Cucumber Growth and Nitrogen Uptake as Affected by Solution Temperature and NO_3^-:NH_4^+ Ratios during the Seedling

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Abstract. The effect of solution temperature and nitrogen form on cucumber (*Cucumis sativus* L.) growth, photosynthesis and nitrogen metabolism was investigated in hydroponic culture. Cucumber plants were grown for 35 days in a greenhouse at three constant solution temperatures (15°C, 20°C, and 25°C) within a natural aerial temperature (15-30°C). Four nitrate:ammonium (NO_3^-:NH_4^+) ratios (10:0, 8:2, 5:5, and 2:8 mmol·L^{-1}) at constant nitrogen (N) concentration of 10 mmol·L^{-1} were applied within each solution temperature treatment. Results showed an increasing solution temperature enhanced plant growth (height, dry weight, and leaf area) in most N treatments. Dry weight accumulation was greatest at the 10:0 NO_3^-:NH_4^+ ratio in the 15°C solution, the 5:5 ratio in the 20°C solution and the 8:2 ratio in the 25°C solution. Photosynthetic rate (Pn) response to solution temperature and NO_3^-:NH_4^+ ratio was similar to that of plant growth. Probably, the photosynthate shortage played a role in the reduced biomass formation. Increasing solution temperature enhanced the nitrate reductase (NR) activity, and further reduced shoots nitrate content. Our results indicate that the optimal ratio of nitrate to ammonium that promotes growth in hydroponic cucumber varies with solution temperature.

Additional key words: *Cucumis sativus* L., dry weight, net photosynthetic rate, nitrate:ammonium ratio, nitrate reductase (NR) activity

Introduction

Nitrogen (N) is one of the major nutrients in plants. Nitrogen form, nitrate (NO_3^-) and ammonium (NH_4^+) play a key role in plant growth and development (Bar-Tal et al., 2001). Excessive nitrogen fertilizer application can result in secondary soil salinisation and high nitrate accumulation in plant tissues (Mengel and Pilbeam, 1992), which are dangerous to human health. Furthermore, when exposed to high ammonium concentration, plants show excessive growth (Gerendás et al., 1997) or can be restrained by ammonium toxicity (Britto and Kronzucker, 2002). So proper NO_3^-:NH_4^+ ratio is important to nitrogen fertilizer utilization.

It has generally been observed that the favorable nitrogen source depends on plant species and environmental factors (Taghavi et al., 2004). The effect of NO_3^-:NH_4^+ ratios on plant growth has been studied among many species such as tomato, Chinese cabbage (Kafkafi, 1990), melon (Feigin, 1990), wheat (Silberbush and Lips, 1991), rape (Ali et al.,

1998; Macduff et al., 1987), coffee (Vaast et al., 1998), lettuce (El-Nemr et al., 2012; Lastra et al., 2009), pepper (Bar-Tal et al., 2001), rice flower (Silber et al., 2004) and so on. For example, the rape shoot dry weight could be maximal in NO_3^-:NH_4^+ ratio of 6:4 (Ali et al., 1998). While, pepper fruit dry weight production could be enhanced with high percentage NO_3^- concentration than NH_4^+ (Bar-Tal et al., 2001). These comparative studies indicate the differences in NO_3^-:NH_4^+ ratios exist among species. In addition, plant growth, nitrogen accumulation and uptake can be largely affected by root-zone temperature (Legros and Smith, 1993). Ganmore-Neumann and Kafkafi (1980) reported the optimal NO_3^-:NH_4^+ ratio for tomato plants growth was 1:1 at wide range of root temperature from 8°C to 34°C.

Cucumber is one of the most important horticultural crops in the world. Excessive accumulation of nitrate inhibits cucumber plant growth and development (Gao et al., 2008). Moreover, cucumber is very sensitive to root temperature (Lee et al., 2005). Therefore, the objective of this work was

to find the optimal $NO_3^-:NH_4^+$ ratio under different root temperatures, toward the growth, photosynthesis and nitrogen composition of cucumber plants, to optimize the nitrogen fertilizer utilization and to minimize the N losses.

