

Influence of Different $\text{NO}_3^-/\text{NH}_4^+$ on Nitrate and Ammonium Uptake Kinetics of Sugar Beet (*Beta vulgaris* L.) Seedlings

Duoying Zhang¹, Fengming Ma¹, Yue Zhao¹, Caifeng Li²

(1. Northeast Agricultural University, Harbin, Heilongjiang 150030, China;

2. Institute of Heilongjiang Academy of Agricultural Sciences, Harbin, Heilongjiang 150030, China)

Abstract: Solution culture experiments were carried out to study the kinetics at cotyledon stage (11-day-old) and the effect of different $\text{NO}_3^-/\text{NH}_4^+$ on NO_3^- and NH_4^+ uptake at seedling stage (31-day-old) with two cultivars of sugar beet, including Tianyan7 and Tianyan8. NO_3^- uptake by sugar beet seedlings at cotyledon stage (11-day-old) reached equilibration after 2 hours of adaptation, and NH_4^+ uptake reached equilibration after 6 hours of adaptation. K_m values of NH_4^+ uptake by Tianyan7 were lower than Tianyan8, and V_{max} values were higher. It was benefit for Tianyan7 to uptake NH_4^+ . The kinetics of NO_3^- and NH_4^+ uptake by the cultivars changed after cultivated in nutrient solution contained different $\text{NO}_3^-/\text{NH}_4^+$ for 20 days. NO_3^- uptake by sugar beet was stimulated by lower concentration of NH_4^+ in the nutrient solution. The NH_4^+ uptake by sugar beet changed complicatedly. Even cultivated in nutrient solution contained pure NH_4^+ , the uptake ability of Tianyan7 was higher. Above all, it showed that NH_4^+ uptake of Tianyan7 was higher than Tianyan8, and when $\text{NO}_3^-/\text{NH}_4^+$ was 1:4 it reached the highest. This experiment provided a theoretical basis to realize the highly effective ammonium assimilation for sugar beet through the experiment. [Nature and Science. 2004;2(3):70-78].

Key words: sugar beet; nitrate; ammonium; uptake kinetics

1 Introduction

Nitrogen (N) is one of the most requisites of all mineral elements and the main restriction factor demanded by higher plants. Nitrogen supply is also critical for crop productivity. Several studies have demonstrated that N fertilization improves grain yield through improved water use efficiently (Shepherd 1987; Palta and Fillery 1995). In contrast, other experiments have reported that N fertilization reduces grain yield. A supra optimal N supply stimulates vigorous, early vegetative growth, resulting in the premature depletion of available soil moisture and reduced grain filling (Cantero-Martinez 1995; Papastyliou 1995; Cirbeeks 1999). In soil, N is available to higher plant in three major forms: NO_3^- , NH_4^+ , and amino acid N. Traditionally, only NO_3^- and NH_4^+ are assumed to be important plant N sources (Nordin 2001). Supra-optima NO_3^- -N in soil improves the product of sugar beet, but makes the attribute and percentage of sugar decrease and leads to lower the utilization rate of nitrogenous fertilizer. Eluviations of NO_3^- aggravate the environment pollution. All

these have become a serious problem in beet sugar industry's progress in China. Since 1993 when Hirel put forward that nitrogen uptake by plant could be improved by the means of gene manipulation, men had paid more attention to the question of high ammonium assimilation. Highly effective ammonium assimilation is the ability of which plants absorb and assimilate ammonium from soil directly (Wu 2001). It can promote the plant salt resistance and cut down the consumption of energy and carbohydrate caused by nitrate reductase and nitrite reductase that are correlated to glycolysis and photorespiration during the course of NO_3^- transformed to NH_4^+ . Most recent studies were concentrated on the effect of NH_4^+ on physiological index and production and quality of sugar beet in different growth periods (Ma and Gao 1996; Ma 1996; Li 2003; Zhang 2001; Zhao 2003), but there was few report about NO_3^- and NH_4^+ uptake by plant seedlings.

