

# Different nitrogen forms on the growth and nitrogen utilization of cucumber

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Accepted 23<sup>rd</sup> March, 2020.

**Abstract.** Different nitrogen sources are effective on plants growth and yield. Ammonium [NH<sub>4</sub><sup>+</sup>-N], nitrate [NO<sub>3</sub><sup>-</sup>-N] and amide [CO (NH<sub>2</sub>)<sub>2</sub>] are the three different nitrogen sources. Here, we studied the effects of 9 ratios of different nitrogen forms with equal nitrogen on dry matter, nitrogen utilization and yield in cucumber. The results demonstrated that the yield of cucumber was enhanced as the proportion of nitrate increased in the ammonium and nitrate treatments except 100% nitrate. Nitrogen utilization efficiency in treatments with added amide were higher than in treatments with added ammonium with equal proportion of nitrate. A correlation analysis was performed; the proportion of nitrate and ammonium was significantly positively related to total dry matter, total nitrogen content and nitrogen utilization efficiency respectively ( $r = 0.919, 0.930, \text{ and } 0.961$ ), and nitrate-amide ratio was related to root-shoot ratio ( $r = 0.934$ ). Additionally, there was a remarkably positive relationship between leaf nitrogen content and yield. Dry matter distribution, yield and root cap index may be regulated by the ratios of nitrogen forms. Moreover, cucumber yield is inseparable from its leaf nitrogen content. In conclusion, the nitrogen consisting of 75% nitrate+25% amide was superior to other treatments in cucumber growth in the present study.

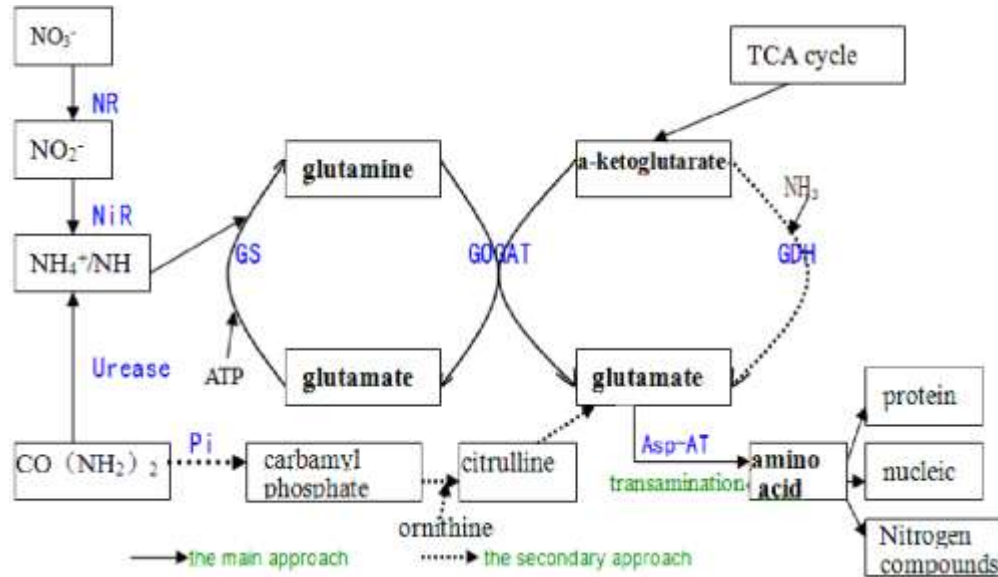
**Keywords:** *Cucumis sativus*, Nitrogen sources, dry matter, nitrogen utilization, yield.

## INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important vegetable that is cultivated for its economic and nutritional benefits throughout the world. One of the most important factors controlling cucumber growth is nitrogen, which is known as the 'life element' (Canfield *et al.*, 2010). Current annual nitrogen fertilizer inputs in cucumber production are excessive; however, its utilization efficiency is low, at only 30 to 35% in developing countries, such as China (Zhao *et al.*, 2015; Wang *et al.*, 2008), and at 40 to 50% in developed countries (Bingham *et al.*, 2012; Li *et al.*, 2014). Since its importance but low utilization efficiency, scientists pay attention to study how to promote the

absorption, distribution and utilization of nitrogen. One of the main research topics is the effects of different N fertilizer forms on plant growth (Yang *et al.*, 2013; Xu *et al.*, 2005).