Materials and Methods

Plants, Growth Conditions, and Experimental Design

Cucumber (*Cucumis sativus* L.) seeds, 'Jinlv 3' (Tianjin Lvfeng gardening new technology development Co., Ltd, China), were germinated in an illuminating incubator at 28°C with a humidity of 80%. After two days of germination, the seeds were transplanted to trays containing a peat-vermiculite mixture (2:1, V/V). Seedlings were chosen after the second set of true leaves emerged and transplanted to a plastic beaker with 1 L of nutrient solutions containing one of the four $NO_3^-:NH_4^+$ ratios (10:0, 8:2, 5:5, and 2:8 mmol·L^{-1}) with a constant total N concentration (10 mmol·L^{-1}) in each solution temperature box (Table 1). Each nutrient treatment also contained 0.1 mmol·L^{-1} of EDTA-Fe-Na, 20 μmol·L^{-1} of H_3BO_3, 5 pmol·L^{-1} of $(NH_4)_6Mo_7O_{24}$, 1 μmol·L^{-1} of $MnSO_4$, 0.2 μmol·L^{-1} of $CuSO_4$, and 1 μmol·L^{-1} of $ZnSO_4$. The nutrient solutions were changed weekly in order to ensure a consistent nutrient supply. The temperature control equipment contained for the nutrient solutions consisted of a polyvinyl chloride box, plastic pots, sand and heating wires. The internal dimensions of the polyvinyl chloride box were 240 (L) × 70 (W) × 20 (H) cm deep. Each box contained 32 pots. The space between pots in the box was filled with sand, in which heating wires (DV, Ningbo China, 800 W 100 m) were immersed. Through the heating wires, heat was delivered to sand, and then to the solutions in the plastic pots. Temperature was regulated by a temperature controller and timing device.

Plant roots were subjected to each treatment from the day they were transferred to the root-containers and allowed to be acclimated to each nutrient concentration for one week, and then exposed to different root temperatures for four weeks. After five weeks from planting, a sample of eight plants was selected from each temperature × N ratio treatment from each box.

Determination of Plant Growth Characteristics

Plant height was measured with a ruler from cotyledon scar to base of petiole on last fully expanded leaf. The initial five euphylla areas on each plant were measured by leaf area meter (Li-3000C, LI-COR, USA). Plants were separated into shoot and root tissues after the whole plants were removed from the pots. The fresh shoots and roots were dried carefully at 105°C for 30 min, and then further dried at 80°C for 24 h.

Determination of Photosynthesis Light−response Curve

Photosynthetic rate (Pn) was measured on clear days from 9:00 to 12:00 after 30 days of temperature treatments, using a portable photosynthetic apparatus (Li-6400, LI-COR, USA), choosing three plants per temperature × N ratio treatment and measuring the third function leaf from bottom. Twelve photosynthetic photon flux density (PPFD) (2000, 1800, 1400, 1000, 500, 200, 150, 100, 50, 20, 10, and 0 μmol·m^{-2}·s^{-1}) were set. Before measuring, leaves were acclimated to high intensity light (1800 μmol·m^{-2}·s^{-1}) for 4 min. Pn-PPFD curves were plotted using the mean values of Pn measured at each PPFD.

Chlorophyll Content

Leaves on the same plants used for Pn measurements were collected for later determinations of chlorophyll contents based on modified measurement of Li et al. (2000). Chlorophyll was extracted by placing 0.5 g leaves in 50 mL mixture of acetone and ethanol (1:1) in the dark for 24 h. Chlorophyll a and b were determined using a UV-visible spectrophotometer (UV-2000, Shanghai, China).

Nitrate (NO_3^-), Nitrite (NO_2^-), and Ammonium (NH_4^+) Concentration

Tissue nitrate (NO_3^-) concentration was determined by the modified method of Li et al (2000). Shoots/roots (2 g) were homogenized in 10 mL distilled water. The extract was centrifuged at 4000 r·min^{-1} for 15 min, the supernatant was used to nitrate measurement. 0.1 mL extract with 0.4 mL 5% salicylic acid sulfate were mixed for 20 min, then added 9.5 mL sodium hydroxide. Nitrogen concentration was determined at 410 nm using a UV-visible spectrophotometer (UV-2000, Shanghai, China).

Table 1. Composition of solutions used to grow cucumber with 10 mmol·L^{-1} N form different ratios of $NO_3^-:NH_4^+$.

