



## Changes of glutamine and asparagine content in cucumber seedlings in response to nitrate stress

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### Abstract

Nitrogen fertilizer application rates in intensive agricultural systems have increased dramatically in recent years, especially in protected vegetable production systems. This excessive use of nitrogen fertilizer has resulted in soil secondary salinity, which has become a significant environmental stress for crops such as cucumber, in the protected farmlands. It is thus necessary to illuminate how crops respond to nitrate stress. The objective of this work was to investigate the influence of three nitrate levels of 14 (CK), 56 (T-1), and 140 (T-2) mmol L<sup>-1</sup> on glutamine and asparagine content in the roots, stems, and leaves of cucumber (*Cucumis sativus* L. cv. Xintaimici) seedlings grown in hydroponic culture. The results showed that glutamine content in the roots, stems, and leaves of T-1 was a little higher than CK. In the roots, stems and leaves of T-2, glutamine content dramatically decreased over treatment course, and at 12 d decreased by 76%, 46% and 68%, respectively, with respect to CK. Asparagine content in the roots, stems, and leaves of T-1 was not significantly different from CK, whereas, in the roots, stems, and leaves of T-2 seedlings asparagine content decreased significantly. At 12 d, asparagine content in the roots, stems, and leaves of T-2 decreased by 78%, 60% and 74%, respectively, with respect to CK. This evidence indicates that the underlying mechanism of nitrate stress might be different from other abiotic ones such as salt stress and drought, which often stimulate the synthesis of amides.

**Keywords:** Asparagine; Cucumber; Glutamine; Hydroponic culture; Nitrate.

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### Introduction

Nitrogen is needed in large amounts by plants because it is a constituent of macromolecules such as proteins. However, the fact is that often less than 50% of nitrogen fertilizer applied to crops ultimately may be utilized by crops because nitrate is highly mobile and is not adsorbed by soil colloids (Allison, 1966). To satisfy the nitrogen demand, agriculturists often add nitrogen in large quantities to maintain adequate levels in the rhizosphere (Zhu et al., 2005). This excessive use of nitrogen fertilizers has resulted in

undesirable conditions such as the accumulation of nitrate in plants and soil. The large accumulation of nitrogen in the soil, on the one hand, has contaminated the ground water (Barker and Mills, 1980), and on the other hand has resulted in soil secondary salinity in the protected farmlands because of a lack of leaching by rainfall and strong evaporation of soil water (Darwish et al., 2005; Kitamura et al., 2006). Secondary salinity is considered to be one of the major factors that limit sustainable development of agricultural production in the greenhouse (Darwish et al., 2002; Yu et al., 2005). According to the previous studies (Yu et al., 2005; Ju et al., 2007), accumulation of ions in protected farmland is greatly different from ordinary soil salinity. In protected farmlands, the main cation and anion are  $\text{Ca}^{2+}$  and  $\text{NO}_3^-$ , respectively, while  $\text{Na}^+$  and  $\text{Cl}^-$  are the main forms of ions in ordinary soil salinity.

In the past several years, lots of researches about salt stress in plants have been done, but most of them have been focused on NaCl effects (Zhu, 2002; Karimi et al., 2009; Cha-um et al., 2010). So far there have been few investigations about nitrate stress to horticultural crops. Cucumber is one of the most important horticultural crops. It has been reported that excessive accumulation of nitrate widely inhibited the growth and development of cucumber in the protected farmlands of China (Yang et al., 2009), while the underlying mechanism has not been well understood.

The amides glutamine and asparagine serve as the major nitrogen transport compounds in higher plants (Urquhart and Joy, 1981). Nitrogen assimilated into glutamine may be converted into asparagine, which plays a prominent role in nitrogen transport and storage in plants because of its high nitrogen:carbon ratio and its stability (Sieciechowicz et al., 1988). High concentrations of asparagine can also be found in various plant tissues under stress conditions such as mineral deficiencies, salt stress, and drought (Moller et al., 2003). However, it is still unclear how glutamine and asparagine content in cucumber seedlings respond to nitrate stress.