In research of absorption character of sugar beet seedlings at cotyledon stage, Ma (1996) found out that the seedlings began to consistently absorb NO_3^- after 2 hours of adaptation. He pointed out

that NO_3^- uptake by seedlings at cotyledon stage constituted unidirectional continuous system through xylem translocation, and kinetic parameters V_{max} and K_m of NO_3^- uptake by different cultivars were obviously different. Despite the potential benefits of NO_3^- -N for the growth of higher plants, especially under anaerobic conditions (Malavolta 1954; Bertani 1986), NH_4^+ is predominant and most readily bioavailable N form in dry soil (Yu 1985). However, compared with the extensive investigations NO_3^- uptake, the kinetics and energetics of NH_4^+ uptake in higher plants have received relatively little attention. Up till now, there was no available report about the changes in NO_3^- and NH_4^+ uptake by sugar beet seedlings after cultivated under the condition contained different $\text{NO}_3^-/\text{NH}_4^+$. The present study tested the kinetics parameters of NO_3^- and NH_4^+ uptake by 11-day-old sugar beet seedlings and the changes in the parameters uptake by 31-day-old sugar beet seedlings after cultivated in the nutrient solution contained different $\text{NO}_3^-/\text{NH}_4^+$. The object of this study was to investigate physiological characteristics of nitrate and ammonium uptake by sugar beet and to provide a theoretical basis to realize highly effective ammonium assimilation for sugar beet through the experiment.

2 Material and Methods

2.1 Plant material

Two sugar beet (*Beta vulgaris* L.) cultivars (Tianyan7 and Tianyan8) of diploid inbred strains and leading cultivars in Heilongjiang Province of China were obtained from Sugar Beet Research Institute, Hulan, Heilongjiang University, China.

2.2 Preparation of seedlings at cotyledon stage

The seeds were surface sterilized in 1% NaOCl solution for 30 min and rinsed several times with deionized water. Seeds were cultivated in clean quartz sand and washed with deionized water in dark condition at 20°C. After 10 days roots of seedlings were washed and the regular seedlings were cultivated in culture flask contained 0.2 mmol/L CaSO_4 and put in Conviron environment controlled chamber where growth conditions were maintained as follows: temperature $20 \pm 2^\circ\text{C}$; RH 75% and flux density $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-2}$. After 24 hours, the seedlings at cotyledon stage (11-day-old seedlings) were used to test relationship between the nitrate and ammonium uptake by seedlings and time, and uptake kinetic parameters of NO_3^- and NH_4^+ .

2.3 Preparation of seedlings at seedling stage

The regular seedlings (10-day-old) were held in lids of 20 L porcelain vats and 20 plants per vat. Cell foam collars, which excluded light from the roots and nutrient solution, were opened. The aerated nutrient solution (Hoagland nutrient solution, adapted from Shanqi and Liu, 1989) was modified. The modification was as follows: KNO_3 was replaced by $\text{Ca}(\text{NO}_3)_2$ and NH_4NO_3 was by $(\text{NH}_4)_2\text{SO}_4$. With constant total nitrogen content (4 mmol/L), ratio of $\text{NO}_3^-/\text{NH}_4^+$ was 4:0, 4:1, 3:1, 1:1, 1:3, 1:4 and 0:4 respectively. Nutrient solution was changed every 7 days. Plants were grown in a temperature controlled glasshouse (20°C day time and 14°C night time). Plants were grown in solution culture for 20 days. On the day prior to uptake measurements, the regular seedlings (30-day-old) were cultivated in culture flask contained 0.2 mmol/L CaSO_4 and put in Conviron environment controlled chamber where growth conditions were maintained as follows: temperature $20 \pm 2^\circ\text{C}$; RH 75% and flux density $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-2}$. After 24 hours, the seedlings at seedling stage (31-day-old) were used to test the relationship between the nitrate and ammonium uptake by the seedlings and time, and NO_3^- and NH_4^+ uptake kinetic parameters.

2.4 The relationship between nitrate uptake and time

Every 3 prepared 11-day-old and 31-day-old seedlings were put into 30 ml 1 mmol/L KNO_3 solutions (pH6.5, 25°C). Uptake time was 1, 2, 3 and 4 hours respectively. Every uptake time was 3 repetitions. The check (CK) was 30 ml 1 mmol/L KNO_3 solution without absorptive seedlings. The nitrate contents were assayed with directly colorimetric method (Cheng 1995). 1.5 ml solution was diluted to 15 ml with distilled water. The absorbance of the solution was measured at 210 nm with Varian Cary 50 ultraviolet-visible spectrophotometer. The absorbed dose was NO_3^- reduced contents in the solution (KNO_3 contents in CK solution — KNO_3 contents in absorbed solution).