Plants have the ability to take up several chemical forms of nitrogen (Helali *et al.*, 2010; Liu *et al.*, 2013). The most common nitrogen forms are: ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) and amide (CO (NH<sub>2</sub>)<sub>2</sub>) (Miller *et al.*, 2006). These forms of nitrogen have common metabolic pathway, the glutamate pathway that nitrogen is homogenized into amino acids (Figure 1), in which glutamine synthesis (GS) and glutamate synthesis (GOGAT) play a role and



**Figure 1.** Nitrogen metabolizing cycle (Celine *et al.*, 2010).

therefore called GS/GOGAT cycle (Chen *et al.*, 2010). In addition, amide nitrogen metabolism has another pathway, namely amino acid pathway. The amino acid metabolic pathway is completely different from the ammonium and nitrate metabolism pathway. Amide is not decomposed to  $\text{NH}_3$  but is assimilated directly into amino acids, and citrulline content reflects the activity of amide nitrogen metabolism in amino acid pathway (Solomon *et al.*, 2010; Liao and Yan, 2003).

In recent years, research has shown that mixed nitrogen forms are beneficial for improving nitrogen utilization efficiency and reducing nitrate pollution (Matteson *et al.*, 2009; Tehryung *et al.*, 2002). These studies focused on the effect of different treatments with different concentrations of ammonium [ $\text{NH}_4^+\text{-N}$ ] and nitrate [ $\text{NO}_3^-\text{-N}$ ] on vegetable growth (Andriolo *et al.*, 2006; Zhang *et al.*, 2016). Some results showed that different nitrogen forms have remarkable effects on the dry matter and yield of cucumber (Roosta *et al.*, 2007; Yan *et al.*, 2013). It was reported that appropriate ratio of  $\text{NO}_3^-\text{-N}$  and  $\text{NH}_4^+\text{-N}$  was favourable for the growth, development and yield of plants, such as tobacco (Xie *et al.*, 2014), ginger (Singh *et al.*, 2016), Chinese cabbage (He *et al.*, 2009), cauliflower (Liu *et al.*, 2013). However, the dry matter distribution under three major nitrogen forms mentioned before is less researched. In addition, few studies on improving cucumber yield and nitrogen utilization efficiency through the composition of different nitrogen forms have been reported.

Therefore, this article studied the effect of three nitrogen forms, including ammonium [ $\text{NH}_4^+\text{-N}$ ], nitrate [ $\text{NO}_3^-\text{-N}$ ] and amide [ $\text{CO}(\text{NH}_2)_2$ ], on dry matter, nitrogen utilization efficiency and yield. This experiment was carried out to study the dry matter, nitrogen content, yield and nitrogen utilization efficiency of plants for improving nitrogen

utilization efficient and reducing nitrogen inputs in cucumber cultivated.

## MATERIALS AND METHODS

### Plant materials and growth conditions

The experiment was implemented in the greenhouse at the Institute of Vegetables and Flowers at the Chinese Academy of Agricultural Sciences in 2018 spring-summer seasons ( $30 \pm 3^\circ\text{C}$  day/ $20 \pm 2^\circ\text{C}$  night, relative humidity of 80%, 13 h light/11 h dark). The cucumber variety “Zhongnong 26” was acquired from Institute of Vegetables and Flowers. Seedlings each with two leaves were transplanted into experimental pots containing 8 L of coir dust (0.25 g N/1 L coir dust), and nutrient solution was applied into substrate regularly. Their vines were hung when the cucumber plants had 8 to 10 leaves. A tray was placed at the bottom of each pot in order to catch and return leaked nutrient solution to the tray to fertilize the substrate in the pot.

### Nitrogen treatment

The nitrogen treatment was characterized by the proportions of different nitrogen forms in the nutrient solution, and the nitrogen treatments were applied to each plant with the corresponding nutrient solution. Nine treatments with the same total nitrogen contents in Table 1 with different ratios of 3 forms of nitrogen ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and  $\text{CO}(\text{NH}_2)_2$ ) on the basis of the Shanqi formula (Guo, 2003), in which the percentage (%) represents one nitrogen form accounts for nitrogen forms composition.