The objective of this work was to examine the effects of nitrate stress on glutamine and asparagine content in cucumber seedlings. Glutamine and asparagine content in the roots, stems, and leaves of cucumber seedlings were investigated under nitrate stress in hydroponic culture.

## Materials and Methods

### *Plant growth conditions and experimental design*

Cucumber (*Cucumis sativus* L. cv. Xintaimici) seeds were sterilized with sodium hypochlorite containing 5% active HClO for 5 min, washed 5 times with deionized water, and thereafter soaked for 12 h in deionized water. The soaked seeds were raised in well-washed quartz sand and irrigated with tap water. The experiments were carried out in the greenhouse of Shandong Agricultural University from March to June in 2007. When plants had one fully expanded leaf, they were removed from the trays, well washed with tap water, and then transplanted into hydroponic boxes (40×30×12 cm, 8 plants per box) containing a complete cucumber nutrient solution (Guo, 2004). The solution was continually aerated with an electric pump and completely renewed every four days. When the seedlings developed three fully expanded leaves, excess nitrate was dissolved in nutrient solution directly. The experiments were carried out in a completely randomized design with a split plot arrangement of three replications, providing 8 plants per replication. Three treatments were applied (Table 1):

(CK) complete nutrient solution (control);  
 (T-1) complete nutrient solution +  $\text{Ca}(\text{NO}_3)_2$  10.5  $\text{mmol}\cdot\text{L}^{-1}$  +  $\text{KNO}_3$  21  $\text{mmol}\cdot\text{L}^{-1}$ ;  
 (T-2) complete nutrient solution +  $\text{Ca}(\text{NO}_3)_2$  31.5  $\text{mmol}\cdot\text{L}^{-1}$  +  $\text{KNO}_3$  63  $\text{mmol}\cdot\text{L}^{-1}$ .

Table 1. Nitrate concentration and osmotic potential of nutrient solutions.

Treatment	$\text{Ca}(\text{NO}_3)_2$ ( $\text{mmol L}^{-1}$ )	$\text{KNO}_3$ ( $\text{mmol L}^{-1}$ )	$\text{NO}_3^-$ ( $\text{mmol L}^{-1}$ )	Osmotic potential before treatment (MPa)	Osmotic potential after treatment for 3 d (MPa)
CK	3.5	7	14	-0.256	-0.218
T-1	14	28	56	-0.341	-0.315
T-2	35	70	140	-0.570	-0.567

At 0, 1, 2, 4, 6, 8, and 12 d after treatment, the second and third fully expanded leaves counted from the top, main stems, and lateral roots of cucumber seedlings were sampled and measured for glutamine and asparagine content.

#### Determination of glutamine and asparagine content

Oven-dried samples (0.5 g) were put in a mortar and ground to a fine powder. The powder was transferred to 100 ml 2% (W/V) trichloroacetic acid and stirred for 1 h on a shaking table at 100  $\text{r min}^{-1}$  at room temperature. The mixture was filtered into a 100-ml beaker.

The extract (2 ml) was used for the determination of ammonia concentration before boiling according to the method described by Tang (1999). The reaction solution included 2 ml ammonia extract, 3 ml ninhydrin reagent solution, and 0.1 ml 1% (W/V) ascorbic acid. The mixture was well stirred and boiled for 15 min. Reagent blank was incubation mixture in which the ammonia extraction was replaced by deionized water. After cooling to room temperature in a cold water bath, the reaction solution was made to 5 ml with alcohol and well stirred. The absorbance was recorded with spectrophotometer (Shimadzu 160A, Japan) at 580 nm.