2.5 The relationship between ammonium uptake and time

Every 3 prepared 11-day-old and 31-day-old seedlings were put to 30 ml 5 mmol/L $(\text{NH}_4)_2\text{SO}_4$, and uptake time was 1, 2, 3, 4, 5, 6, 7 and 8 hours respectively. Every uptake time was 3 repetitions. The CK was 30 ml 5 mmol/L $(\text{NH}_4)_2\text{SO}_4$ solution

without absorptive seedlings. The ammonium contents were assayed with distillation method (Liu 1996). 25 ml solution with 2 g $MgCl_2$ was put in distilled tube, then distilled. Boric acid solution with 2 drops of indicator was under the condenser for absorption. When absorbed solution was 30~40 ml, it was titrated with hydrochloric acid standard solution. The absorbed dose was NH_4^+ reduced contents in the solution ($(NH_4)_2SO_4$ contents in CK solution — $(NH_4)_2SO_4$ contents in absorbed solution). The instrument was VELP azotometer.

2.6 Test of nitrate and ammonium uptake kinetic parameters

Nitrate and ammonium uptake kinetic parameters were assayed according to Yang (1991) with followed modification. Every 3 prepared seedlings (11-day-old or 31-day-old) was put to 30 ml 0.05, 0.1, 0.2, 1, 2, and 5 mmol/L KNO_3 or $(NH_4)_2SO_4$ solution respectively with 3 repetitions. The CK was 30 ml 0.05, 0.1, 0.2, 1, 2, and 5 mmol/L KNO_3 or $(NH_4)_2SO_4$ solution without absorptive seedlings, respectively. The uptake time was 4 hours for KNO_3 and 7 hours for $(NH_4)_2SO_4$. The absorbed dose was NO_3^- or NH_4^+ reduced contents in the solution. Kinetic parameters V_{max} and K_m were calculated with Eisenthal graphical analysis methods (Eisenthal and Athel 1974).

3 Results and Discussion

3.1 The relationship between nitrate or ammonium uptake and time

Sugar beet seedlings at cotyledon stage began

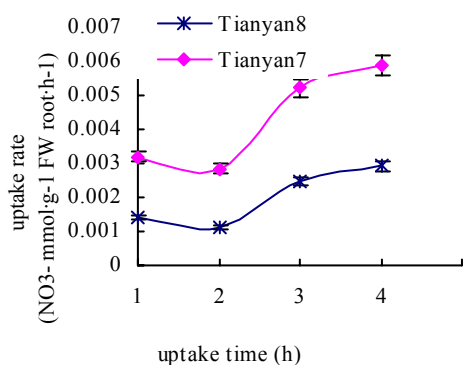


Figure 1. The relationship between absorption rate of NO_3^- uptake by sugar beet and time.

Sugar beet seedlings (11-day-old) were grown in 1 mmol/L KNO_3 solution. Uptake time was 1, 2, 3 and 4 hours respectively. Temperature was 25°C

to continuously absorb NO_3^- after 2 hours according to Ma (1996). In the present study, 11-day-old sugar beet seedlings began to uptake NO_3^- steadily after 3 hours (Figure 1). NH_4^+ uptake by sugar beet seedlings at cotyledon stage (11-day-old) was more complex than NO_3^- (Figure 2). It showed first decrease followed by a little increase and absorbed stably 6 hours later. NH_4^+ is absorbed reversing electrochemical gradient and blocked by metabolic inhibitor (Wu 2001). After 24 hours $CaSO_4$ starvation treated, NH_4^+ in the solution stimulated ammonium uptake by sugar beet, so the uptake rate in the first hour was significant high. Since NH_4^+ concentration of solution decreased, the seedlings adapted to environment step by step and NH_4^+ absorption caused pH around rhizoplane to decrease, stimulated extending of cell wall and built electrochemical gradient, the uptake rate increased then reached equilibration 6 hours later. The uptake rate of Tianyan8 was a little higher than that of Tianyan7.

As shown in Figure 1 and Figure 2, it was ascertained that the 11-day-old seedlings of sugar beet absorbed NO_3^- steadily after 3 hours of adaptation and absorbed NH_4^+ after 6 hours. The absorption curve of 31-day-old seedlings was similar to that of 11-day-old seedlings (Figures were not presented). So the absorbed time was 4 hours when test the NO_3^- uptake kinetics and 7 hours when test that of NH_4^+ .

