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Rootzone temperature on nitrogen absorption and some physiologcal traits in cucumber

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Abstract

Increasing nitrogen absorption efficiency reduces use of excessive application of N. The effect of rootzone temperature on nitrogen absorption needs clarification. This experiment was conducted to investigate low (15°C, RTZ1), high (35°C, RTZ2) and optimum (25°C, RTZ3) root zone temperatures and nitrogen 52.5 (ND1), 78.75 (ND2) and 105 (ND0) mg·L^{-1} levels on cucumber (*Cucumis sativus* L.), cv. Super N3, cultured in Johnson nutrient solution. Shoot and root fresh and dry weights, greenness, maximum photochemical quenching (Fv/Fm), antioxidant activity, total phenol and nitrate reductase (NR) activity increased with N. Shoot fresh and dry weights, greenness, total phenol, antioxidant and NR activity have reduced at high and low root zone temperature compared to the optimum temperature. Shoot fresh and dry weights increased in RTZ1 and RTZ2 for the ND2 and ND0 treatments. The SPAD value increased in RZT2 at all nitrogen levels. The highest Fv/Fm occurred at ND0 at all temperature levels. Antioxidant activity increased for the ND0 and ND2 treatments with increasing root zone temperature. Total phenol content increased in ND1 and ND2 at low and high temperatures compared to the optimum temperature, and increased with increasing temperature level in ND0 treatment. The NR activity increased at the high root zone temperature in ND2 and ND0 treatments. The ND0 and ND2 treatments alleviated the root zone temperature effect on cucumber grown in hydroponic culture.

Keywords: *Cucumis sativus*, Antioxidant activity, Fv/Fm, Hydroponic, Nutrient fertilizer

Introduction

Temperature is an abiotic factor that can limit plant growth and productivity (Allakhverdiev *et al*., 2008). Nxawe *et al*. (2009) reported that plant growth was limited at low root temperature.

Cucumber (*Cucumis sativus* L.) has originated in the subtropics and is sensitive to chilling temperature (Miao *et al.*, 2007) and low root temperatures may seriously affect the performance of its seedlings (Lee *et al*., 2005). A temperature of 7- 9°C in a hydroponic nutrient solution is considered critical for the root function (Calatayud *et al*., 2008). Low temperature affects nutrient uptake by plants (Pregitzer and King, 2005). The low root temperature at night induces greater H_2O_2, malondialdehyde (MDA) and soluble sugar content in cucumber at low root temperature also leads to plasma membrane damage and inhibition of growth (Qiu-yan *et al*., 2013). High temperature can cause biochemical, physiological, and molecular change in plant metabolism (Gulen and Eris, 2004) and limited growth and productivity of plant (Weih and Karlsson, 1999). Low or high temperature in the root zone could limit photosynthesis through stomatal closure (Nada *et al*., 2003).

Nitrogen is an essential nutrient and consists 3-4% of dry matter, and when present in insufficient supply can become a limiting factor for plant growth (Makhziah *et al*., 2013). Nitrogen is important for plant growth and it is the basic elements for the synthesis enzyme and chlorophyll and involved in cell division and growth (Liu *et al*., 2013).

The effect of different root temperatures (10, 17, 25 and 32 °C) and NO^{-3}/NH^{+4} ratios (0⁄7, 2⁄5, 3.5⁄3.5, 5⁄2, 7⁄0) was studied in strawberry nutrient solution. Results revealed that strawberry grows well at 25°C root temperature at both N forms at equal concentration. Increasing root temperature decreased total cation concentration (K^+, Na^+, Mg^{2+}), and increased Ca^{2+} at NO^{-3}-fed plants. High root temperature reduced inorganic anion concentration in the roots at NH^{+4} treated plants (Ganmore-Neumann and Kafkafi, 1984).

To the best of our knowledge, there is no systematic empirical research on the effect of root zone temperature on N absorption and physiology in cucumber. This project was undertaken to determine the effects of root zone temperature and nitrogen level on cucumber growth, photosynthesis, antioxidant and NR activity in hydroponic conditions.