**Table 1.** Compositions of nitrogen treatments in terms of different nitrogen forms.

Treatments	NO <sub>3</sub> <sup>-</sup> + NH <sub>4</sub> <sup>+</sup>	Treatments	NO <sub>3</sub> <sup>-</sup> + CO(NH <sub>2</sub> ) <sub>2</sub>
N100A	100%NO <sub>3</sub> <sup>-</sup>	N100C	100%NO <sub>3</sub> <sup>-</sup>
N75A	75%NO <sub>3</sub> <sup>-</sup> + 25%NH <sub>4</sub> <sup>+</sup>	N75C	75%NO <sub>3</sub> <sup>-</sup> + 25% CO(NH <sub>2</sub> ) <sub>2</sub>
N50A	50%NO <sub>3</sub> <sup>-</sup> + 50%NH <sub>4</sub> <sup>+</sup>	N50C	50%NO <sub>3</sub> <sup>-</sup> + 50% CO(NH <sub>2</sub> ) <sub>2</sub>
N25A	25%NO <sub>3</sub> <sup>-</sup> + 75%NH <sub>4</sub> <sup>+</sup>	N25C	25%NO <sub>3</sub> <sup>-</sup> + 75% CO(NH <sub>2</sub> ) <sub>2</sub>
N0A	0%NO <sub>3</sub> <sup>-</sup> +100%NH <sub>4</sub> <sup>+</sup>	N0C	0%NO <sub>3</sub> <sup>-</sup> + 100% CO(NH <sub>2</sub> ) <sub>2</sub>

N--nitrate (NO<sub>3</sub><sup>-</sup>), A—ammonium (NH<sub>4</sub><sup>+</sup>), C--amide (CO (NH<sub>2</sub>)<sub>2</sub>) in the treatments; N100A= N100C; the number after N indicates the percentage of NO<sub>3</sub><sup>-</sup>-N in the treatment.

All treatments were 14 mM N element which NH<sub>4</sub><sup>+</sup> as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> or NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, NO<sub>3</sub><sup>-</sup> as Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O or KNO<sub>3</sub>; In all the N treatments, other nutrients compositions were the same: 3.5 Ca, 6.0 K, 2.0 Mg and 1.0 P (mmol L<sup>-1</sup>), and 110 Fe, 20.6 B, 0.16 Cu, 5.3 Mn, 0.49 Mo and 0.34 Zn (μmol L<sup>-1</sup>). For all the N treatments, solution pH was adjusted to 5.5 before the addition to the substance. Each experimental pot was fertilized with 1 L nutrient solution every five days until the harvest of the experimental plants; therefore, the applied nitrogen could be calculated from the nutrient solution input per cucumber plant throughout its growth period. Nine treatments with 162 pots were employed in the experiment, and there were 18 pots for each treatment in three replicates.

### Determination of index

#### Yield estimation

After cucumber fruits reached harvest standard which was usually 15 days after pollination, fruits were harvested and weighed separately, and we measured the yield of each treatment of plant growth.

#### Dry matter estimation

The cucumber plants were harvested when growth stage entered a later period of fruiting, which was about 40 days after flowering. Firstly, the different parts (leaf, fruit, stem and root) of plants were separated, and fresh weight was measured after cleaning with distilled water. Secondly, enzymes were inactivated at 110°C for 15 min in an oven. Finally, the tissues were dried to a constant weight at 75°C in an oven, and the dry weight was measured.

#### Nitrogen content estimation

Dried plant were crushed with a mill and sieve through 60-mesh net and then digested with H<sub>2</sub>SO<sub>4</sub> - H<sub>2</sub>O<sub>2</sub>. Then, the nitrogen content of different tissues were measured with a Gerhardt analyser (KDY-9810), and the nitrogen contents were calculated (Liao and Yan, 2003). Nitrogen

utilization efficiency (NUE) = plant nitrogen content (g.plant<sup>-1</sup>)/applied nitrogen quantity (g.plant<sup>-1</sup>).

### Citrulline measurement

The third top leaf at 16 leaves stage was taken from cucumber, immediately frozen in liquid nitrogen, and stored at -80°C for citrulline content analysis. Citrulline content was measured by the HPLC method (Cheng *et al.*, 2010).

### Statistical analysis

The analysis of the variance was conducted by ANOVA with Tukey multiple range test at P < 0.05 in different N-forms. Analyses were conducted in three replicates per treatment. Pearson's correlation was carried out using SPSS 22.0 software.

## RESULTS

### Effect of different nitrogen sources on plant parameters in cucumber

#### Dry matter weight

The N75C and N75A treatment gives the highest values to total dry matter weight in individual plant, compared to other treatments, the N0A and N25A treatment gives the lowest values of dry matter in individual plant, as well as in the leaves and fruits, and they were significantly lower than other nitrogen treatments (Table 2, Figure 2a). As the proportion of NO<sub>3</sub><sup>-</sup>-N < 50%, total dry matter in treatments with added NH<sub>4</sub><sup>+</sup>-N was lower than that in treatments with added CO(NH<sub>2</sub>)<sub>2</sub> with equal proportion of nitrate. The dry matter of the total plant increased as the proportion of NO<sub>3</sub><sup>-</sup>-N increased in the NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N treatments except N100A treatment.