Salt (mmol·L^{-1})	$NO_3^-:NH_4^+$ ratio treatment			
	10:0	8:2	5:5	2:8
$Ca(NO_3)_2·4H_2O$	4	4	2	1
KNO_3	2	-z	1	-
KH_2PO_4	1	1	1	1
$(NH_4)_2SO_4$	-	1	2.5	4
K_2SO_4	1.5	2.5	2	2.5
$CaCl_2$	-	-	2	3
$MgSO_4·7H_2O$	2	2	2	2

[z]The treatment did not contain this salt.

Plant nitrite concentration was measured based on Fox et al. (1981). Shoots/roots (2 g) were homogenized in 10 mL distilled water and filtered. Nitrite concentration was determined colorimetrically at 520 nm (UV-visible spectrophotometer UV-2000, China) by adding 2 mL sulfanilic amide (SAN) and 2 mL 1-naphthylamine (1-NA).

Ammonium (NH_4^+) concentration was measured according to Li et al. (2000). Shoots/roots (1.0 g) were homogenized in 5 mL ethanoic acid and diluted with distilled water to 100 mL constant volume. 1.0 mL filtrate and 1.0 mL distilled water were added to tube, then added 3.0 mL ninhydrin and 0.1 mL ascorbic acid. Ammonium concentration was determined at 570 nm using a UV-visible spectrophotometer (UV-2000, Shanghai, China).

Nitrate Reductase (NR) Activity

Nitrate reductase (NR) activity was determined with modified methods described by Hageman and Hucklesby (1971). Four parts of disks (0.5 g) with a diameter of 1 cm were punched out from mature leaves and placed to four centrifuge tube containing 9 mL of 100 mmol·L^{-1} potassium phosphate buffer (pH 7.5) and 100 mmol·L^{-1} KNO_3. One of the tubes was with 1 mL 30% trichloroacetic acid (TCA) except the 9 mL buffer solution, as the control. Then the tubes were placed in a dryer, the air between leaf tissues was removed using a vacuum pump. When leaves sunk to the tube bottom, 1 mL TCA was added to the test tubes to stop the enzymatic reaction. Nitrite reductase was determined colorimetrically at 540 nm (UV-visible spectrophotometer UV-2000, China) by adding 1% sulphanilamide and 0.02% (w/v) naphthalene base ethylene amines.

Statistical Analysis

Analysis of variance (ANOVA) was performed using the general linear model univariate procedure from the SPSS (17.0) software based on the main effects of root zone temperature and NO_3^-:NH_4^+ ratio treatments with Duncan's multiple range test (DMRT) at the 5% level.

Data are means of three replicates.

Results

Growth Characteristics

Elevated solution temperature increased plant height in most N ratio treatments with the exception of the NO_3^- treatment (10:0) at 15°C and 20°C (Fig. 1A). Different performances were obtained among variance NO_3^-:NH_4^+ ratios at each solution temperature. At 15°C, plant height reduced with the increasing NH_4^+ concentration. Plant height in all

the nutrient treatments was higher at 25°C than that at 20°C. The maximal height at 20°C and 25°C was obtained when the NO_3^-:NH_4^+ ratio was 5:5 (Fig. 1A).

The effect of solution temperature on leaf area was similar to that on plant height (Fig. 1B). With the increasing solution temperature, leaf area increased except a treatment with the ratio of 10:0 at 15°C than that at 20°C. At 15°C, leaf area was inhibited by the increasing NH_4^+ concentration. At 20 and 25°C, the maximal leaf area was in the ratio of 8:2. Percentage of NH_4^+ concentration (larger than 20%) inhibited leaf area performance (Fig. 1B).

