

Effects of foliar spraying kinetin on growth parameters and photosynthesis of tomato under different levels of drought stress

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Abstract

The effects of 2 concentrations of kinetin, i.e., 2 and 4 mM together with 2 levels of drought stress, i.e., moderate (35% soil saturation capacity) and severe (20% soil saturation capacity) were investigated on the growth parameters of tomato including fresh and dry leaf weight, leaf number, leaf surface area, fruit number, fruit weight, and enzymatic antioxidants. To this end, first tomato plants cultivar super strain were planted in pot culture. During the early growth stage, the plants were subjected to 2 drought stress levels (35% and 20%) as well as normal irrigation (control: 50% soil saturation capacity). Also the plants were treated with foliar spraying of kinetin for 60 days. There were a total of 9 treatments in the experimental study based on a completely randomized design with 4 repetitions. The finding suggested that spraying kinetin at both levels of moderate and severe drought stress improved growth parameters in tomato. While soluble sugars increased under drought stress, the insoluble sugars content decreased. Application of kinetin on the other hand reduced soluble sugars and increased insoluble sugars content under various levels of drought stress. Finally, chlorophyll a and b contents were reduced under drought and kinetin application had no significant effect on plant pigments.

Keywords: kinetin; growth parameters; chlorophyll; soluble sugars; insoluble sugars

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Introduction

Biotic and abiotic stresses make changes in natural physiological performance of the plants. These stresses reduce the plant biosynthesis capabilities and cause serious damages. Drought is an important stress that inhibits full realization of genetic potentials in agricultural plants and therefore, reduces yields in crops. During drought stress, biochemical, molecular, and physiological responses are observed in plants that prepare them to adapt to the restricted environmental conditions (Basra et al., 2002; Mundree et al., 2002). Generally, reduction of water content in plant tissues under drought conditions limits their growth. Studies show that the stress caused by water shortage reduces growth and leaf surface area, damages and reduces photosynthesis process, damages cell membranes, damages and reduces proteins and enzymes, hurts pigments and plastids, and

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reduces chlorophyll and root growth (Levitt, 1980).

Water shortage stress can directly affect photosynthesis related biochemical processes while it indirectly reduces CO₂ molecules entering stomata which are closed due to water shortage. As a result, transportation of photosynthetic substances are affected by water shortage and leaves become saturated which in turn may further limit photosynthesis. Limitation in photosynthesis substances under water shortage will obviously reduce plant growth and eventually its photosynthesis and performance (Blum, 1996).

Effective natural adjustment of leaf surface can have a tremendous effect in reaction to drought stress (Lakso, 1985). The capability of adjusting leaf surface area is an important mechanism utilized by plants in managing water consumption (Blum, 1996). Reduction in leaf surface area is reported in onion and basil (Alkhire et al., 1993; Hassani and Omid Beighi, 2002; Moshtaghi Niaki, 2008). Rapid and temporary reduction in leaf surface area was also reported after sudden increase in drought stress level in maize, wheat, and Oat (Basra et al., 2002). Reduction of leaf dry matter in plants subjected to drought stress is probably directly related to reduction in their soluble sugar content. This in turn is attributed to reduction in photosynthesis which is reduced under drought stress (Rabiee, 2003). Jones et al. (1985) in their study found that relative water content in leaves is a very useful index of the plant status to the point that it could be used as an index of drought tolerance by the plant. In response to drought stress, a change is observed in the relationships between water potential and pressure potential. This can occur by osmotic adjustment (osmotic accumulation of active substances), increase in cell wall elasticity, or increase in apoplastic water (Pospisilova et al., 2000).

Soluble sugars are among adaptive osmolytes that accumulate in the plants under drought stress and may act as osmotic agent or protectant. As an osmotic agent, sugars increase because of stress through osmotic adjustment and maintaining turgescence while as a protectant, they stabilize cell membranes and proteins (Bohnert et al., 1995). Many parameters

for soluble are proposed increasing carbohydrates. There are some reports showing drought that under stress, complex carbohydrates may break down into simple carbohydrates. Also under drought stress conditions, increase in the ratio of sucrose to insoluble sugars, decomposition of insoluble sugars, and reduction in transfer of sucrose from leaves result in an increase in carbohydrates content in plants (Pereira et al., 1993) which in turn plays an important role in adjusting osmotic pressure of leaves in response to drought stress in many plants (Watanabe et al., 2000; Morgan, 1984). Researchers have attributed the increase in soluble sugars of the drought-stressed plants to increase in decomposition of insoluble carbohydrates and therefore increase in soluble sugars level, synthesis of osmotic compounds through processes other than photosynthesis, cessation in growth, reduction in the speed of transfer of substances, and increase in sucrose synthesis due to activation of sucrose phosphate synthetase enzyme(OLiviera-Nato, 2009).