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Materials and methods

The experiment was conducted in a greenhouse in the Department of Horticulture, Isfahan University of Technology, Iran, at 30-35°C and 30-35% relative humidity. This study was arranged as a factorial experiment based on a completely randomized design (CRD) with 3 replicates. Seeds of cucumber, cv. Super N3, were germinated in peat at 25°C. Seedlings of uniform size were transplanted to 3L plastic pots containing sand and pots were placed in a water bath. The root zone temperature in the water baths was adjusted to low (15°C, RTZ1), high (35°C, RTZ2) and optimum (25°C, RTZ3). The nitrogen was placed into the Johnson nutrient solution (N) (Jones, 1930) at 3 levels so that treatments were 52.5 $mg\cdot L^{-1}$ (50% of N, ND1), 78.75 $mg\cdot L^{-1}$ (75% of the N, ND2) and 105 $mg\cdot L^{-1}$ (100% of N, ND0).

At the end of the experiment after almost 1 months after transplanting, plants were harvested and washed. Shoots were separated from roots using a steel blade, and dried in a conventional oven at 70°C for 2 days to obtain a constant weight. The fresh weight (FW) and dry weight (DW) of shoots and roots were determined. Greenness was measured using a chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ). The ratio of Fv/Fm was measured by chlorophyll fluorescence (OS-30, Minolta Corp.) after 3 weeks. Nitrate reductase (NR) activity was determined according to Sagi *et al*. (1997). Total phenol content of leaves was determined by mixing fresh tissue with 5 mL Folin-Ciocaltea and measured with a spectrophotometer at 765 nm (McDonald *et al.*, 2001).

The antioxidant activity of cucumber leaves was estimated according to Koleva *et al*. (2002). 3 mg of the sample was dissolved in 5 mL of methanol stock; Then, 1.4 mL of this solution was blended with 0.6 mL of antioxidants solution. After 30 mins, the absorbance of the solution was recorded at 515 nm with a spectrophotometer (V-530, JASCO, Hitachi, Japan). Growth response to root zone temperature for the shoot and root was calculated using a modified formula (Planchette *et al*., 1983) which indicates the dependency of yield to N.

Nitrogen dependency (ND) and root (NDR) were calculated by the following modified formula:

$$ND= \frac{\text{FWS } \textit{full nitrogen} - \text{FWS } \textit{nitrogen deficit}}{\text{FWS } \textit{full nitrogen}}$$

FWS= Fresh weight of shoot

Shoot nutrient efficiency was calculated with the formula of Haghighi *et al*. (2014).

Data were analyzed with Statistix 8 (Tallahassee, FL). All data were subjected to two-way ANOVA. The significant differences between the means of treatments were determined by the least significant difference, i.e. LSD at $P < 0.05$.

Results

Main effect of ND (Nitrogen dependency) on FWR, RDW, Fv/Fm, antioxidant activity, total phenol and NR activity were significant (Table 1). Root zone temperature application affected all parameters except Fv/Fm significantly. Interactive effect of ND×RTZ showed significant effect in all parameters except STI (stress tolerance index), MP (mean productivity) and YSI (yield stability index) (Table 1).

The fresh weight shoot (FWS) and fresh weight rhoot (FWR) increased with increase of N concentration of nutrient solution in ND2 and ND0 respectively. Shoot Dry Weight (SDW) and FV/FM decreased in ND1 and ND2 and root dry weight (RDW) and greenness decreased in ND1. Antioxidant activity and NR activity decreased in ND2 and ND1 respectively. Phenol content of leaves increased in ND2 and ND1, respectively (Table 2). GMP, MP and YSI did not change with ND treatment significantly (Data was not shown).

FWS and SDW and greenness decreased in RTZ1 and RTZ3. FWR and RDW decreased in RTZ3. FV/FM did not change between treatments. Antioxidant activity increased in RTZ2 and RTZ3 compare to RTZ1 respectively. Both high and low root zone temperature decreased phenol level and this decrease were more in RTZ3. NR activity was the highest in RTZ2 and was lowest RTZ1 (Table 3).

Growth dependency to Nitrogen (GDN) of root was not significant conversely the GDN shoot was significant and it was highest in ND1. NE of root was significant and it was higher for ND2 than ND1 but it was not significant for shoot nutrient efficiency (SNE). GDRTZ of root was higher in ND1 than ND2 and it was not significant for shoot in different root zone temperature (Table 4).

GDRTZ of ND1 was significant and it was higher in root than shoot and NUE was significant in ND2 between root and shoot and it was higher in the shoot than root (Table 5).T-test value showed significant differences between GDRTZ and NUE of root and shoot (Table 5).