Another 50 ml of the extract was transferred into 100-ml kjeldahl flask with 2 ml 98% sulphuric acid and boiled on reflux exchanger for 3 h. Ammonia concentration after boiling was determined with the same method above as well. Glutamine and asparagine content was calculated by the equations as follows:

$$C_{\text{Gln}} = 10.42 \times (C_{\text{NH}_3'} - C_{\text{NH}_3}) \times V \div \text{DW},$$

$$C_{\text{Asn}} = 9.42 \times (C_{\text{NH}_3'} - C_{\text{NH}_3}) \times V \div \text{DW},$$

where  $C_{\text{NH}_3}$  and  $C_{\text{NH}_3}'$  represent ammonia concentration in the extract before and after boiling, respectively; V represents volume of the extract; DW represents dry weight of the sample (Tang, 1999).

#### Statistical Analysis

Data were analyzed with OriginPro8 (Version8E, OriginLab Corporation, Massachusetts, USA) and presented as means of three replicates  $\pm$  standard errors.

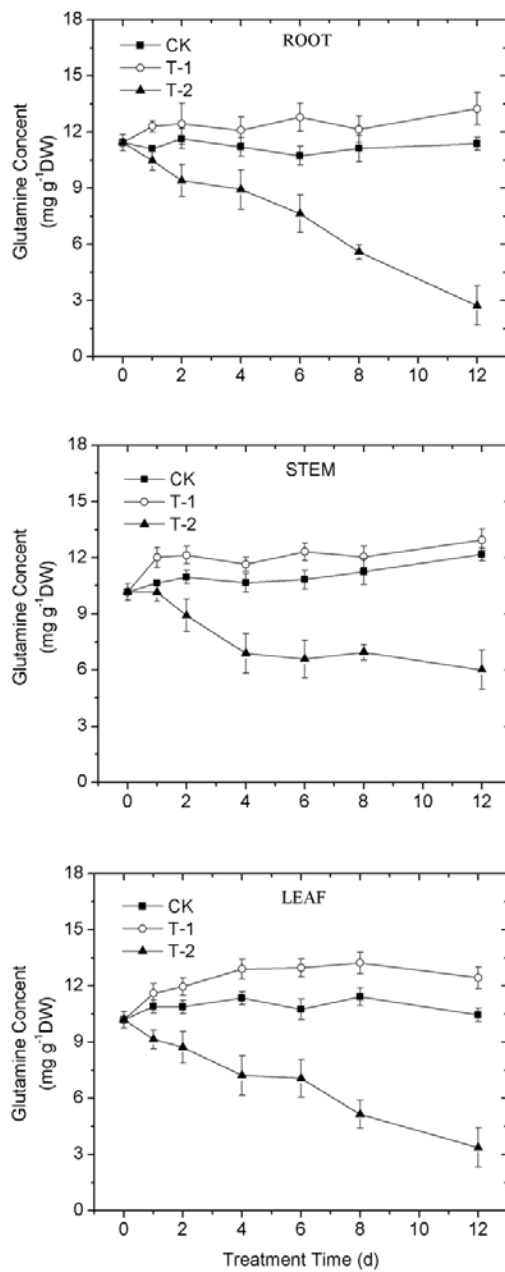


Figure 1. Changes of glutamine content in the roots, stems and leaves of cucumber seedlings under nitrate stress. Plants were grown in nutrient solutions containing 14 (CK), 56 (T-1), and 140 mmol L<sup>-1</sup> (T-2) nitrate, respectively, during 12 d. Vertical bars represent the standard errors (n=3).