Cytokinins are special chemical messengers in plants (hormones) that play pivotal role in managing plant cell life cycle and many more processes. The first known cytokinin was a derivative of adenine (6-furfury) amino purine) separated as a byproduct during decomposition of DNA. These hormones affect the general plant metabolism, specially enzymes and coenzymes (Shudo, 1994). Cytokinin modifies many physiological activities induced by drought stress (Werner et al., 2010). Drought stress usually speeds up withering process in leaves whereas cytokinin postpones this process. Application of cytokinin can reverse the process of shedding leaves and fruits induced by ABA or drought stress. The antagonism between cytokinin and ABA could be due to metabolic interactions. Cytokinin partly shares its biosynthesis process with ABA. Cytokinin can directly affect photosynthesis parameters such as chlorophyll, and synthesis and decomposition of photosynthetic proteins, chloroplast compounds and macrostructures, electron transfer, and enzyme activities in plants (Pospisilova et al., 2000). The reason why cytokinin is reduced under drought stress is the reduction in cytokinin biosynthesis and also its decomposition (Nagvi,

1994). Reduction in cytokinin and accumulation of abscisic acid (ABA) in plants subjected to drought stress result in high ratio of ABA/CKs (Shudo, 1994).

Tomato (*Solanum lycopersicum* L) is a dicotyledonous plant belonging to potato family. It is an important vegetable with different applications in human daily life. It also has high nutritional values and is rich with antioxidants and vitamins specially vitamin C.

Various studies show that drought stress negatively affects plants including tomatoes and reduce yields in crops. Application of hormones and growth regulators on the other hand, improves growth parameters in the plants under drought stress. Therefore, in the present study effects of spraying kinetin are investigated on growth parameters, chlorophyll pigments, and soluble and insoluble sugars as photosynthetic indexes in tomatoes under 2 levels of drought stress

Materials and Methods

Pot culture

As the study necessitated controlling for soil moisture, pot culture was used and 36 plastic pots (10 kg) were obtained and filled with the farm soil and sand (3:1). Following the recommendation of the agricultural technicians at Kannoosh Tomato Paste Incorporation, tomato plants cultivar super strain were used for the experimentation as this cultivar is grown in Eastern Mazandaran. Young plants at 2-4 leaf stage were obtained from Kamnoosh Tomato Paste Incorporation farm which were then planted in pots in late April, 2011.

Treatments

Nine treatments including different levels of irrigation and spraying kinetin at 2 levels, namely 2 and 4 mM were applied (Table 1). Before treatments, pots were irrigated every 5 days by 50% soil saturation capacity. This was followed by foliar spraying of 2 levels of kinetin (2 and 4 mM) twice a week for 3 weeks. Finally, 2 levels of mild and severe drought stress including irrigation at 35% and 20% of the soil saturation capacity together with foliar spraying of kinetin

Table 1 Treatments of the study

- 1 irrigation 50% soil saturation capacity (control)
- 2 irrigation 50% soil saturation capacity + kinetin 2 mM
- 3 irrigation 50% soil saturation capacity + kinetin 4 mM
- 4 irrigation 35% soil saturation capacity (moderate drought stress)
- 5 irrigation 35% soil saturation capacity (moderate drought stress) + kinetin 2 mM
- 6 irrigation 35% soil saturation capacity (moderate drought stress) + kinetin 4 mM
- 7 irrigation 20% soil saturation capacity (severe drought stress)
- 8 irrigation 20% soil saturation capacity (severe drought stress) + kinetin 4 mM

were applied for 60 days.

Growth parameters measurement

In order to measure morphological and physiological parameters, plants were carefully removed from the pots and thoroughly washed with distilled water 60 days after plantation. Shoots and roots lengths were measured using a metal measurement ruler. Leaves and roots of each plant were cut and weighed by a digital scale to find their fresh weight. In order to measure dry weight of leaves and roots, the samples that were obtained for fresh weight measurement were kept in an oven at 75 °C for 72 h and then weighed. Leaves from similar positions on the stems of the plants under study were used to measure their surface area using a planimeter.