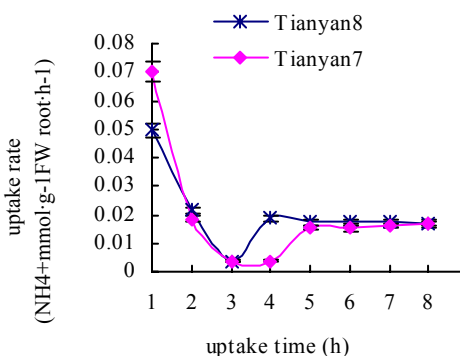


Figure 2. The relationship between absorption rate of NH_4^+ uptake by sugar beet and time.

Sugar beet seedlings (11-day-old) were grown in 5 mmol/L $(NH_4)_2SO_4$. Uptake time was 1, 2, 3, 4, 5, 6, 7 and 8 hours respectively. Temperature was 25°C

3.2 Nitrate and ammonium uptake kinetic parameters of 11-day-old sugar beet seedlings

The values of NO_3^- and NH_4^+ uptake rate of sugar beet conformed to Michaelis—Menten kinetics (Figure 3, Figure 4). NO_3^- uptake rate of Tianyan8 was a little higher than Tianyan7 (Figure 3), but NH_4^+ uptake rate by Tianyan7 was significant higher than Tianyan8 (Figure 4).

The reciprocal of K_m may represent the ion avidity to absorptive site of roots. The lower K_m values are, the higher the avidity. V_{max} values are useful to be used as an indicator of the maximum velocity of ion uptake. The higher V_{max} values are, the higher internal ion absorptive potentiality. They correlated with the amount of carriers in root cell

and carriers transport efficiency (Larsson 1989).

Values of V_{max} and K_m for NO_3^- uptake by Tianyan7 were almost similar to that of Tianyan8 in Table1. It showed that there was slight difference in uptake avidity and potentiality between Tianyan7 and Tianyan8. But there was significant difference in NH_4^+ uptake between these two cultivars. V_{max} value for NH_4^+ uptake by Tianyan7 was 13.317 mmol/L. That was higher than Tianyan8 (6.738 mmol/L). K_m value for Tianyan7 was 0.512 mmol/L, and lower than Tianyan8 (0.941 mmol/L). Statistical analyses revealed that NH_4^+ avidity absorptive potentiality of Tianyan7 was higher than that of Tianyan8. Maybe that was beneficial for Tianyan7 to uptake NH_4^+ .

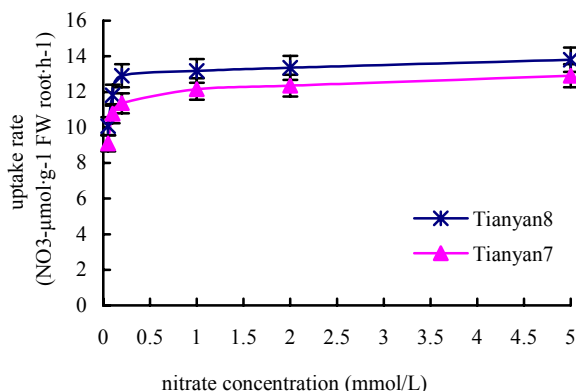


Figure 3. Absorption rate of NO_3^- uptake by sugar beet

Sugar beet seedlings (11-day-old) were grown at 0.05, 0.1, 0.2, 1, 2 and 5 mmol/L KNO_3 solution respectively.

Each data point is the mean of 3 replicates with SE values shown as vertical bars.

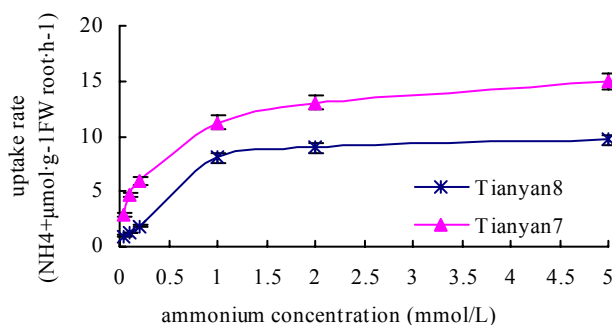


Figure 4. Absorption rate of NH_4^+ uptake by sugar beet

Sugar beet seedlings (11-day-old) were grown at 0.05, 0.1, 0.2, 1, 2 and 5 mmol/L $(\text{NH}_4)_2\text{SO}_4$ solution respectively.