FWS and SDW were increased in RTZ1 and RTZ2 in both ND2 and ND0. Shoot fresh and dry weight were lower in high zone temperature in all ND levels (Figure 1, 2) as well as in ND1×RTZ1 in the root dry weight (Figure 2).

FWR was lowest in RTZ3 in all ND levels and ND1×RTZ1. The highest FWR was in ND0 in RTZ1 and RTZ2 and ND2×RTZ2 (Figure 3).

Root dry weight was higher in ND1 and ND2 when the root zone was optimum. Although when the N of nutrient solution completed at the RTZ1 the RDW was highest (Figure 4).

The high greenness was in ND0×RTZ2 (Figure 5). Fv/Fm increased in ND0 at all RTZ levels and decreased in all other treatments (Figure 6).

Antioxidant activity increased with increasing root temperature in RTZ1, RTZ2 and RTZ3, respectively in both ND2 and ND0 (Figure 7).

Total phenol increased in both high and low temperatures in ND1 and ND2 compared with optimum

Table 1-Analysis of variance of nitrogen and temperature on some parameters of cucumber

Source	df	FWS (g/plant)	FWR (g/plant)	SDW (g/plant)	RDW (g/plant)	Chloro-phyll content (SPAD value)	Fv/Fm
ND	2	1.16 ns	0.57 *	0.002 ns	0.001 *	21.50 ns	0.007 **
RTZ	2	40.43 **	2.04 **	0.20 **	0.002 *	63.08 **	0.0005 ns
ND × RTZ	4	1.40 *	0.07 *	0.003 *	0.0005 *	71.46 **	0.0002 *
Error	16	0.56	0.11	0.004	0.0003	8.31	0.0002
CV	26	13.80	32.28	17.99	32.14	22.98	35.12

ns, *, ** not significant or significant at 5% or 1%

Continue of table 1-

Source	df	Antioxidant activity (% inhibition)	Total phenol content (mg GAE/g FW)	STI	GMP	MP	YSI	NR activity (mg/ 100 g FW)
ND	2	0.001 **	333.43 **	0.01 ns	0.0001 ns	0.0005 ns	0.01 ns	3.11 **
RTZ	2	0.001 **	1318.3 **	0.91 **	0.01 **	0.05 **	0.91 **	6.98 **
ND × RTZ	4	0.005 **	529.86 **	0.01 ns	0.0001 ns	0.0008 ns	0.01 ns	2.77 **
Error	16	0.00001	0.02	0.02	0.0002	0.001	0.02	1.26
CV	26	2.58	0.19	18.52	17.93	8.05	18.52	1.80

ns, *, ** not significant or significant at 5% or 1%

Table 2- The main effect of different N levels of nutrient solution on some parameters of cucumber.

ND	FWS (g/plant)	FWR (g/plant)	SDW (g/plan)	RDW (g/plan)	Greenness (SPAD value)	Fv/Fm	Antioxidant activity (% inhibition)	Total phenol content (mg GAE g^{-1} FW)	NR activity (mg per 100 g FW)
ND1	5.78 c	0.95 c	0.30 b	0.04 b	11.12 b	0.03 b	0.10 c	83.65 a	0.057 c
ND2	6.50 b	1.35 b	0.38 b	0.06 ab	12.35 ab	0.03 b	0.11 b	75.44 b	0.061 b
ND0	7.07 a	1.88 a	0.57 a	0.07 a	14.19 a	0.08 a	0.13 a	71.76 c	0.068 a

‡Within a column means followed by the same letter are not significantly different at P<5% according to the least significant different test.
†† ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = 0% nitrogen dilution (optimum)

Table 3- The main effect of root zone temperature on some characteristics of cucumber

RTZ	FWS (g/plant)	FWR (g/plant)	SDW (g/plan)	RDW (g/plan)	Greenness (SPAD value)	Antioxidant activity (% inhibition)
RTZ1	4.61 b	2.17ab	0.36 b	0.05 b	9.7 b	0.10 c
RTZ2	6.00 a	2.54 a	0.51 a	0.06 a	15.7 a	0.11 b
RTZ3	3.74 b	1.47 b	0.38 b	0.04 b	12.0 b	0.12 a

