Root Vigor and Kinetic Characteristics and Nitrogen Use Efficiencies of Different Potato (*Solanum tuberosum* L.) Cultivars

S. L. Zheng¹, H. Cheng¹, P. H. Li², and J. C. Yuan¹*

ABSTRACT

In the present study, the root vigor and kinetic characteristics of nitrogen absorption in potato cultivars was investigated through sand cultivation and pot tests with nutrient solution. Four cultivars of potatoes, namely, Feiwuruita, Yunshu301, Liangshu97, and Chuanyu56 were grown in nutrient solution matrix cultures. The result showed that: (1) Nitrogen uptake rates differed significantly among cultivars with different nitrogen efficiencies; (2) cultivars with High nitrogen-utilization-rate showed significantly higher root vigor, the average root vigor values of cultivars with the high nitrogen-utilization were 115% at seeding stage, 53% at tuber-bulking stage, and 18% at mature stage, respectively, higher than those of cultivars with the low nitrogen-utilization; and (3) Root vigor varied according to growth stage and nitrogen supply i.e. cultivars with high nitrogen-utilization-rate showed enhanced root vigor at the seedling stage under lownitrogen conditions, while cultivars with low nitrogen-utilization rate showed reduced root vigor at the tuber-bulking and mature stages under high-nitrogen conditions. The kinetic characteristics of nitrogen uptake differed significantly among cultivars: (1) In comparison with cultivars with the low, high nitrogen-utilization showed lower K_m and higher $V_{\rm max}$ values; (2) The $K_{\rm m}$ value for nitrate nitrogen uptake was higher than that for ammonium nitrogen uptake, especially in cultivars with low nitrogen-utilization, than those of ammonium nitrogen; (3) In cultivars with low nitrogen-utilization, the $V_{\rm max}$ value for nitrate nitrogen was lower than that for ammonium nitrogen; and (4) In cultivars with high nitrogen-utilization, the V_{max} value for nitrate nitrogen was the opposite. The study provides a basis for breeding high nitrogen-utilization cultivars, thereby improving the efficiency of nitrogen fertilization.

Keywords: High nitrogen utilization, Kinetics of absorption, Nitrogen form.

INTRODUCTION

Nitrogen is one of the most highly demanded mineral elements, with considerable effect on crop productivity, and represents a crucial means of improving plant yield, quality (Matson *et al.*, 2002), and resistance ability. For example, waterlogging damage was alleviated by the application of nitrogen fertilizer in winter rape (Zhou *et al.*, 1997). In China, a main

problem is the low utilization rate of nitrogen fertilizer, which results in wasted resources and environmental pollution. The average nitrogen utilization rate for the main food crops in China is estimated to be only 35%, which is considerably lower than the 40–60% reported for other countries (Zhang et al., 2008). Thus, improving the utilization rate of nitrogen fertilizer is a key agricultural issue (Presterl et al., 2003). Nitrogen utilization rate differ significantly among different cultivars (Inthapanya et al., 2000)

¹ Potato Research Center, College of Agronomy, Sichuan Agricultural University, Wenjiang 611130, People's Republic of China.

^{*}Corresponding author; e-mail: yuanjichao5@163.com

² Department of Agronomy, Xi Chang College, Xichang 615000, People's Republic of China.



and between different crops (Cheng et al., 2007; Sun et al., 2006; Zhao et al., 2006). Therefore, cultivars with high nitrogenutilization rate represent an important means of reducing nitrogen supply and improving the utilization rate of nitrogen fertilizer. Nitrogen utilization rates vary according to the uptake rates of root systems. The kinetic characteristics of nitrogen absorption have been extensively studied in a variety of root systems (for example, rice (Zhao et al., 2006), wheat (Sun et al., 2006; Wang et al., 2010), and corn (Cheng et al., 2007; Huang et al., 2001; Wang et al., 2011). Some studies of nitrogen utilization rate for crops showed that the nitrogen utilization was affected by many factors, such as irrigation which has significant impact on the nitrogen accumulation, remobilization partitioning of rice, and then the yield (Lin et al., 2006). The moisture conditions was another factor. In this regard, the nitrogen use efficiency was affected greatly by the applied nitrogen amount: the uptake of nitrogen increased with increase in the level of nitrogen applied up to the highest level, but the reverse was true for nitrogen recovery (Badr et al., 2012); and increasing nitrogen or water input showed inconsistent impact on dry matter production per unit of applied water (Darwish et al., 2006). Changing the nitrogen sources also showed impact on the nitrogen use efficiency, nitrogen yield efficiency and N physiological efficiency in rice (Hosseiny et al., 2008). There were some differences of nitrogen utilization in different cultivars. Late maturing sweet potato tended to have higher nitrogen recovery and physiological efficiency than early maturing cultivars (Ankumah et al., 2003). Sweet potato had a higher nitrogen utilization than taro due to a higher above-ground biomass production (Hartemink et al., 2000). These results suggested that cultivar in maturity should play an important part in nitrogen fertilizer recommendations. All of these studies focus on the absorption and recovery of nitrogen with utilization of nitrogen, but seldom on the root absorption of nitrogen efficiencies.

Up to date, however, fewer studies have focused on the kinetic characteristics of nitrogen absorption in cultivars with different nitrogen utilization rate, nitrogen utilization in potatoes. Therefore, how to improve the nitrogen utilization of potatoes is an important problem.

The purpose of this study was: (1) To investigate the root vigor and kinetic characteristics of nitrogen absorption in different potato cultivars, and (2) To provide a basis for breeding high nitrogen-utilization rate cultivars, thereby improving the utilization of nitrogen fertilizer in potatoes.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

For the present study cultivars with, high nitrogen-utilization-rate were Feiwureita and Yunshu301, and cultivars with the low nitrogen-utilization-rate were Liangshu97 and Chuanyu56 (Cheng, 2010).