At low solution temperature of 15°C, an increased percentage of NH_4^+ in the solution significantly ($P < 0.05$) restrained shoot and root dry matter production, but slightly increased shoot:root ratio, as shown in Table 2. Different from that at 15°C, proper addition of NH_4^+ promoted shoot and root dry weight at 20 and 25°C. In general, at 20°C and 25°C,
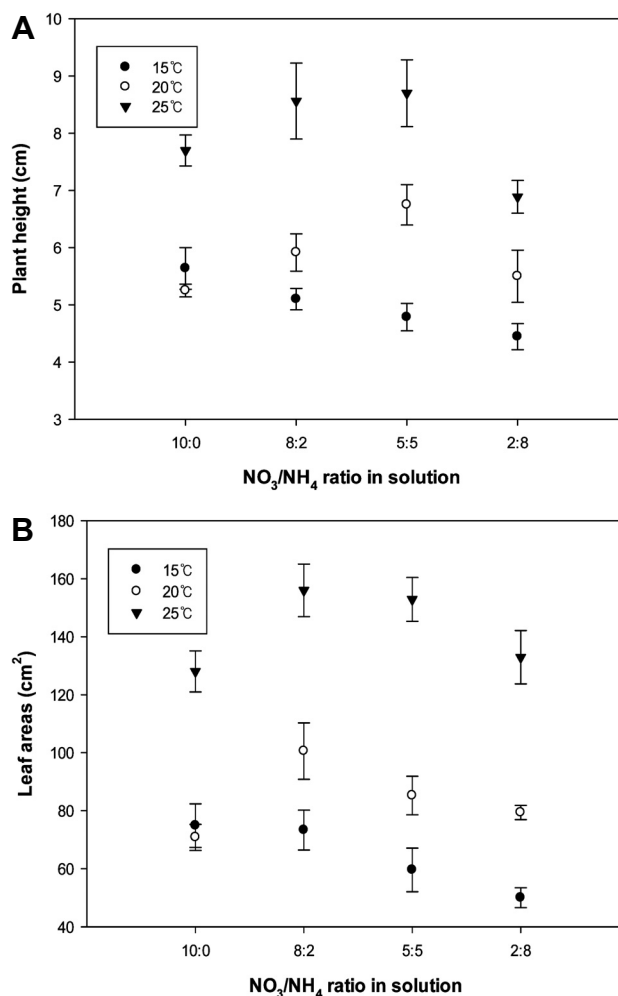

Fig. 1. Effects of solution temperature on plant height (A) and leaf areas (B) of 35-days-old cucumber seedlings grown with solutions containing different NO_3^-:NH_4^+ ratios. Data are means of three replicates, with standard (n = 3) deviations shown by vertical bars.

Table 2. Effects of solution temperature on dry weight (DW) of 35-days-old cucumber seedlings grown with solutions containing different $NO_3^-:NH_4^+$ ratios (unit: g).

$NO_3^-:NH_4^+$	Shoot DW			Root DW			Shoot : root ratio		
	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
10:0	0.961 a[z]	0.595 b	1.322 b	0.231 a	0.076 b	0.154 b	4.503 b	7.784 a	8.584 a
8:2	0.561 b	0.964 ab	1.948 a	0.103 b	0.132 a	0.317 a	5.420 b	7.275 a	6.135 a
5:5	0.569 b	1.226 a	1.491 b	0.069 c	0.240 a	0.206 ab	8.187 a	5.110 b	7.220 a
2:8	0.450 b	0.553 b	0.829 c	0.049 c	0.061 b	0.096 c	9.101 a	9.073 a	8.640 a

[z]Mean values followed by different letters within a row line indicated significant differences at $P < 0.05$.

solutions with $NO_3^-:NH_4^+$ ratios of 5:5 and 8:2 have greater shoot and root dry weights. Elevating solution temperature enhanced shoot dry matter production in the same $NO_3^-:NH_4^+$ ratio except a relatively lower value in higher $NO_3^-:NH_4^+$ ratio at 20°C. Shoot dry weight increased by 71.8% (elevating solution temperature from 15 to 20°C) and 102.1% (from 20 to 25°C) in $NO_3^-:NH_4^+$ ratio (8:2), 115.4% and 21.6% in the ratio of 5:5, 22.8% and 49.9% in the ratio of 2:8, individually.

Photosynthesis and Chlorophyll Content

Net photosynthetic rate (Pn) increased with the increased photosynthetic photon flux density (PPFD) (Fig. 2). Net photosynthesis rate curve (Pn-PPFD) showed Pn was increased with the increasing NO_3^- concentration at low solution temperature of 15°C. At 20°C, the Pn value with the ratio of 10:0 was lower than the other N ratio treatments, whereas Pn decreased with the increasing NH_4^+ concentration in treatments when the mixture of NO_3^- and NH_4^+ was used. At 25°C, Pn was, in decreasing order: 8:2 > 10:0 > 5:5 > 2:8 ($NO_3^-:NH_4^+$ ratios).