†Within a column means followed by the same letter are not significantly different at P<5% according to the least significant different test.
††RTZ1 = root zone temperature in15°C; RTZ2 = root zone temperature in 25°C (optimum); RTZ3 = root zone temperature in 35°C

Continue of table 3-

RTZ	FV/FM	STI	GMP	MP	YSI	NR activity (mg per 100 g FW)	Total phenol content (mg GAE g^{-1} FW)
RTZ1	0.05 a	0.97 a	0.11 a	0.47 a	0.97 a	0.05 c	76.9 b
RTZ2	0.04 a	0.44 b	0.05 b	0.34 b	0.44 b	0.07 a	89.0 a
RTZ3	0.04 a	1.01 a	0.11 a	0.48 a	1.01 a	0.06 b	64.8 c

†Within a column means followed by the same letter are not significantly different at P<5% according to the least significant different test.
††RTZ1 = root zone temperature in15°C; RTZ2 = root zone temperature in 25°C (optimum); RTZ3 = root zone temperature in 35°C

Table 4- The effect of ND and RTZ on GDN, NUE of roots and shoots

	GDNR	GDNS	SNUE	RNUE		GDRTZ-S	GDRTZ-R
ND1	0.22	4.66	93.30	69.07	RZT1	-0.58	0.29
ND2	4.84	2.75	100.62	70.55	RZT3	-1.70	-1.11
T-test	0.49	0.058	0.20	0.08	T-test	0.18	0.025

†ND1 = nitrogen dilution 50%; ND2= nitrogen dilution 25%
††RTZ1 = root zone temperature in15°C; RTZ3 = root zone temperature in 35°C

Table 5- The T-test value between GDN, GDRTZ and NE of roots and shoots

	GDN	GDRTZ	NUE
ND1	0.27	0.05	0.12
ND2	0.38	0.32	0.05
T-test	0.12	0.03	0.09

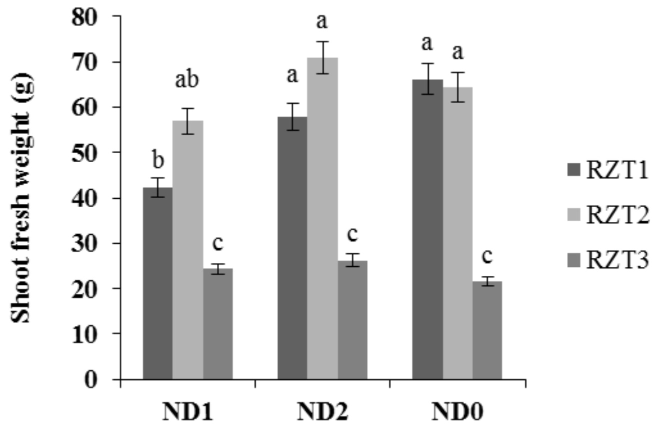

Figure 1- The effect of different levels of ND and RTZ on SFW of cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature in 15°C; RTZ2 = root zone temperature in 25°C; RTZ3 = root zone temperature in 35°C.

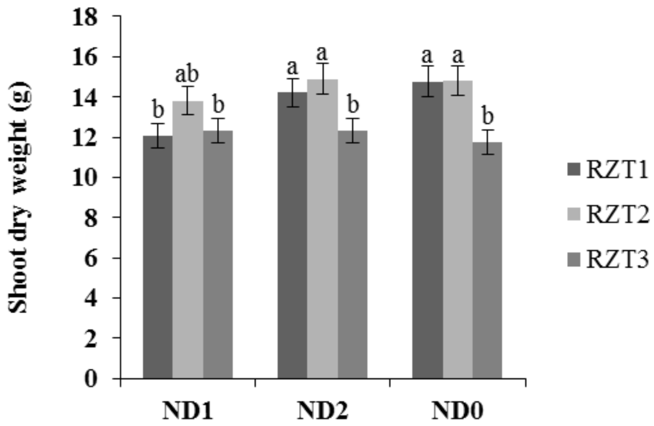

Figure 2. The effect of different levels of ND and RTZ on SDW of cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature in15°C; RTZ2 = root zone temperature in 25 °C; RTZ3 = root zone temperature in 35°

temperature of root zone. The highest phenol in ND0 was in RTZ3 and the lowest in RTZ1 (Figure 8).