Each data point is the mean of 3 replicates with SE values shown as vertical bars.

Table 1. Kinetic parameters of NO₃⁻, NH₄⁺ uptake by seedlings of different Sugar beet cultivars at cotyledon stage (11-day-old)

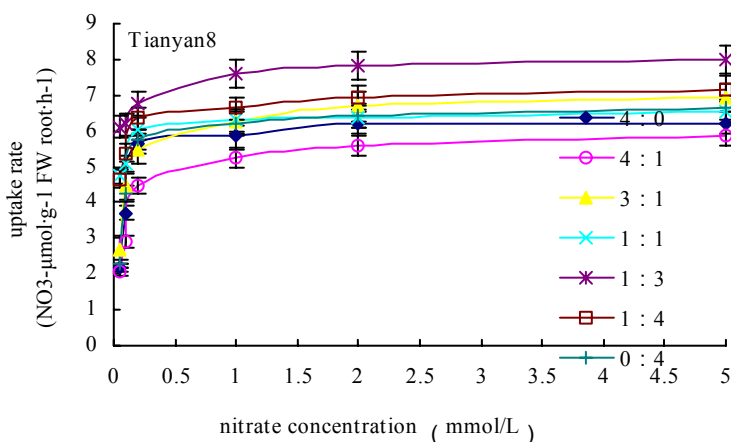
Cultivars	Kinetic parameters of NO ₃ ⁻ uptake		Kinetic parameters of NH ₄ ⁺ uptake	
	V _{max}	K _m	V _{max}	K _m
	(NO ₃ ⁻ μ mol • g ⁻¹ FW root • h ⁻¹)	(mmol/L)	(NH ₄ ⁺ μ mol • g ⁻¹ FW root • h ⁻¹)	(mmol/L)
Tiyanan7	12.601 ± 0.64	0.018 ± 0.04	13.317 ± 0.48	0.512 ± 0.07
Tiyanan8	13.698 ± 0.51	0.016 ± 0.03	6.738 ± 0.28	0.941 ± 0.02

Each datum is the average of 3 replicates. Values of K_m and V_{max} were obtained by Eisenthal transformation.

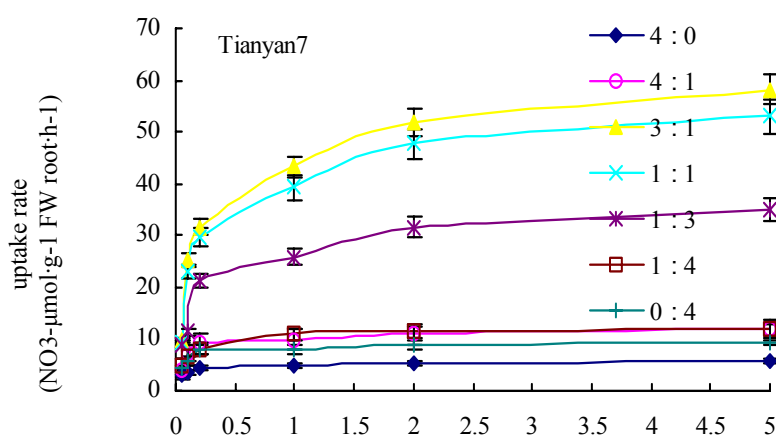
3.3 Influence of different NO₃⁻/NH₄⁺ on nitrate uptake kinetics of 31-day-old sugar beet seedlings

The values of NO₃⁻ uptake rate of sugar beet conformed to Michaelis—Menten kinetics (Figure 5). After cultivated in different NO₃⁻/NH₄⁺ nutrient solution, the difference in uptake rate by Tiyanan7 was as follows: 3:1 > 1:1 > 1:3 > 1:4, 4:1 > 0:4 > 4:0. The difference in Tiyanan8 was less than Tiyanan7, that was as follows: 1:3 > 1:4 > 3:1 > 0:4, 1:1 > 4:0 > 4:1. Supra optimal NO₃⁻ or NH₄⁺ concentration lowed nitrate uptake rate, so optimal NO₃⁻/NH₄⁺ in nutrient solution could enhance nitrate uptake rate. NO₃⁻ uptake kinetic parameters of 31-day-old sugar beet were changed significantly after cultivated in different NO₃⁻/NH₄⁺ nutrient solution for 20 days. Kinetics of Tiyanan8 changed less than that of Tiyanan7. V_{max} values for NO₃⁻ uptake by Tiyanan7 showed a first increase, and followed by a decrease along with the enhanced ammonium