#### Nitrogen content

Table 3 and Figure 2b show that there was lower nitrogen

**Table 2.** Effect of different nitrogen forms on dry matter weight of cucumber (g/plant).

Treatments	Total	Leaf	Stem	Fruit	Root
N0C	232.20 <sup>b</sup>	80.13 <sup>b</sup>	23.63 <sup>bc</sup>	124.50 <sup>a</sup>	3.93 <sup>b</sup>
N0A	143.81 <sup>c</sup>	54.33 <sup>c</sup>	18.67 <sup>c</sup>	67.97 <sup>b</sup>	4.11 <sup>b</sup>
N25C	238.46 <sup>b</sup>	79.27 <sup>a</sup>	27.36 <sup>ab</sup>	126.54 <sup>a</sup>	5.29 <sup>ab</sup>
N25A	164.48 <sup>c</sup>	59.37 <sup>c</sup>	20.93 <sup>c</sup>	79.42 <sup>ab</sup>	4.76 <sup>ab</sup>
N50C	233.30 <sup>b</sup>	84.63 <sup>a</sup>	31.03 <sup>a</sup>	111.54 <sup>ab</sup>	6.10 <sup>ab</sup>
N50A	227.75 <sup>b</sup>	78.10 <sup>b</sup>	27.67 <sup>ab</sup>	114.49 <sup>ab</sup>	7.49 <sup>a</sup>
N75C	253.32 <sup>a</sup>	86.33 <sup>a</sup>	30.93 <sup>a</sup>	129.85 <sup>a</sup>	6.20 <sup>ab</sup>
N75A	251.72 <sup>ab</sup>	85.77 <sup>a</sup>	31.67 <sup>a</sup>	127.62 <sup>a</sup>	6.67 <sup>ab</sup>
N100A(C)	241.47 <sup>b</sup>	79.13 <sup>b</sup>	28.83 <sup>ab</sup>	126.14 <sup>a</sup>	7.37 <sup>a</sup>

N - nitrate (NO<sub>3</sub><sup>-</sup>), A - ammonium (NH<sub>4</sub><sup>+</sup>), C - amide (CO(NH<sub>2</sub>)<sub>2</sub>) in the treatments, and the number after N indicates the percentage of NO<sub>3</sub><sup>-</sup>-N in the treatment. Different letters after the number indicate significant differences at P < 0.05 according to Tukey's multiple comparison test.

**Table 3.** Effect of different nitrogen forms on nitrogen content of cucumber (g/plant).

Treatments	Total	Leaf	Stem	Fruit	Root
N0C	9.15 <sup>b</sup>	3.28 <sup>a</sup>	0.75 <sup>bcd</sup>	4.98 <sup>a</sup>	0.15 <sup>bc</sup>
N0A	5.26 <sup>d</sup>	1.88 <sup>c</sup>	0.57 <sup>d</sup>	2.72 <sup>b</sup>	0.13 <sup>c</sup>
N25C	8.87 <sup>b</sup>	2.82 <sup>ab</sup>	0.80 <sup>abc</sup>	5.06 <sup>a</sup>	0.19 <sup>abc</sup>
N25A	6.20 <sup>c</sup>	2.25 <sup>bc</sup>	0.63 <sup>cd</sup>	3.18 <sup>ab</sup>	0.15 <sup>bc</sup>
N50C	9.04 <sup>b</sup>	3.40 <sup>a</sup>	0.95 <sup>a</sup>	4.46 <sup>ab</sup>	0.23 <sup>ab</sup>
N50A	8.49 <sup>b</sup>	2.87 <sup>ab</sup>	0.79 <sup>abc</sup>	4.58 <sup>ab</sup>	0.26 <sup>a</sup>
N75C	9.66 <sup>a</sup>	3.38 <sup>a</sup>	0.88 <sup>ab</sup>	5.19 <sup>a</sup>	0.21 <sup>abc</sup>
N75A	9.30 <sup>ab</sup>	3.06 <sup>a</sup>	0.94 <sup>a</sup>	5.10 <sup>a</sup>	0.20 <sup>abc</sup>
N100A(C)	9.06 <sup>b</sup>	2.87 <sup>a</sup>	0.88 <sup>ab</sup>	5.05 <sup>a</sup>	0.25 <sup>ab</sup>