NR activity increased in ND0 in all root zone temperatures as well as ND2×RTZ3 (Figure 9).

Discussion

Heat stress is usually defined as high temperatures causing injury to plant function, physiology or development (Smirnoff, 1993). It seems that high

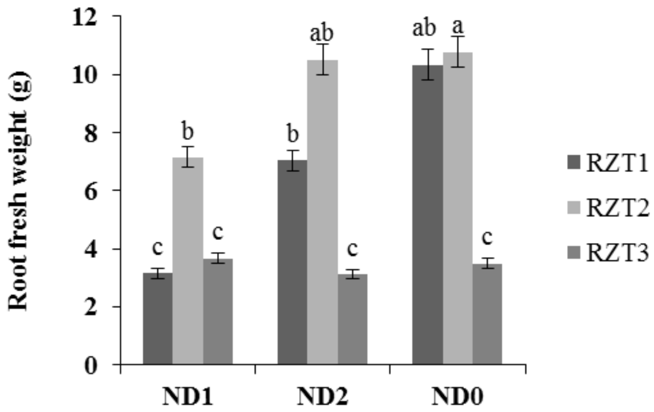

Figure 3- The effect of different levels of ND and RTZ on RFW in cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature at15°C; RTZ2 = root zone temperature at 25 °C; RTZ3 = root zone temperature at 35°C

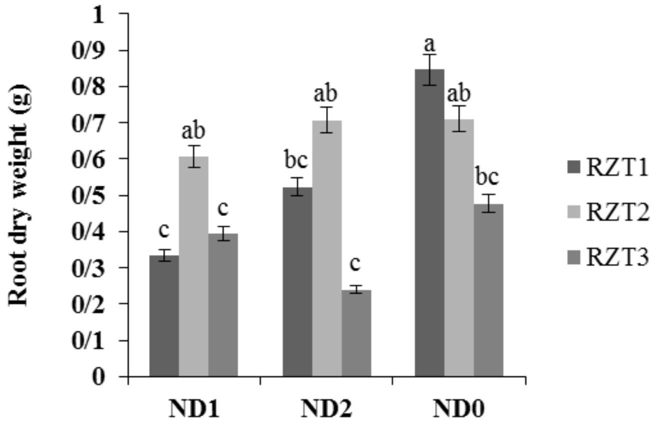

Figure 4- The effect of different levels of ND and RTZ on RDW in cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature at15°C; RTZ2 = root zone temperature at 25 °C; RTZ3 = root zone temperature at 35°C

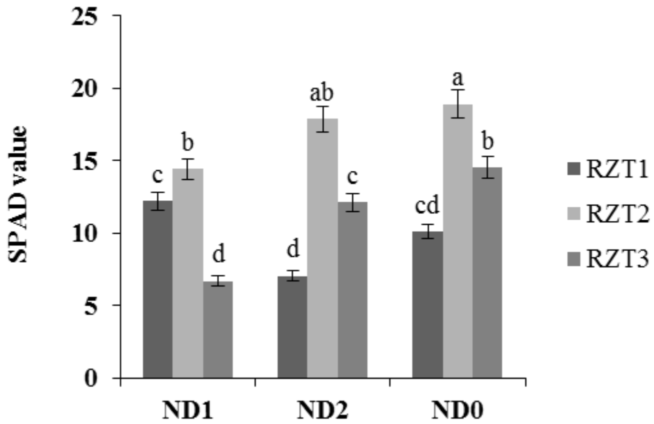

Figure 5- The effect of different levels of ND and RTZ on SPAD in cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature at15°C; RTZ2 = root zone temperature at 25 °C; RTZ3 = root zone temperature at 35°C

temperature has more deleterious effect on shoot growth than low temperature stress. Also, in low N level the low temperature can be harmful too (Figure 1, 2).