Photosynthesis pigments measurement

One gram of leaf was ground with 10 mL acetone. The solution was then filtered through Whatman 2 paper and adding acetone 80%, 20 mL solution was obtained. Absorbance of the solution was read at 645 nm and 663 nm using the control by spectrophotometry. Chlorophyll content was then calculated in mg/g fresh weight using the following formulas (Jenson, 1987):

Chl (a) =0.0127A₆₆₃ - 0.00269A₆₄₅

Chl (b) =0.0229A₆₄₅ - 0.00468A₆₆₃

Chlt (a+b) =0.0202A₆₄₅ + 0.00802A₆₆₃

Soluble and insoluble sugars measurement

Plant samples were first dried at 90 °C in the oven for 48 h. Then these samples were weighed and after adding with 10 mL ethanol 70%, they were kept in refrigerator for 1 week. One mL of the supernatant was then added with 1 mL distilled water. Then 1 mL phenol 50% and 5 mL pure sulfuric acid were added to the solution and the absorbance was read spectrophotometrically against control at 485 nm. To obtain the concentration of soluble sugars using standard curve, different (c), concentrations of glucose were used. The sugars content was measured per gram dry weight.

The samples used for obtaining soluble sugars were also used to obtain their insoluble sugars. The samples were ground with 10 mL distilled water before they were boiled for 10-15 mins and filtered. Nine mL distilled water was added to 1 MI filtered solution. One mL ethanol 70% and 1 mL phenol were then added to 1 mL of the solution. Afterwards, 5 mL pure sulfuric acid was added to the resulting solution and after it was cooled, the absorption was read at 476 nm using a spectrophotometer (Kochert, 1987).

Statistical analysis

were compared with SPSS using Duncan's test for the 4 replicates and the graphs were drawn with Excel.

Results

Shoot and root length

A significant difference ($P \le 0.05$) was observed in shoot lengths between moderate and severe drought stress (Table 2). Also, the difference between severe drought stress and combination of severe drought stress and kinetin 2 and 4 mM was significant ($P \le 0.05$). Moreover, a significant difference was observed between moderate drought stress and combination of moderate stress and kinetin 4 mM ($P \le 0.05$)..

Fresh and dry weights of leaf and root

Severe drought stress together with kinetin 4 mM treatment resulted in meaningful increase in fresh leaf weight in comparison with severe drought stress alone. A significant difference ($P \le 0.05$) was observed in dry leaf weight between severe drought stress treatment and combination of drought stress and kinetin 2 and 4 mM. Severe drought stress meaningfully reduced dry leaf weight in comparison with the control (Table 2).

As Table (2) suggests, fresh root weight under moderate and severe drought stresses significantly reduced in comparison with control

Table 2

Effects of drought stress (20% and 35% saturation capacity of soil, moderate and severe stress, respectively) and normal irrigation (50% saturation capacity of soil: control) together with application of kinetin (2 and 4 mM) on the growth parameters of tomato plants cultivar super strain.

Treatment	Shoot	Root	Leaf Fresh	Root Fresh	Leaf Dry	Root Dry	Leaf	Leaf	Fruit
	Length	Length	Weight	Weight	weight	weight	Number	Surface	Number
	(cm)	(Cm)	(g)	(g)	(g)	(g)			
Control	50.25 a	35 a	20.5 bc	9.72 a	2.72bc	1.36 a	55 b	18.75 a	3 ab
Kinetin 2 mM	47.5 ab	31.12ab	22.87ab	7.64 ab	2.76ab	0.83 cd	61.5 a	20 a	2.25 bc
Kinetin 4mM	43.2 bc	30 ab	25 a	7.99 ab	2.97 a	0.93 cd	65.25 a	20.5 a	3 ab
Drought 35%	33 e	23.5 cd	15.62def	6.90 bc	2.45 c	1.02 bcd	47.5cd	14.5 bc	3.25 ab
35% + Kin 2	37.2 de	27.87bc	17.62cde	7.37 bc	2.63bc	1.11 abc	52.25 bc	16.75abc	4 a
35% + Kin 4	39.75cd	30 ab	18.37cd	7.87 ab	2.66bc	1.25 ab	54.75 b	17.75 ab	2.75 ab
Drought 20%	26.25 f	20.12d	11.87 f	3.97 d	1.50e	0.52 e	38.75 e	10.75 d	1.25 c
20% + Kin 2	36.25de	22.75cd	14.25 ef	5.23 cd	1.86d	0.73 de	41.5 e	13.5 cd	2.25 b
20% + Kin 4	38.5cde	24.12cd	16.75cde	5.78 bcd	1.93d	0.95bcd	43.25de	14.375bc	2.25 b

The same letters indicate no meaningful difference at P \leq 0.05.