N - nitrate (NO<sub>3</sub><sup>-</sup>), A - ammonium (NH<sub>4</sub><sup>+</sup>), C - amide (CO(NH<sub>2</sub>)<sub>2</sub>) in the treatments, and the number after N indicates the percentage of NO<sub>3</sub><sup>-</sup>-N in the treatment. Different letters after the number indicate significant differences at P ≤ 0.05 according to Tukey's multiple comparison test.

content in the equal-proportion NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N treatment than that in the equal-proportion NO<sub>3</sub><sup>-</sup>-N and CO(NH<sub>2</sub>)<sub>2</sub> treatment in individual plant, as well as in the leaves and fruits, when the proportion of NO<sub>3</sub><sup>-</sup>-N < 50%. The treatments N0A and N25A manifested significantly lower nitrogen content than the other treatments in cucumber plant. The results demonstrated that the influence of different nitrogen forms did not agree with the nitrogen content of cucumber.

### Yield of plants

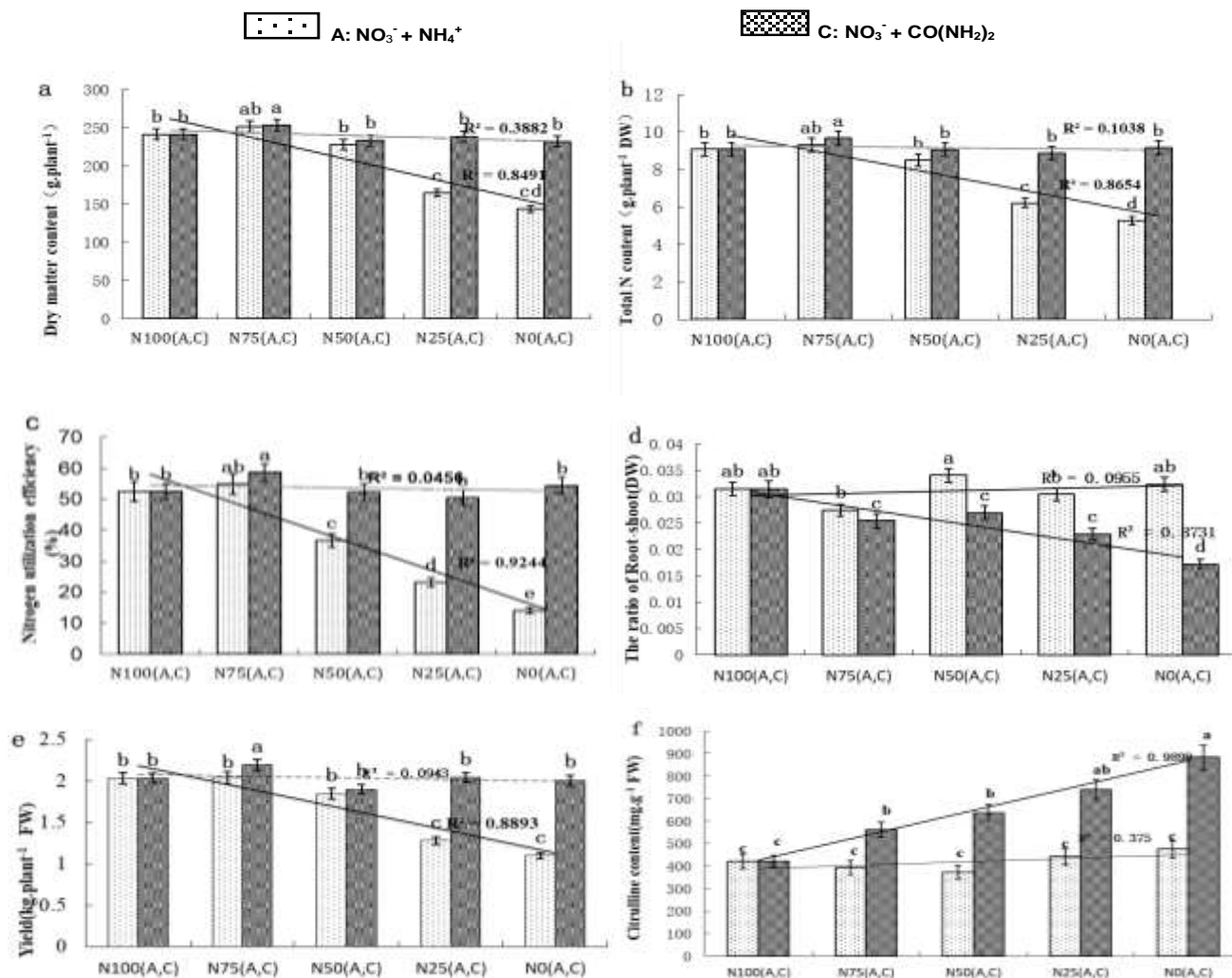
The N75C treatment had the highest yield among the 9 treatments. When applied as one nitrogen form, the yield was CO(NH<sub>2</sub>)<sub>2</sub> > NO<sub>3</sub><sup>-</sup>-N > NH<sub>4</sub><sup>+</sup>-N. In treatments with NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, the yield of cucumber was in 75%NO<sub>3</sub><sup>-</sup> > 100%NO<sub>3</sub><sup>-</sup> > 50%NO<sub>3</sub><sup>-</sup> > 25%NO<sub>3</sub><sup>-</sup> > 0%NO<sub>3</sub><sup>-</sup> (Figure 2e), demonstrating that cucumber yield increased as the proportion of NO<sub>3</sub><sup>-</sup>-N increased and was reduced as the proportion of NH<sub>4</sub><sup>+</sup>-N increased except N100A treatment.