Total chlorophyll (Chl) concentration was not significantly affected by the $NO_3^-:NH_4^+$ ratio at 15°C and, but significant ($P < 0.05$) differences existed at 20°C and 25°C (Table 3). The ratio of $NO_3^-:NH_4^+$ in solution had no influence on Chl a/Chl b at 20°C and 25°C. At 15°C, plants grown in 10:0 ratios had the highest Chl a/Chl b values.

Nitrogen Metabolism and Nitrate Reductase (NR) Activity

Roots nitrate concentration decreased with an increasing NH_4^+ proportion regardless of solution temperatures (Fig. 3A). Shoot nitrate contents showed diverse patterns at different solution temperature in the order of $NO_3^-:NH_4^+$ ratios (8:2 > 5:5 > 10:0 > 2:8) at 15°C, 8:2 > 10:0 > 5:5 > 2:8 at 20°C, 10:0 > 8:2 > 5:5 > 2:8 at 25°C, respectively. Regarding the ratio of 10:0, shoot nitrate contents were greater at 20°C and 25°C than that at 15°C, whereas root nitrate uptake was highest at 25°C. With respect to other $NO_3^-:NH_4^+$ ratio treatments (8:2, 5:5 and 2:8), shoot nitrate content reduced with the increasing solution temperature. In contrast, root
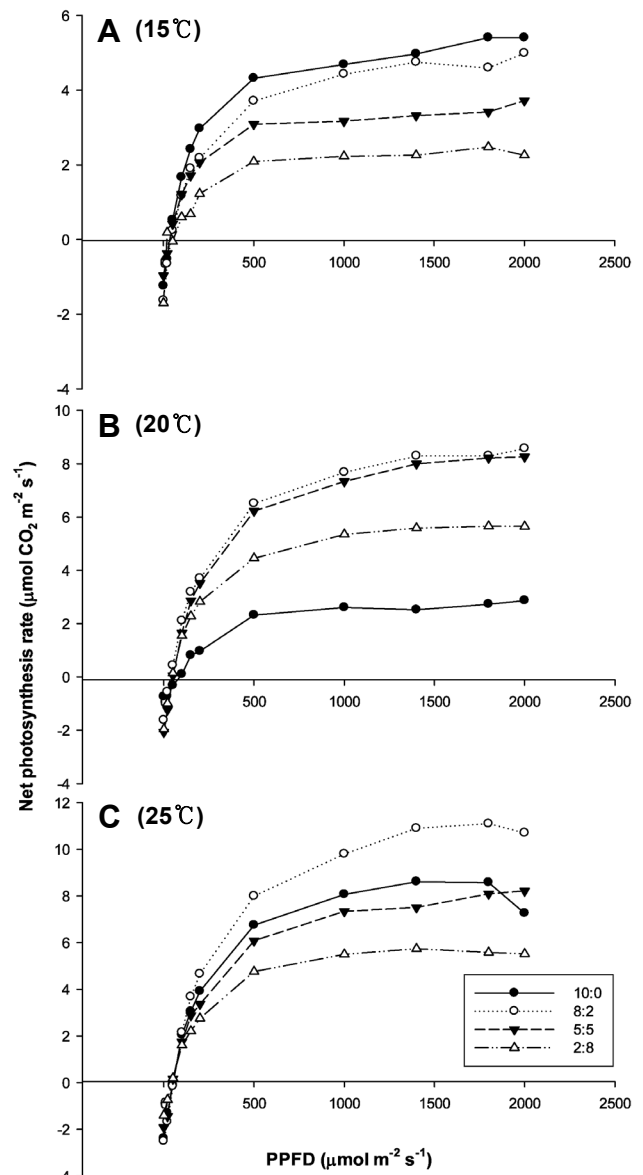

Fig. 2. Effects of solution temperature on net photosynthesis rate - photosynthetic photon flux density (PPFD) curve of 35-days-old cucumber seedlings grown with solutions containing different $NO_3^-:NH_4^+$ ratios. Values are mean of two replicates (n = 2).

nitrate contents were enhanced slightly with the increasing solution temperature except 2:8 ratio.