concentration in nutrient solution. K_m values for NO₃⁻ uptake by Tiyanan7 changed complicatedly. Because K_m values for NO₃⁻ uptake by Tiyanan7 were significantly low and V_{max} values were high when NO₃⁻/NH₄⁺ was 3:1, avidity and absorptive potentiality of NO₃⁻ uptake were the highest in Tiyanan7. One explanation was that the existence of NH₄⁺ in nutrient solution promoted NO₃⁻ absorption by sugar beet made the values obviously dominant. Since the absorption has reached the saturation point, the avidity and absorptive potentiality of NO₃⁻ uptake decreased with the NH₄⁺ concentration in the nutrient solution enhanced. When NO₃⁻/NH₄⁺ was 1:3, the K_m values reached the highest. This was a critical value for NO₃⁻ uptake by Tiyanan7. K_m values decreased along with an increase of NH₄⁺ concentration continuously because the lack of NO₃⁻ stimulated its avidity, but limited the absorptive potentiality.



(a)



(b)

Figure 5. Absorption rate of NO₃⁻ uptake by Tianyan7 and Tianyan8 respectively

Sugar beet seedlings (31-day-old) were grown at 0.05, 0.1, 0.2, 1, 2 and 5 mmol/L KNO₃ solution respectively.

Each data point is the mean of 3 replicates with SE values shown as vertical bars.

V_{max} values were higher and K_m values were lower as NO₃⁻/NH₄⁺ was 1:4. This meant that avidity and absorptive potentiality of NO₃⁻ uptake by Tianyan8 was higher at this ratio. The existence of NH₄⁺ in the nutrient solution enhanced the NO₃⁻ uptake by Tianyan8. The Table 2 showed that K_m values of NO₃⁻ uptake by Tianyan8 was lower than Tianyan7 when NO₃⁻/NH₄⁺ was the same, which

meant that the NO₃⁻ avidity of Tianyan8 was higher than that of Tianyan7. The reason might be that the existence of NH₄⁺ in solution changed permeability of cell membrane of different sugar beet cultivars and verified the nitrate transform velocity. The present study revealed that Tianyan8 absorbed NO₃⁻ more effectively in the lower NO₃⁻ concentration solution than Tianyan7.

Table 2. Kinetic parameters of NO₃⁻ uptake by seedlings of different sugar beet cultivars at seedling stage (31-day-old) after cultivated in different NO₃⁻/NH₄⁺

NO ₃ ⁻ /NH ₄ ⁺ in nutrient solution	Kinetic parameters of NO ₃ ⁻ uptake by Tianyan7		Kinetic parameters of NO ₃ ⁻ uptake by Tianyan8	
	V _{max}	K _m	V _{max}	K _m
	(NO ₃ ⁻ μ mol · g ⁻¹ FW root · h ⁻¹)	(mmol/L)	(NO ₃ ⁻ μ mol · g ⁻¹ FW root · h ⁻¹)	(mmol/L)
4:0	5.158 g	2.011 d	6.166 e	0.0674 d
4:1	11.727 d	0.141 c	5.802 f	0.0109 a
3:1	57.124 a	0.035 a	6.734 c	0.0450 b
1:1	52.140 b	0.153 c	6.463 d	0.0592 c
1:3	32.148 c	5.069 e	7.956 a	0.0701 d
1:4	11.172 e	0.070 cd	6.887 b	0.0093 a
0:4	9.510 f	0.055 cd	6.505 d	0.0086 a

Values of K_m and V_{max} were obtained by Eisenthal transformation. Each datum in the Table is the average of three replications using SPSS analyses system. Different letters mean significant difference at p<0.05.