Two-factor variance analysis and comparison of means were carried out. Means

($P \le 0.05$). Moreover, severe drought stress together with kinetin 4 mM increased dry root

weight in comparison with severe drought stress alone.

Leaf number and leaf surface area

The findings showed that application of kinetin 4 mM under moderate drought stress increased leaf number in comparison with moderate drought stress alone and the increase was statistically significant (P \leq 0.05). On the other hand, combination of Kinetin 4 mM and severe stress treatment increased leaf surface area compared with severe stress condition (Table 2).

Number of fruits

As Table (2) suggests, application of kinetin 2 mM in moderate drought stress treatment did not result in significant difference in the number of fruits in comparison with the plants treated with moderate drought stress alone. However, a different pattern was observed under severe drought stress and the combination of kinetin and severe drought stress significantly increased fruit number in comparison with the tomatoes treated with drought stress

alone(P≤0.05).

Chlorophyll a, b, and a/b

Table (3) summarizes the findings regarding chlorophyll content of the plants under different treatments. Moderate and severe drought stresses reduced chlorophyll a and b in comparison with control. These pigments were also increased when kinetin was applied under severe drought stress and the increase was statistically significant ($P \le 0.05$). On the other hand, chlorophyll a/b did not show meaningful variation under different treatments of the study.

Soluble and insoluble sugars

Severe drought stress caused a meaningful increase in soluble sugars compared with control (Table 3). On the other hand, application of kinetin in severe drought stress conditions reduced soluble sugars. No significant difference was observed in root soluble sugars when various drought stress and kinetin treatments were applied.

Table 3

Effects of drought stress (20% and 35% saturation capacity of soil, moderate and severe stress, respectively) and normal irrigation (50% saturation capacity of soil: control) together with application of kinetin (2 and 4 mM) on the chlorophyll a, b, and a/b contents, soluble and insoluble sugars of leaves and roots of tomato plants cultivar super strain.

Treatment	Chl a (mg.g ⁻¹ fw)	Chl b (mg.g ⁻¹ fw)	Chl a/b	Leaf soluble sugars (mg.g ⁻¹ dw)	Root soluble sugars (mg.g ⁻¹ dw)	Leaf starch (mg.g⁻¹dw)	Root starch (mg.g ⁻¹ dw)
Control	0.258 a	0.469 a	0.551 a	0.0252 bc	0.0117 b	0.01315 ab	0.0115 d
Kinetin 2 mM	0.232 ab	0.428 ab	0.542 a	0.0215 c	0.0105 b	0.0151 ab	0.0119 cd
Kinetin 4 mM	0.206 bc	0.373 bc	0.551 a	0.02 c	0.00925 b	0.01705 a	0.0121 bcd
Drought 35%	0.197 bcd	0.358 bcd	0.551 a	0.0287 bc	0.0137 b	0.010525 b	0.0129 bc
35% + Kin 2	0.199 bcd	0.362 bcd	0.551 a	0.0227 c	0.012 b	0.014325 ab	0.0122 bcd
35% + Kin 4	0.219 bc	0.398 bc	0.549 a	0.0167 c	0.0095 b	0.01425 ab	0.0147 a
Drought 20%	0.164 d	0.299 d	0.55 a	0.0492 a	0.0197 b	0.010225 b	0.0129 bc
20% + Kin 2	0.191 c	0.346 c	0.551 a	0.0372 b	0.014 b	0.011375 b	0.0126 bc
20% + Kin 4	0.186 c	0.338 c	0.550 a	0.0272 bc	0.0095 b	0.012425 ab	0.0133 b

The same letters indicate no meaningful difference at P \leq 0.05.

Discussion

Shoot length in tomato plants was decreased under drought stress while kinetin treatment increased this trait under severe drought stress. The findings also showed that drought stress reduced root length while kinetin application increased this trait under moderate drought stress. It is reported that the halt in growth under water shortage can be attributed to preserving carbohydrates for sustainable metabolism and providing energy for long term (Osario et al., 1998). In Fenugreek (Trigonella foenum – graecum)drought stress increased root length while it decreased stem length (Reddy, 2003). Under drought stress conditions, dehydration and reduction in shoot cell mass is more pronounced than in roots, i.e., under drought condition shoot growth is affected more than in roots. It seems that under drought conditions, more photosynthetic products are provided for roots. Increase in root-shoot ratio under water shortage was also reported in basil, maize (Sharp and Davies, 1979; Taylor et al., 1982), and tomato (Hassani and Omid Beigi, 2002).