At 15°C, highest nitrite content in shoot occurred in NO_3^-:

Table 3. Effects of solution temperature on chlorophyll (Chl) concentration in leaves of 35-days-old cucumber seedlings grown with solutions containing different NO_3^-:NH_4^+ ratios.

NO_3^-:NH_4^+	Total Chl (a + b) concentration (mg·g^{-1})			Chl a/Chl b		
	15°C	20°C	25°C	15°C	20°C	25°C
10:0	1.184 ± 0.004 a[z]	0.830 ± 0.078 c	1.094 ± 0.026 bc	3.131 ± 0.008 a	2.882 ± 0.067 a	3.167 ± 0.056 a
8:2	0.990 ± 0.083 a	1.126 ± 0.006 a	1.207 ± 0.027 a	2.975 ± 0.024 b	3.082 ± 0.033 a	3.201 ± 0.045 a
5:5	1.038 ± 0.066 a	1.074 ± 0.039 ab	1.137 ± 0.021 ab	3.000 ± 0.043 b	3.178 ± 0.000 a	3.167 ± 0.016 a
2:8	1.007 ± 0.075 a	0.933 ± 0.029 bc	1.009 ± 0.013 c	3.015 ± 0.095 b	2.506 ± 0.642 a	3.228 ± 0.016 a

[z]Mean ± Standard deviation (n = 3).
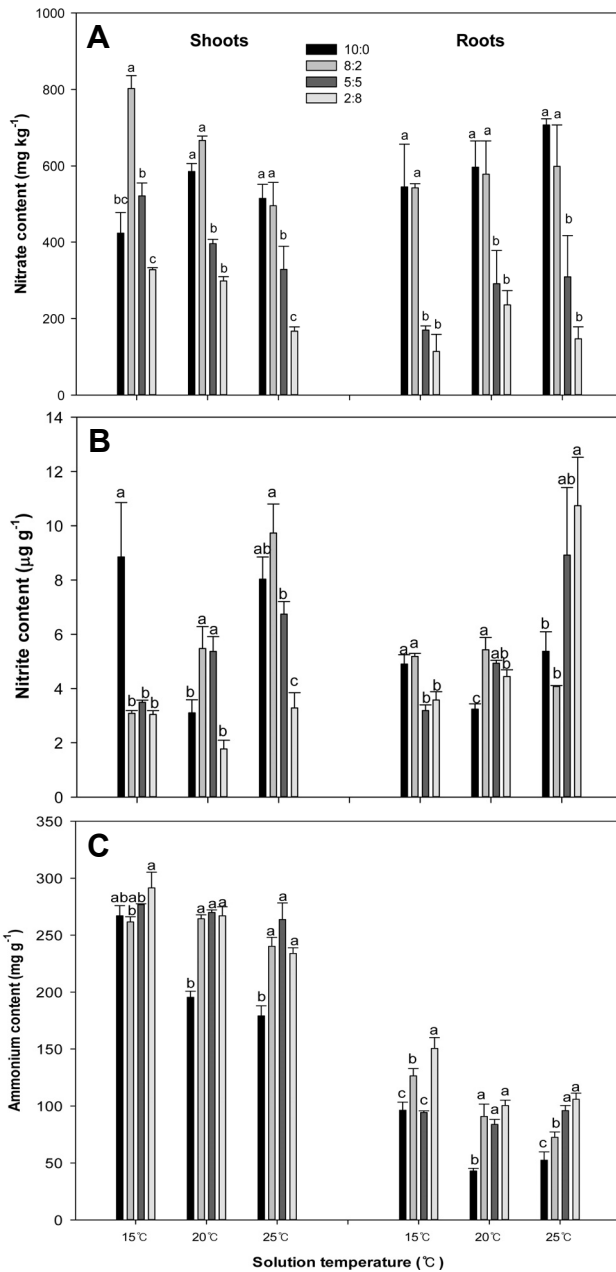Values followed by different letters within a column indicated significant differences at $P < 0.05$.