Many recent studies proposed that the existence of NH₄⁺ in solution inhibited NO₃⁻ uptake by king rice, but less affected nonglutinous rice and

played effective roles sometimes (Feng 2001; Feng 2003; Wang 2003). Youngdahl (1982) pointed out that NH₄⁺ strongly affected V_{max} values for NO₃⁻

uptake but less influenced Km values. But there was no report about the influence of different NO₃⁻/NH₄⁺ nutrition on higher plant uptake kinetics. In the present study, it showed that after 20 days ion absorption, low NH₄⁺ concentration increased Vmax values of NO₃⁻ uptake by sugar beet. Tianyan7 was more affected than Tianyan8. Vmax and Km values for NO₃⁻ uptake by sugar beet were both significantly improved after cultivated in different NO₃⁻/NH₄⁺ nutrient solution for 20 days. Tianyan7 was more influenced than Tianyan8. There are two reasons for NO₃⁻ uptake by sugar beet increased: firstly, promoted carrier on root cell membrane conjugated NO₃⁻ and enhanced the gene expression of synthetic nitrate carrier protein; second, the existence of NH₄⁺ changed membrane potential, membrane polarized degree and membrane structure.

3.4 Influence of different NO₃⁻/NH₄⁺ on ammonium uptake kinetics of 31-day-old sugar beet seedlings

The values of NH₄⁺ uptake rate of sugar beet conformed to Michaelis-Menten Kinetics (Figure 6). After cultivated in different NO₃⁻/NH₄⁺ nutrient solution, the difference in NH₄⁺ uptake rate by Tianyan7 was as follows: 1:3> 3:1> 1:1> 4:1> 4:0> 0:4> 1:4. The difference in Tianyan8 was different from Tianyan7, it was as follows: 1:3, 0:4> 3:1> 4:0> 1:4> 1:1> 0.4:0.5. All these confirmed that optimal NO₃⁻/NH₄⁺ in nutrient solution could

enhance nitrate uptake rate. Values of Vmax and Km of NH₄⁺ uptake by 31-day-old sugar beet seedlings changed irregularly after cultured in different NO₃⁻/NH₄⁺ concentration nutrient solution for 20 days (Table 3). When NO₃⁻/NH₄⁺ was 1:4, Km value of NH₄⁺ uptake by Tianyan7 was the lowest (0.312 mmol/L), and the Vmax value was the highest (3.54 μmol·g⁻¹FW root·h⁻¹). So the NH₄⁺ avidity was the highest, but absorptive potentiality was the lowest. When even seedling was cultivated in pure NH₄⁺ solution (NO₃⁻/NH₄⁺=0:4) for 20 days, Tianyan7 had higher NH₄⁺ avidity. For Tianyan8, the avidity was the highest after cultivated in pure NO₃⁻ solution (NO₃⁻/NH₄⁺=4:0) for 20 days. It showed firstly decrease followed by an increase along with the increased NH₄⁺ concentration in the nutrient solution. When NO₃⁻/NH₄⁺ was 1:3, Km of NH₄⁺ uptake by Tianyan8 was the highest. This meant that NH₄⁺ avidity uptake by Tianyan8 was the lowest but the absorptive potentiality was the highest. The avidity increased and absorptive potentiality decreased along with NH₄⁺ concentration in the nutrient solution enhanced. NH₄⁺ avidity uptake by Tianyan7 changed less than Tianyan8. Adequate evidence confirmed that Tianyan7 did more endurance to NH₄⁺ than Tianyan8.

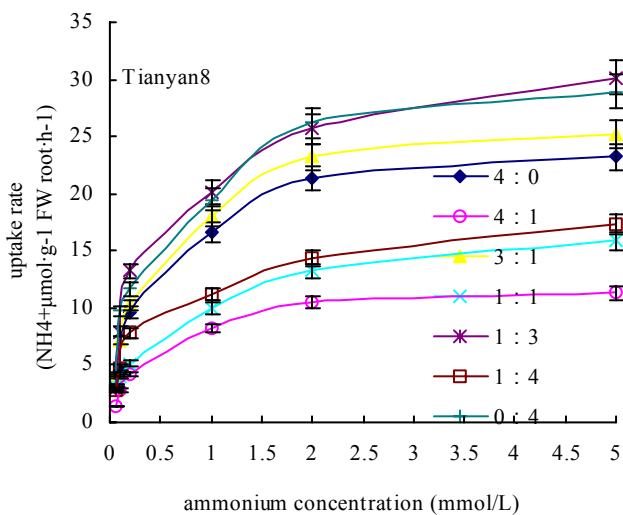


Figure 6. Absorption rate of NH₄⁺ uptake by sugar beet

Sugar beet seedlings (31-day-old) were grown at 0.05, 0.1, 0.2, 1, 2 and 5 mmol/L (NH₄)₂SO₄ solution respectively.