In the present study it was revealed that drought stress reduced fresh weights of leaves and roots while kinetin application under severe stress increased fresh weights and this was consistent with the findings of Naeem et al. (2004) on lentil.

The reduction in leaf dry matter under drought stress is probably directly related to the reduction in insoluble sugars. Reduction in insoluble sugars in turn is due to reduced photosynthesis as drought reduces photosynthesis (Rabiee, 2003). There are reports on reduction of dry matter (%) under drought in onion (Moshtaghi Niaki, stress 2008; Kazemipour and Arvin, 2002), basil (Hassani and Omid Beighi, 2002), and eggplant (Kirnak et al., 2001).

Moderate and severe drought stress reduced leaf number and leaf surface area and application of kinetin particularly at high concentration increased both these traits. Adjustment in leaf area can be a natural reaction to stress (Lakso, 1985). The ability to adjust leaf surface is an important mechanism through which a plant under drought stress controls water consumption (Blum, 1996). Reduction in leaf surface under drought stress was reported in basil and onion by Hassani and Omid Beigh (2008) and Moshtaghi Niaki (2002), respectively.

Kinetin was reported to increase leaf and stem fresh weight in *Menthaspicata* and *Salvia officinalis* (Karanov et al., 1992). Fatma et al. (2007) reported an increase in branches under benzyl adenine treatment in *Viciafaba*.

The study showed that severe drought stress reduced fruit numbers while kinetin treatment increased this trait. This is consistent with the findings of Ibrahim et al. (2007) in *Viciafaba* and Guo et al. (1995) in maize. In a study on maize, application of cytokinins was reported to improve seed yield (Guo et al., 1995). In rice, cellular division in endosperm is closely related with endogenous concentration of cytokinins and exogenous application of kinetin can increase the number of endosperm cells (Yang et al., 2003).

Results of the present study showed that drought stress significantly reduced severe chlorophylls a and b while application of kinetin had a meaningful effect on the contents of these pigments. This confirms the findings of Kaya et al. (2010) in maize, Ibrahim et al. (2007) in Viciafaba, and Rivero et al. (2010) in tobacco. These researchers found that kinetin increased photosynthesis pigments and leaf chlorophyll contents in the plants under study. Drought withers the plants, decomposes chloroplasts, and reduces chlorophylls. Unger drought stress, formation of new plastids, chlorophylls a, and b are reduced and the ratio of chlorophyll a to chlorophyll b is changed (Heidari-Sharifabad, 2001). Increase in chlorophylase and peroxidase under drought stress are the effective factors in reduction of chlorophyll. Keeping the photosynthesis ability and preservation of leaf chlorophyll under stress condition are among indexes of resistance. Reduction in leaf chlorophyll content under drought stress is reported in many plants including basil, onion, mint (Hassani and Omid Beighi, 2002; Moshtaghi Niaki, 2008).

Increase in the concentration of chlorophyll in the plants treated with kinetin can minimize electrolytic leakage (Kaya et al., 2010;

Chen et al., 1991). Some researchers have attributed this to the effect of kinetin on membrane osmosis (Williams and Hester, 1983). There are also some reports about the effect of kinetin on increasing the chlorophyll content of plants (Gadallah, 1995a, 1995b).

The results of the present study showed that drought stress particularly severe one increased soluble sugars whereas exogenous kinetin application reduced leaf soluble sugars.

Accumulation of soluble carbohydrates in response to abiotic stresses is associated with osmotic adjustment or keeping cellular membranes. Soluble sugars content could be an effective way for selection of salt resistant cultivars (Kerepesi, 1998). Alcoholic sugars (such as glycerol, inositol, and pinitol), simple sugars (fructose and glucose), and compound sugars (such as trehalose and raffinose fructans) are produced by plants as adaptation regulators (Bohnert et al., 1996).

Generally, increase in the level of soluble sugars during drought stress can be attributed to collapse of insoluble carbohydrates which result in an increase in soluble sugars and synthesis of these compounds through the processes other than photosynthesis and cessation of the growth (Hissao et al., 1973). Some carbohydrates have been found to modify the restrictive effects of drought on duplication of photosynthetic genes (Koch, 1996). Studies on peas (Clive, 1984) and various genotypes of wheat (Martin, 1993) revealed that soluble sugars of roots and shoots increase under drought stress. Results of the present study suggested that drought stress increased root insoluble sugars while application of kinetin increased insoluble sugar content of roots under moderate drought stress.

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