### Leaf citrulline content

The result showed that citrulline content in the nitrate-amide ratio was higher than that in the nitrate-ammonium ratio of the equal-proportion treatment, which is similar to total nitrogen content of cucumber. Moreover, citrulline content increased as the proportion of CO(NH<sub>2</sub>)<sub>2</sub> increased, and it was reduced as the proportion of NO<sub>3</sub><sup>-</sup>-N increased in the NO<sub>3</sub><sup>-</sup>-N and CO(NH<sub>2</sub>)<sub>2</sub> treatments (Figure 2f).

### Effect of different nitrogen sources on the nitrogen utilization efficiency and root-shoot ratio in cucumber

The N75C treatment makes the most of nitrogen utilization efficiency, and the N0A treatment exhibited the lowest nitrogen utilization efficiency. As the proportion of NO<sub>3</sub><sup>-</sup>-N changed, nitrogen utilization efficiency was in 75%NO<sub>3</sub><sup>-</sup> > 100%NO<sub>3</sub><sup>-</sup> > 50%NO<sub>3</sub><sup>-</sup> > 25%NO<sub>3</sub><sup>-</sup> > 0%NO<sub>3</sub><sup>-</sup> in NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N treatments. Moreover, there was lower nitrogen utilization efficiency in treatments with



**Figure 2.** Effect of different nitrogen forms on the index of cucumber growth.  $\text{NO}_3^- + \text{NH}_4^+$ :  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N treatments;  $\text{NO}_3^- + \text{NH}_2$ :  $\text{NH}_4^+$ -N and  $\text{CO}(\text{NH}_2)_2$  treatments. The slash represents the trend line. a. Effect of nitrogen forms on total dry matter; b. Effect of nitrogen forms on total nitrogen; c. Effect of nitrogen forms on nitrogen utilization efficiency; d. Effect of nitrogen forms on root-shoot ratio; e. Effect of nitrogen forms on cucumber yield; f. Effect of nitrogen forms on citrulline content. Bars represent the standard error. Different letters above the bars indicate significant differences at  $P < 0.05$  according to Tukey's multiple comparison test.

added  $\text{NH}_4^+$ -N than in treatments with added  $\text{CO}(\text{NH}_2)_2$  with equal proportions of  $\text{NO}_3^-$ -N (Figure 2c).

Contrary to nitrogen utilization efficiency, the root-shoot ratio was higher in the equal-proportion of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N treatment than that in the equal-proportion of  $\text{NO}_3^-$ -N and  $\text{CO}(\text{NH}_2)_2$  treatment, which was shown in  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N treatments promoting the growth of roots, but the  $\text{NO}_3^-$ -N and  $\text{CO}(\text{NH}_2)_2$  treatments could promote the growth of shoots of cucumbers (Figure 2d).

### Correlations among nitrogen form, dry matter, nitrogen content, root-shoot ratio and yield of cucumber

Data presented in Table 4, the ratio of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N was significantly correlated with total dry matter, total

nitrogen content, nitrogen utilization efficiency and yield of cucumber; their correlation coefficients were 0.919, 0.930, 0.961 and 0.943, respectively. In line with their relationships, dry matter, nitrogen utilization and yield characteristics of cucumber could be regulated by changing the proportion of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N.

There was also a significant positive correlation between the ratio of  $\text{NO}_3^-$ -N and  $\text{CO}(\text{NH}_2)_2$  and the root-shoot ratio ( $r = 0.934$ ), indicating that the ratio of  $\text{NO}_3^-$ -N and  $\text{CO}(\text{NH}_2)_2$  had a close relationship with the root-shoot ratio under the same nitrogen supply; thus, balanced root and shoot growth can be adjusted using the nitrogen supply's proportion of  $\text{NO}_3^-$ -N and  $\text{CO}(\text{NH}_2)_2$ .