Nitrogen is the mineral that most often limits plant growth because large quantities of nitrogen are required to produce organic compounds of plant that are crucial for plant growth and development, such as proteins, nucleic acids, biochemical substances and some plant

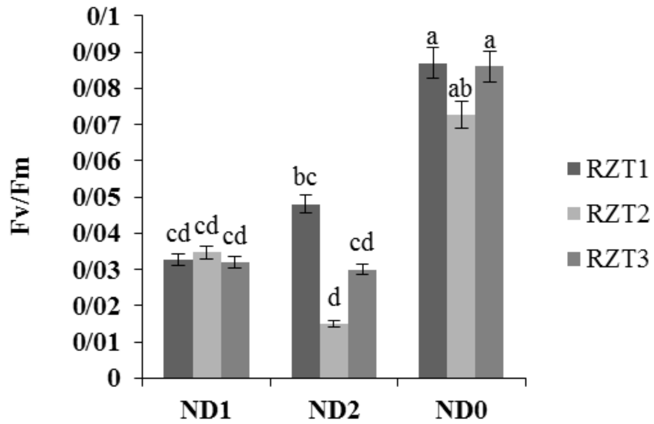

Figure 6- The effect of different levels of ND and RTZ on Fv/Fm in cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature at15°C; RTZ2 = root zone temperature at 25 °C; RTZ3 = root zone temperature at 35°C

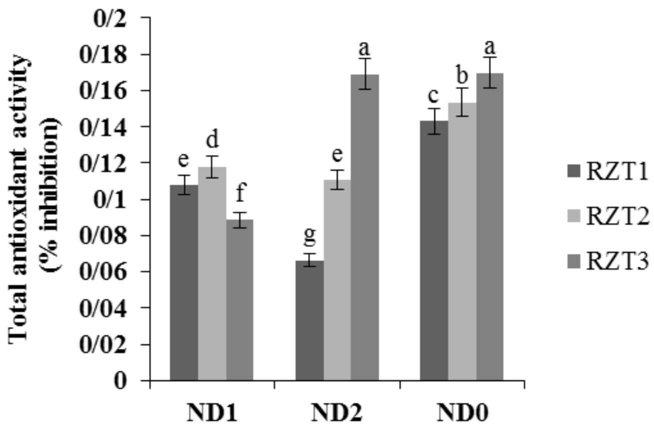

Figure 7- The effect of different levels of ND and RTZ on antioxidant activity in cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature at15°C; RTZ2 = root zone temperature at 25 °C; RTZ3 = root zone temperature at 35°C

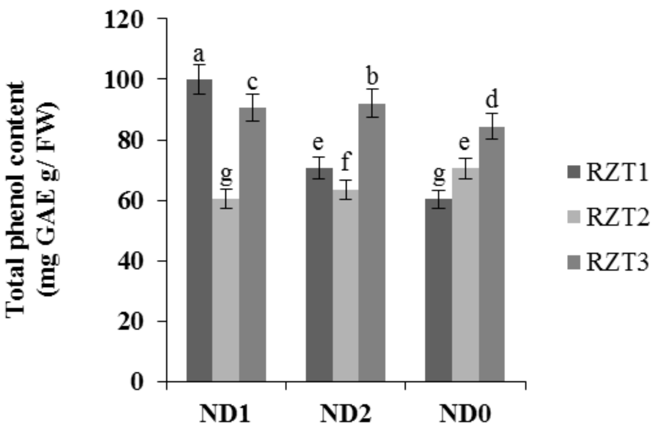

Figure 8- The effect of different levels of ND and RTZ on total phenol in cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature at15°C; RTZ2 = root zone temperature at 25 °C; RTZ3 = root zone temperature at 35°C

hormones (Pessarakli, 2002). Tawfik *et al*. (1996) suggested that plants receiving N fertilization during heat stress tolerate more compared to plants receiving N fertilization before heat stress by changes fresh and dry weights, and significantly higher membrane thermo stabilities. A more recent study reported that adequate N maintains higher N-use efficiency, and photosynthesis in maize (*Zea mays* L.) under heat stress (Wang *et al*.,