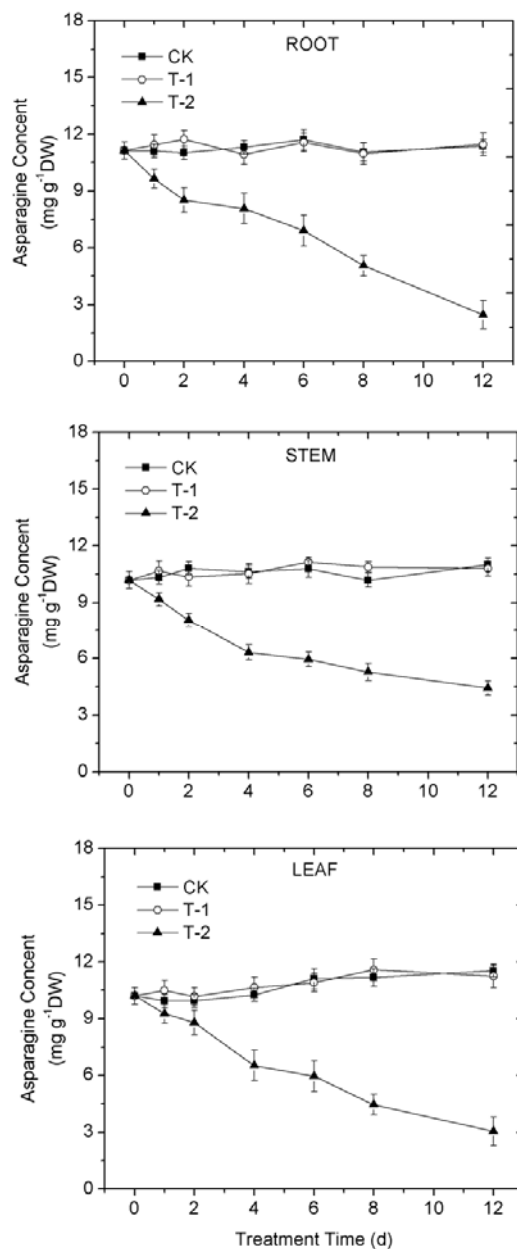


Figure 2. Changes of asparagine content in the roots, stems, and leaves of cucumber seedlings under nitrate stress. Plants were grown in nutrient solutions containing 14 (CK), 56 (T-1), and 140 (T-2) mmol L<sup>-1</sup> nitrate, respectively, during 12 d. Vertical bars represent the standard errors (n=3).

## Results and Discussion

Figures 1 and 2 showed changes of glutamine and asparagine contents in the roots, stems, and leaves of cucumber seedlings under three nitrate levels. Glutamine content in the roots, stems, and leaves of T-1 was a little higher than CK across all treatments. In the roots, stems, and leaves of T-2 seedlings, glutamine content dramatically decreased. At 12<sup>th</sup> d, glutamine content in the roots, stems, and leaves of T-2 decreased by 76%, 46% and 68%, respectively, with respect to CK. Asparagine content in the roots, stems, and leaves of T-1 seedlings had no significant differences from CK over the treatment course. In the roots, stems and leaves of T-2 seedlings, asparagine content decreased significantly with the increase of the treatment time. At 12 d, asparagine content in the roots, stems, and leaves of T-2 decreased by 78%, 60% and 74%, respectively, with respect to CK.

Unfavorable growth conditions can reduce the photosynthetic capacity of plants, resulting in a decreased carbon availability and an enhanced protein degradation, which lead to internal ammonia accumulation (James et al., 1993). The synthesis of amides, especially asparagine, is stimulated by some environmental stresses (Rabe, 1990; Chaffei et al., 2004). Accumulation of asparagine in response to abiotic stresses could be an ammonia detoxification mechanism and a way to store nitrogen when protein synthesis is inhibited by stress conditions (Herrera-Rodríguez et al., 2007).

However, high nitrate stress resulted in severe reduction of glutamine and asparagine content in cucumber seedlings (Figures 1 and 2), indicating a different mechanism might be involved in nitrate stress. Yang et al. (2010) has reported that high nitrate stress caused significant reduction of glutamine synthetase, glutamate synthase, and glutamate dehydrogenase activities. Glutamine synthetase is a principal enzyme responsible for catalyzing the incorporation of ammonia into glutamate, producing glutamine. The reduction of glutamine synthetase activity, on the one hand, may result in significant accumulation of toxic ammonia (Yang et al., 2010), and on the other hand may cause severe reduction of glutamine content in cucumber seedlings treated with 140 mmol L<sup>-1</sup> of nitrate (Figure 1). The reduction of asparagine content in the seedlings treated with 140 mmol L<sup>-1</sup> of nitrate (Figure 2) may be caused by inhibited synthesis of glutamine because synthesis of glutamine is the essential step for the synthesis of asparagine (Mayumi et al., 2005).

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