor cell protection, increasing accumulation can lead to increased various stress tolerance (Ashraf and Foolad, 2007). One of the non-enzymatic defense mechanisms to deal with induce oxidative stress in plants is the accumulation of phenolic compounds. Phenolic compounds act as free radical receivers and cause plants to resist induced oxidative stress. According to Rivero et al. (2001), heat stress causes the production and accumulation of phenolic compound in watermelon and prevention of oxidation. Phenolic accumulation might be due to oxidation reaction reduction or stimulating the production of Glutamate or increasing protease enzyme activity. In the present experiment, it was observed that with increased temperature, K accumulation was significantly decreased. Based on hormonal changes in plants under heat stress, it can be assumed that the changes in the ABA and ethylene level would be called stress hormones. This two hormones act as signals in many physiological processes (Larkindale and Huang, 2005). When plants encountered environmental stresses, ABA concentration increased. ABA prevents α-Amylase activity and prevents the conversion of starch to sugar. Finally, it prevents K absorption by stomatal cells.

The Effect of BA on Heat Stress

Different reports were given on the stimulating or inhibitory actions of CK in different processes, like the growth of roots and shoots, control of apical dominance in shoot and control of leaf senescence (Werner et al., 2001). These results are in agreement with those reported by Abdel-Aziz et al. (2007) that BA causes the increase in the number of shoots, fresh and dry weight of shoot and root in the croton plant. BA led to a significant increase in the amount of total soluble solids and soluble carbohydrates and therefore this led to the turgor and increase in plant growth and finally, increase in plant's weight. Also, the increase in the cell's volume and the adjustment of osmotic potential led to an increase in fresh weight of plant. Furthermore, the application of BA led to a general significant increase in the sugar beet leaf weight and leaf surface and also increase in the growth characteristics and flower induction and development on Tomato plants (Haroun et al., 2011). Moreover, Rylott and Smith (1990) stated that the application of CK on flowers led to the active cell division, and thus caused the assimilation absorption from other plant parts to the new developing flower buds. The application of BA on the orchid flower led to an increase in diameter of flower (Blanchard and Runkle, 2008). Hare et al. (1997) concluded that the application of CK causes a rapid recovery of plant in the confrontation of heat stress. Furthermore, it was reported that the application of BA on the maize shoot led to the increase in heat tolerance and maize kernel dry weight (Hare et al., 1997). With similar results, Liu et al. (2002) also reported the rising effect of CK on the growth characteristics of bentgrass (*Agrostis palustris* L.) under the heat stress.

Plants responses to plant growth regulator could be due to plant species, variety, plant age, environmental condition, physiological and nutritional status and hormone balance is different. In this study, unlike the BA role in maintaining the integrity of membrane, an increase in EL was seen in plants under heat stress compared with the control plants, which was possibly related to the hormone density levels than stress intensity. Effects expressed about the effects of CK is likely due to increased cell division and cell enlargement and thus prevent aging (Liu et al., 2002). In addition, in this study, similar to the conducted test on *Mesembryanthemum crystallinum.* L. (Thomas et al., 1992), it was shown that CK such as BA is so under stress and lack of stress causes an increase in the accumulation of proline. Proline is also effective to increase Antioxidant content (Amirjani, 2010). Also, this finding is in line with DiCosmo and Towers' (1984) test results which showed that the density and type of growth regulators affect the production rate and the secretion of phenolic compound in vitro condition. Available information indicates that some molecules may increase the cell Antioxidant content (Wahid et al., 2007). Also, Nguyen et al. (2010) have confirmed the positive relationship between the content of phenolic compounds and accumulation of antioxidant content. Eid and Abou-Leila (2006) found the same results in croton plant as they showed spraying plants with different BA, increases the concentration of mineral elements such as K+ in comparison with the control plant. According to the information obtained, it is expected that changes in the concentration of elements in tomato stems and roots in response to different treatments of BA are related to its effect on the protein synthesis of membrane (Haroun et al., 2011). But, according to the results of this study, concentrations of BA did not change significantly on K concentrations.