Each datum point is the mean of 3 replicates with SE values shown as vertical bars.

In present study, Vmax and Km values were influenced significantly after cultivated in different NO₃⁻/NH₄⁺ nutrient solution for 20 days. The

effects differed between two cultivars. High NH₄⁺ concentration in the nutrient solution enhanced NH₄⁺ uptake by Tianyan7 but inhibited Tianyan8.

This could be due to the difference of characteristic factors between these two cultivars and the increased nitrate absorption inhibited NH_4^+ uptake by Tianyan8 after different $\text{NO}_3^-/\text{NH}_4^+$ treatment.

NH_4^+ uptake reverses electrochemical gradient and is blocked by metabolic inhibitor, so it is a course of thermodynamic active absorption.

Table 3. Kinetic parameters of NH_4^+ uptake by seedlings of different sugar beet cultivars at seedling stage (31-day-old) after cultivated in different $\text{NO}_3^-/\text{NH}_4^+$

$\text{NO}_3^-/\text{NH}_4^+$ in nutrient solution	Kinetic parameters of NH_4^+ uptake by Tianyan7		Kinetic parameters of NH_4^+ uptake by Tianyan8	
	Vmax ($\text{NH}_4^+ \mu \text{ mol} \cdot \text{g}^{-1}\text{FW root} \cdot \text{h}^{-1}$)	Km (mmol/L)	Vmax ($\text{NH}_4^+ \mu \text{ mol} \cdot \text{g}^{-1}\text{FW root} \cdot \text{h}^{-1}$)	Km (mmol/L)
4:0	9.873 e	0.524 b	22.350 d	0.879 a
4:1	12.649 d	1.085 c	12.540 g	1.089 b
3:1	61.213 b	3.343 d	24.000 c	3.893 d
1:1	41.098 c	7.113 e	14.900 f	4.915 f
1:3	67.504 a	8.361 f	29.970 a	14.699 g
1:4	3.540 g	0.312 a	16.110 e	1.645 c
0:4	5.160 f	0.417 ab	27.830 b	4.182 e

Values of Km and Vmax were obtained by Eisenthal transformation. Each datum in table is the average of three replications using SPSS analyses system. Different letters mean significant difference at $p < 0.05$.

4 Conclusion

There were many discussions on the effect of NO_3^- -N and NH_4^+ -N on plant growth. Several studies have demonstrated that there were many advantages to use NH_4^+ -N as nitrogen source, such as: reduced environment pollution, enhanced iron uptake, cut down consumptions of proton (absorbing NH_4^+ directly by plants saves the energy which is needed for nitrate reduced). But most studies believed that the combination NO_3^- -N and NH_4^+ -N was better for plant growth than sole NO_3^- -N or sole NH_4^+ -N (Alfoldi and Pinter 1992; Bloom 1993). Up to now, many studies were on the effect of different $\text{NO}_3^-/\text{NH}_4^+$ nitrogen nutrition on NO_3^- uptake, assimilation and plant growth vigor, but little work on NH_4^+ uptake by plants. Our study showed that it was beneficial for Tianyan7 to absorb NO_3^- when $\text{NO}_3^-/\text{NH}_4^+$ was 3:1, and to absorb NH_4^+ when that was 1:4. When $\text{NO}_3^-/\text{NH}_4^+$ in nutrient solution was 1:4, it was beneficial for Tianyan8 to uptake NO_3^- ; and when that was 4:0, it was beneficial to uptake NH_4^+ . The values of Vmax of Tianyan7 were significantly higher than Tianyan8. All these indicated that high NH_4^+ contents as nitrogen source were good for Tianyan7

to uptake ammonium but not good for Tianyan8.

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Correspondence to:

Fengming Ma
Gongbin Road, Xiangfang District
Harbin, Heilongjiang 150030, China
Telephone: 01186-451-5519-0298
Cellular phone: 01186-451-5519-1012
E-mail: fengming_ma@sohu.com

Duoying Zhang
59 Wood Street, Gongbin Road, Xiangfang District
Harbin, Heilongjiang 150030, China
Telephone: 01186-451-5517-4385
Cellular phone: 01186-13845080402
E-mail: duo0314@yahoo.com.cn

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