Moreover, there was a significant positive correlation between leaf nitrogen content and yield ( $r = 0.877$ ), revealing that cucumber yield is inseparable from its leaf nitrogen content.

**Table 4.** Correlation between different parameters

r	Total dry matter	Total N content	Yield	NUE	Citrulline content	Root/shoot ratio	Fruit dry matter	Leaf dry matter
NO <sub>3</sub> <sup>-</sup> -N/NH <sub>4</sub> <sup>+</sup> -N	0.919*	0.930*	0.943*	0.961**	0.375	-0.309	0.941*	0.879*
NO <sub>3</sub> <sup>-</sup> -N/CO(NH <sub>2</sub> ) <sub>2</sub>	0.623	0.322	0.146	0.318	0.989**	0.934*	0.147	0.969*
Leaf N content	-	-	0.877**	-	-	-	-	0.931**

NO<sub>3</sub><sup>-</sup>-N/NH<sub>4</sub><sup>+</sup>-N represents the percentage (%) of NO<sub>3</sub><sup>-</sup>-N accounts for nitrogen forms composition in five NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N treatments respectively. Similarly, NO<sub>3</sub><sup>-</sup>-N/CO(NH<sub>2</sub>)<sub>2</sub> represents the percentage (%) of NO<sub>3</sub><sup>-</sup>-N accounts for nitrogen forms composition in five NO<sub>3</sub><sup>-</sup>-N and CO(NH<sub>2</sub>)<sub>2</sub> treatments respectively. For example, the value of NO<sub>3</sub><sup>-</sup>-N/NH<sub>4</sub><sup>+</sup>-N is 75% in the 75%NO<sub>3</sub><sup>-</sup>+25%NH<sub>4</sub><sup>+</sup>(N75A) treatment. There are different values of NO<sub>3</sub><sup>-</sup>-N/NH<sub>4</sub><sup>+</sup>-N in different treatments, which was made correlation analysis with other index.

\* Indicates significance at the 0.05 probability levels.

\*\*Indicate significance at the 0.01 probability levels.

## DISCUSSION

### Cucumber growth response to nitrate-ammonium ratio

The ratios of nitrogen forms play an important role in growth regulation, material distribution, yield and quality improvement in cucumber (Dong *et al.*, 2017). In this study, the yield of cucumber was enhanced as the proportion of nitrate increased in the ammonium and nitrate treatments except 100% nitrate. The results were consistent with the study on spinach by Wang *et al.* (2007), but contrary to the study on chives by Lu *et al.* (2009). Furthermore, the same trend as yield was found in dry matter, total nitrogen content and nitrogen utilization efficiency of cucumber in this experiment. The reason may be due to that NO<sub>3</sub><sup>-</sup>-N is the most effective nitrogen form for cucumber growth (Gangwar and Singh, 2011). These findings are also consistent with a study on the effects of N forms on dry matter distribution in muskmelon (Xu *et al.*, 2005).

Although cucumber prefers nitrate nitrogen to ammonium nitrogen (Dong *et al.*, 2017), the experiment showed the 100%NO<sub>3</sub><sup>-</sup> treatment had not exhibited the maximum dry matter, nitrogen content and yield. The reason can be explained from other scholars' findings. It was reported that excessive energy was used for the transfer of NO<sub>3</sub><sup>-</sup>-N or NH<sub>4</sub><sup>+</sup>-N when the proportion of one form was too high in mixed nitrogen forms, to result in the reduction of carbohydrate synthesis or maladjustment of hormone imbalance in plants, which reduced nitrogen assimilation capacity furtherly. This affected crop yield (Tabatabaei *et al.*, 2008; Walch and Engels, 2000). Our findings are consistent with previous reports about ammonium nitrate ratio, such as cucumber (Zhang *et al.*, 2016), muskmelon (Xu *et al.*, 2005), Chinese cabbage (Hu *et al.*, 2015) and cauliflower (Liu *et al.*, 2013).

The experiment found that both 100 and 75% ammonium nitrogen treatments resulted in significantly lower dry matter contents than other nitrogen forms in different cucumber organs, which resulted in leaf wilting and stunted growth. The reason may be due that too much ammonium nitrogen can inhibit the absorption of K<sup>+</sup>

and Ca<sup>2+</sup> through ammonia toxicity occurrence (Cao *et al.*, 2009; Botella *et al.*, 1997), which was similar to the ammonia harm in other studies on nitrogen utilization of cucumbers (Roosta *et al.*, 2007; Zhang *et al.*, 2016).