Fig. 3. Effects of solution temperature on cucumber nitrate (A), nitrite (B), ammonium (C) concentration of 35-days-old cucumber seedlings grown with solutions containing different NO_3^-:NH_4^+ ratios. Data are means of three replicates, with standard deviations (n = 3) shown by vertical bars. Bars sharing different letters indicate significant differences at $P \leq 0.05$.

Fig. 4. Effects of solution temperature on nitrate reductase (NR) activity of 35-days-old cucumber seedlings grown with solutions containing different NO_3^-:NH_4^+ ratios. Data are means of three replicates, with standard deviations (n = 3) shown by vertical bars. Bars sharing different letters indicate significant differences at $P \leq 0.05$.
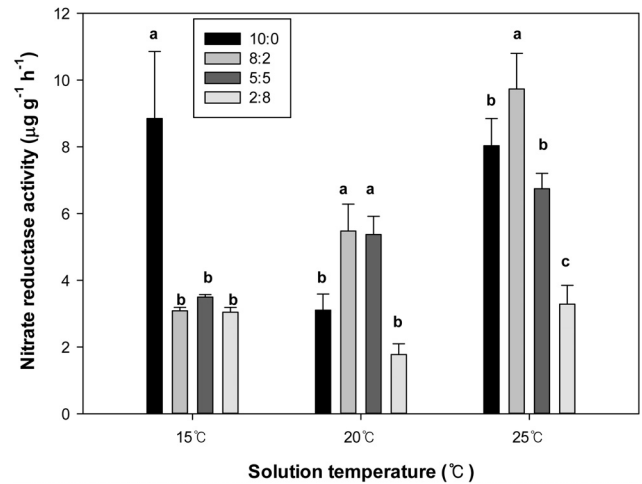
NH_4^+ ratios of 10:0 (Fig. 3B). At 20°C, shoots and roots nitrite content were relatively high in NO_3^-:NH_4^+ ratios (5:5 and 8:2). At 25°C, shoot nitrite contents followed the order of NO_3^-:NH_4^+ ratios (8:2 > 10:0 > 5:5 > 2:8), and root nitrite contents showed the reverse results. Lower shoot nitrite contents were observed in NO_3^-:NH_4^+ ratios (10:0 and 2:8) at 20°C with comparison to 15°C and 25°C, but those in NO_3^-:NH_4^+ ratios (8:2 and 5:5) increased with the increasing solution temperature. Root nitrite content at 20°C in ratio of 10:0 was lower than that at 15°C and 25°C. And in ratio of 8:2, root nitrite content at 25°C was lower than that at 15°C and 20°C. Root nitrite contents in ratios of 5:5 and 2:8 increased as the solution temperature increased.

At 15°C, shoot ammonium content increased with the increasing NH_4^+ concentration, whereas those in root followed the order of NO_3^-:NH_4^+ ratios (2:8 > 8:2 > 10:0 > 5:5) (Fig. 3C). At 20°C, no significant differences were found in most nutrient treatments except a relatively low shoot and root ammonium content in the highest NO_3^- percentage treatment.

When exposed to 25°C solution temperature, root ammonium content was promoted as the NH_4^+ concentration increased, and shoot ammonium content followed the order of $NO_3^-:NH_4^+$ ratios (5:5 > 2:8 > 8:2 > 10:0). Generally, ammonium content in shoot and root decreased with the increasing solution temperature in all $NO_3^-:NH_4^+$ ratios treatments.

The leaf nitrate reductase (NR) activity in highest NO_3^- treatments (10:0) was significantly higher than those in the other $NO_3^-:NH_4^+$ ratios treatments at 15°C (Fig. 4). At 20°C, the NR activity in $NO_3^-:NH_4^+$ ratios 8:2 and 5:5 were significantly higher than those in ratios 10:0 and 2:8. At 25°C, NR activity was maximum in $NO_3^-:NH_4^+$ ratios 8:2 and minimum in the 2:8 ratio treatments. Both the treatments of $NO_3^-:NH_4^+$ ratios 10:0 and 2:8 showed a relatively lower NR activity at 20°C than those at 15°C and 25°C, while the NR activities increased with the increasing solution temperature in ratios 8:2 and 5:5.