The Effect of K_2sio_3 on Heat Stress

Wang and Galletta (1998) reported that spraying K2SiO3 solution on strawberry led to the increase in plant growth and a significant increase in the root dry material of the treatment plant than the control. Furthermore, Ma (2004) observed the beneficial effects of Silicon (Si) on the plants under stress. Silicone improves the growth under stress condition (Eraslan et al., 2008). Most of the important effects of Silicon appear from the sediment in leaf, shoot and skin, which var among the plant species (Ma, 2004). One of the obvious effects of K on the resistance to the heat stress can be the adjustment of Osmotic potential, hydraulic conductivity of plant and making change in the water absorption of the plant. Moreover, K is very necessary for transferring the photosynthetic assimilations in the root growth.

According to Kamenidou et al. (2010), K2SiO3 had a positive impact on the concentration of K in plant. K uptake is probably due to the increasing activity of the root plasma membrane pump ATP ase H+ by silicon (Pei et al., 2009). K is a very important element in maintaining water balance, turgor creating pressure and opening and closing of stomata and accumulation and transfer produced carbon hydrate and causes more tolerance to stress condition. Silicate had no influence on other physiological parameters, which can be attributed to insufficient concentration of K2SiO3 or the usage of SI in this experiment, and duration of experiment and variety and plant condition.

CONCLUSIONS

All in all, it can be concluded that it seems that K_2SiO_3 is less efficient compared with BA in decreasing harmful effects of heat stress. Applying 0.6 ppm BA, compared with K_2SiO_3, improved height 10.26%, shoot fresh weight 94.07%, root fresh weight 62.28%, root dry weight 37.5% and decreased abscission 29.25%, respectively. Stress indices like antioxidant content, prolin and phenol increased with heat stress.

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بنزیل آدنین نسبت به سیلیکات پتاسیم در کاهش اثرات مضرتنش گرما بر فلفل موثرتر است

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چکیده- تنش گرما موجب ریزش گل و میوه در فلفل می‌گردد. این پژوهش در گلخانه‌های دانشگاه صنعتی اصفهان به منظور ارزیابی تاثیر کاربرد برگی بنزیل آدنین (BA) و سیلیکات پتاسیم (K_2SiO_3) تحت شرایط تنش گرما بر فلفل دلمه‌ای انجام شد. دو آزمایش فاکتوریل بر اساس طرح کاملا تصادفی با چهار غلظت BA (۰، ۰/۰۶، ۰/۶و ۶ پی پی ام) و دومین بار با دو سطح K_2SiO_3 (۰ و ۵ میلی‌مولار) در دو تیمار دمایی(اپتیمم (۲±۲۵) و دمای بالا (۲±۳۵)) با شش تکرار انجام شد. نتایج پژوهش نشان داد که استفاده BA (بخصوص غلظت ۶ پی‌پی‌ام) فاکتورهای رشد را بهبود می‌بخشد، پرولین، میزان فنول و محتوای آنتی‌اکسیدان را افزایش می‌دهد. همچنین کاربرد BA با غلظت ۶ پی‌پی‌ام پایداری غشا سلولی را بهبود می‌بخشد یا نشت یونی را در فلفل دلمه‌ای را کاهش می‌دهد و همچنین ریزش گل را کاهش می‌دهد. بنابراین کاربرد BA اثرمنفی تنش گرما را کاهش می‌دهد. BA با غلظت ۶ پی‌پی‌ام، ارتفاع گیاه و ریزش گل را بهبود می‌بخشد، کاهش وزن تر و خشک شاخساره و ریشه، پرولین و فنول کل را کاهش می‌دهد. وزن تر ریشه و غلظت پتاسیم را افزایش و ریزش گل را کاهش می‌دهد. محتوای آنتی‌اکسیدان با تنش گرما در همه سطوح BA افزایش می‌یابد. نتایج پژوهش نشان می‌دهد که وزن تر و خشک ریشه و غلظت پتاسیم با غلظت سیلیکات پتاسیم ۵ میلی‌مولار افزایش می‌یابد. همچنین، در غلظت ۵ میلی‌مولار سیلیکات پتاسیم ، وزن تر ریشه و غلظت پتاسیم بهبود و محتوای آنتی‌اکسیدان تحت تنش گرما افزایش می‌یابد.