### Cucumber growth response to nitrate-amide ratio

There are fewer reports about the effect of amide nitrogen on cucumber growth. We found innovatively that the treatment of 75%NO<sub>3</sub><sup>-</sup>-N + 25%CO(NH<sub>2</sub>)<sub>2</sub> had the highest dry matter, nitrogen content, nitrogen utilization efficient and yield of cucumber, which proved that the addition of amide nitrogen could promote the absorption and utilization of nitrate nitrogen. Furthermore, we also found that dry matter and nitrogen utilization efficiency in the nitrate-amide ratio were higher than those in the nitrate-ammonium ratio of the equal-proportion treatment, which may be due to the multi-pathway of amide metabolism. Amide nitrogen metabolism mainly has urease pathway and amino acid pathway. In the urease pathway, amide is decomposed into NH<sub>3</sub> and CO<sub>2</sub> and then enters into the GS/GOGAT pathway, which is the same as ammonium assimilation (Cao *et al.*, 2009; Solomon *et al.*, 2010). The amino acid metabolic pathway is completely different from the ammonium and nitrate metabolism pathways, in which amide is not decomposed to NH<sub>3</sub> but is assimilated directly into amino acids. Firstly, CO(NH<sub>2</sub>)<sub>2</sub> is transferred into the cell by transport molecules and is combined with phosphate groups, then they are synthesized into citrulline. After that, citrulline goes into the urea cycle and synthesizes the amino acids that plants need. Thus, citrulline content reflects the activity of amide nitrogen metabolism in amino acid pathway (Liao and Yan, 2003). We found that citrulline content in the nitrate-amide ratio was higher than that in the nitrate-ammonium ratio of the equal-proportion treatment in the experiment, which is similar to total nitrogen content and nitrogen utilization efficiency of cucumber. It could be concluded amino acid pathway promoted amide nitrogen metabolism in NO<sub>3</sub><sup>-</sup>-N and CO(NH<sub>2</sub>)<sub>2</sub> treatments, furtherly improved dry matter and nitrogen utilization efficiency, resulting in the higher yield



with treatments of  $\text{NO}_3^-$ -N and  $\text{CO}(\text{NH}_2)_2$ .

### Correlation between nitrogen forms and cucumber growth

The relationship between nitrogen forms and cucumber growth was revealed furtherly by correlation analysis in the experiment. The ratio of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N has significant positive correlations with total dry matter, total nitrogen content, nitrogen utilization efficiency and yield. On the other hand, the ratio of  $\text{NO}_3^-$ -N and  $\text{CO}(\text{NH}_2)_2$  has a significant positive correlation with the root-shoot ratio. These correlations indicated that different nitrogen forms influenced nitrogen utilization, thereby regulating the growth process of cucumber (Lange *et al.*, 2013). Therefore, dry matter and nitrogen utilization in cucumber can be regulated by changing the proportion of nitrate nitrogen and ammonium nitrogen, and the root-shoot ratio can be adjusted through the proportion of nitrate nitrogen and amide nitrogen. Besides, according to the significant positive correlation between the leaf nitrogen content and yield in cucumber, leaf nitrogen content could be considered an important reference element for estimating cucumber yield.

### CONCLUSIONS

The aim of the study was to analyze the impact of different nitrogen sources on growth of cucumber. The results indicated that dry matter, nitrogen content and nitrogen utilization efficiency in treatments with added amide were higher than in treatments with added ammonium with equal proportions of nitrate. Treatments of 75% $\text{NO}_3^-$ -N + 25%  $\text{CO}(\text{NH}_2)_2$  can be the first recommended ratio of nitrogen, which has the highest yield, dry matter, nitrogen content and nitrogen utilization efficiency of cucumber in the experiment. Meanwhile, correlation analysis further showed that total dry matter, total nitrogen content and nitrogen utilization efficiency were significantly positively related respectively to the proportion nitrate and ammonium, as were as between the root-shoot ratio and nitrate-amide ratio. Additionally, there was a remarkably positive relationship between leaf nitrogen content and yield. Therefore, dry matter distribution, yield and root cap index may be regulated by the ratio of nitrogen forms; moreover, cucumber yield is inseparable from its leaf nitrogen content.

### ACKNOWLEDGEMENTS

This work was supported by Youth Fund of Guizhou Academy of Agricultural Science [2018(84)] by the National Natural Science Foundation of China [31760596].

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