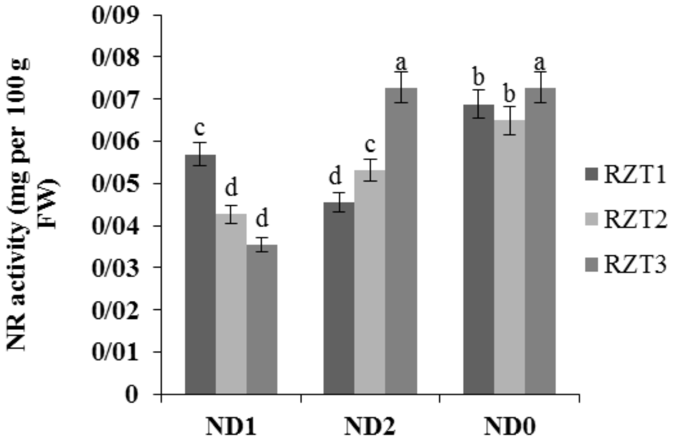

Figure 9- The effect of different levels of ND and RTZ on NR activity in cucumber. ND1 = nitrogen dilution 50%; ND2 = nitrogen dilution 25%; ND0 = nitrogen dilution 100%; RTZ1 = root zone temperature at 15°C; RTZ2 = root zone temperature at 25 °C; RTZ3 = root zone temperature at 35°C

2008). In present study it seems that in low N of nutrient solution both high and low root temperature cause the decrease in root growth. On the other hand in medium N deficiency and optimum N, high root temperature result in more deleterious effect on root growth than low temperature (Figure 3, 4). Tachibana (1987) reported that the best root temperature for cucurbits plants was15°C and when its falls below the plant growth reduced. In the present study also RFW and SFWdecreased more under RTZ3.

Antioxidant response was suggested as a possible mechanism for the reductions of deleterious effect of in heat injury (Liu and Huang, 2002). Similar to the present study antioxidant activity increased more in RTZ3 than other root zone temperature (Figure 7). In creeping bentgrass antioxidant compound concentration increased as a response to the increasing temperature in many researches (Liu and Huang, 2002; Jiang and Huang, 2001; He *et al*., 2005; Xu *et al*., 2006). Antioxidant activity of cucumber significantly increased under suboptimumtemperature stress (Chen *et al*., 2013).

In present study, antioxidant activity increased at optimum N level together with high root zone temperature. In contrast with the present study, increasing N supply significantly reduced antioxidant activity of chrysanthemum flower (Liu *et al*., 2010). Liu *et al*. (2010) reported that an excess N supply negatively affected the antioxidant activity and, thereby, reduced the quality of chrysanthemum.

Nitrate reductase (NR) activity of cabbage, spinach, and grape significantly increased with high N supplementation (Chen *et al*., 2004). In this study, NR activity increased at high root zone temperature in ND2 and ND0.

In the present experiments, total phenol content of cucumber shoot has increased at low and high root zone temperature in ND1 and ND2. In agreement with present study, Giorgi*et et al*. (2009) showed that the nitrogen deficit reduced plant growth, total nitrogen, chlorophyll, and carotenoids, while diminishing nitrogen significantly increased total phenol compound and antioxidant contents of the roots and leaves of yarrow (*Achillea collina* Becker ex Rchb.) compared to the normal nitrogen supply condition (Giorgi *et al*., 2009).

Similar to the present study under low nitrogen in sorghum, leaf area, photosynthesis rate, Greenness, and biomass production significantly reduced (Zhao *et al*., 2005). Nitrogen deficiency induces the chloroplast disintegration and loss of chlorophyll and maybe this is the cause of low chlorophyll concentration under N starvation condition (Forde, 2000). Heat stress inhibition of photosynthesis in chloroplasts maybe because of an imbalance of the electron-transfer chain and producing reactive oxygen species, like singlet oxygen (O_2), superoxide radical (O_2^-), and hydrogen peroxide (H_2O_2) (Smirnoff, 1993). Some other researchers believe that ROS can function as signal molecules for plant. On the other hand, ROS can cause the autocatalytic peroxidation of membrane lipids, leading to loss of membrane permeability and modified its function (Wahid *et al*., 2007). Likewise, Panuccio *et al*. (2001) reported that under nitrogen starvation plant growth, the synthesis of enzymes, the greenness and photosynthesis reduced with the same results was observed from the present study. Zhao *et al*. (2005) found that leaf area, greenness, photosynthesis rate, and biomass production in sorghum reduced with decreasing nitrogen application.

Greenness increased in RZT2 (optimum temperature) at all nitrogen levels. Antioxidant and NR activity increased in ND0 and ND2 with increasing root zone temperature. Total phenol content increased in ND1 and ND2 at low and high temperature stress compared with optimum temperature. Based on our results, we suggest that complete Johansson nutrient solution could not be the best N supply but %0 of N (ND2) in the optimum RTZ2 could be more effective.

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