Discussion

Results of this study showed that plant growth was inhibited under high NH_4^+ concentration at low solution temperature of 15°C, but the negative effects of NH_4^+ on plant growth could be relieved at higher solution temperature of 20°C and 25°C. Therefore, high NH_4^+ concentration at low solution temperature might be unfavorable for cucumber growth. As previously reported, low root temperature (8°C and 16°C) inhibited tomato plant growth in high percentage of NO_3^-, but was favorable at high root temperature (24°C and 34°C) (Ganmore-Neumann and Kafkafi, 1980). In this current study, plants grown with $NO_3^-:NH_4^+$ ratio 2:8 generally grew less, regardless of solution temperatures. Generally, it is better that ammonium percentage does not exceed more than 50% of total nitrogen in the nutrient solution (Taghavi et al., 2004). Bybordi (2011) also reported high quality canola growth when ammonium and nitrate at 50:50 ratios. Compared to 28.2°C, maize yield at 14.8°C were greatly reduced in different form nitrogen solution, especially those in high NH_4^+ percentage (Moraghan and Porter, 1975). Ammonium results in lower root biomass and leaf area, which may contribute to the low carbon gain and then inhibit plant growth (Guo et al., 2007). Low root temperature (12°C and 16°C) significantly reduced the dry weight of cucumber plants (Lee et al., 2005). Similar response of the rape plants to root temperature was reported by Ali et al. (1998). Dry weight of cucumber shoots showed direct relationship with roots dry weight in response to root temperature and $NO_3^-:NH_4^+$ ratios (Table 2). It is possible that the relative size of root enable to assimilate nutrients required by shoots.

The $NO_3^-:NH_4^+$ ratios and root temperature had less sig-

nificant effect on the cucumber leaf chlorophyll content (Table 3). Similar effect on pepper was reported by Bar-Tal et al. (2001). Root temperature effects on cucumber plants photosynthesis were significant, similar to shoot dry weight biomass. It indicates that Chl content was not the crucial factor for Pn, while affected by solution temperature and $NO_3^-:NH_4^+$ ratios. With regard to each nutrient treatment, low solution temperature inhibited the rate of CO_2 uptake, while photosynthesis rate could be improved as solution temperature increased. Similarly, Vapaavuori et al. (1992) reported the CO_2 uptake of conifer was inhibited at low root temperature. Pn of cucumber seedlings grown with high percentage NH_4^+ was lowest at most times, this is according with Guo et al. (2006) study on tobacco. Therefore, cucumber grown with appropriate mixture of NO_3^- and NH_4^+ can increase Pn, and this effect can be promoted by elevated solution temperature.

In general, plant tissues contained higher nitrate concentrations compared to the nitrite and ammonia concentrations (Fig. 3). Chantarotwong et al. (1976) also reported that plants can accumulate nitrate in large amounts, whereas usually very low concentrations of nitrite and ammonium are presented. Ammonium concentrations in shoots were higher than those in roots, and both NH_4^+ concentration in shoots and roots increased as NH_4^+ percentage increased. NO_3^- concentration in shoots and roots, however, did not increase due to the sufficient substrate of NH_4^+ for nitrification. It showed that NO_3^- percentage in the solution was the primary cause of NO_3^- uptake in plant tissues. Addition of NH_4^+ could decrease NO_3^- uptake and decrease nitrate accumulation. The first step of nitrogen absorption is the reduction of nitrate to nitrite, in which nitrate reductase (NR) take the key effect on the process (Kaiser et al., 1999; Toselli et al., 1999). As previously reported, wheat NR activity increased at low temperature treatment (Yaneva et al., 2002). In our study, NR activity in ratios of 8:2 and 5:5 increased with the increasing solution temperature, which indicates that nitrate assimilation in plants can be improved at suitable root temperature (20°C and 25°C).

It is concluded that optimal nitrate:ammonium ratio for cucumber growth during solution temperature of 15°C, 20°C, and 25°C was 10:0, 5:5 and 8:2, respectively. Ammonium applied at the rate of 20% to 50% at higher solution temperature of 20°C and 25°C showed well plant growth, while harmful effects on plant growth occurred once ammonium percentage exceed 50%. Elevating solution temperature inhibited nitrate accumulation in plants. It is further concluded that both ammonium and nitrate are useful for cucumber growth while the suitable $NO_3^-:NH_4^+$ ratio for growth depend upon root temperature.

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