Sand cultivation test and pot tests with nutrient solution (based on the Murashige and Skoog culture medium, without agar powder) were used. Each pot measured 60 cm in length, 25 cm in width, and 20 cm in height and was filled with 20 kg of dry river sand, which contained 0.76 g kg⁻¹ of organic matter, 0.6 g kg⁻¹ of available nitrogen, 1.2 g kg⁻¹ of available phosphorus, and 12.9 g kg⁻¹ of available potassium. Murashige and Skoog composition was used as the basic nutrient solution, with the addition of 3 different levels of nitrogen (nitrate to ammonium nitrogen ratio of 0.52, pH 6.5): No (0 mM); N1 (0.03 mM); and N2 (0.06 mM). The study involved 4 cultivars and three N treatments, with each treatment replicated 4 times. Thus, the total number of trial pots was 48.

On August 15, 2011, 50 potato seed tubers were planted in each pot. To ensure consistent crop quality, and thereby reduce experimental error, the whole seed potatoes were selected for sowing. The 4 types of trial cultivars were pre-elite seeds of virus-

free potato, each weighing 5 g. After germination, the plants were irrigated every 4 days with 1,000 mL of nutrient solution per pot. All other conditions were identical to those in the environment.

Root Vigor

At the seedling, tuber-bulking, and mature stages, 5 strains of the main root were selected from each treatment using TTC method for determine root vigor (Zou, 1993). At first, the 0.5 g apical roots were weighed and put into graduated test tubes, followed by addition of 5 mL 0.4% TTC solution and 5 mL M/15 phosphate buffer. Later, the root was fully immersed in the liquid, maintained for 1 hour at 37 °C, then, 2 mL of 1 mol L⁻¹ sulfate was terminate to the reaction. Afterwards, the roots were taken out and wiped dry, adding 4 mL ethyl acetate and a little quartz sand in the mortar and grinded into homogenate. Then, the clear liquid was poured into 10 mL tube. Colorimetric method was used at 485 nm wavelength by photoelectric colorimeter to get the absorbance values. The TTC concentration was obtained from the standard curve. The strength of root reductive TTC (root vigor) determined as follows:

Root vigor= The reducing amount of TTC (ug)/Root weight (g)×Time (h)

Kinetic Parameters of Nitrogen Absorption

The Kinetic parameters were carried out on the 20th day after germination, by using the depletion method (Tian *et al.*, 2001). Plants of approximately the same size were selected, and removed from the pots. Next, the seed potatoes were removed, and washed first with running water and then with ionized water. During the following 2 days, they were placed in the Murashige and Skoog basic nutrient

solution, supplemented with calcium sulfate (0.2 mM). The $(NH_4)_2SO_4$ the source of ammonium nitrogen, and NaNO₃, the source of nitrate nitrogen, were used at 6 different nitrogen concentrations (0.05, 0.10, 0.20, 0.50, 1.00, and 2.00 mM). The pH of the nutrient solution was 6.5, and the experiments were carried out in triplicates. All of the procedures were carried out during daylight. First, 500 mL of each nutrient solution were poured into 700-mL containers, in which 3 plants with dry surfaces were inserted. After 2 hours, the roots were cut off and weighed. Next, the nutrient solution of the six concentrations was collected immediately, then, the contents of ammonium nitrogen and nitrate nitrogen in the nutrient solution were measured by using the indophenol blue colorimetric method and salicylic acid colorimetric method, respectively. Based on the changes in the contents of ammonium nitrogen and nitrate nitrogen, the sole uptake rate i.e., the absorption content of each unit fresh weight root in each unit time was calculated, according to the Michaelis-

Menten Equation:
$$V = \frac{V_{\text{max}} C}{K_m + C}$$

Where, V= The uptake rate, C= The density of ammonium nitrogen or nitrate nitrogen, $V_{\text{max}}=$ The maximum rate, and $K_{\text{m}}=$ The apparent Michaelis constant.

experiment was designed The Completely Randomized Design (CRD). Each variety had three replicates for each treatment. All data were expressed as means ± standard deviation from triplicate samples and were analyzed using Microsoft Excel 2003, significant differences between treatments assessed according to the LSD test at 5% level, using the DPS 7.05 statistical software, and the experimental results of the nitrogen uptake rates were analyzed **Systat** Software Inc., 2010 (SigmaPlot 12.0).



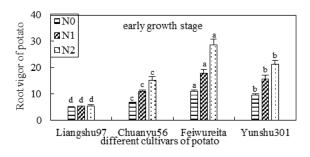
RESULTS

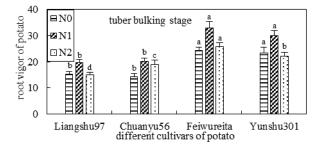
Differences in Root Absorption among Potato Cultivars

An appropriate supply of nitrogen enhanced the root vigor of potato plants (Figure 1), especially at the seedling stage. At early growth stage, root vigor increased with an increase in nitrogen supply, to reach the maximum in the N2 treatment. Meanwhile, at the tuber-bulking and mature stages, root vigor first rose, then fell, with the maximum value in the N1 treatment. Thus, the excess of nitrogen suppressed root vigor, especially in high cultivars with

nitrogen-utilization. The root vigor differed significantly between the high and low nitrogen-utilization cultivars. At all three growth stages, root vigor values of cultivars with the high nitrogen-utilization (Feiwureita and Yunshu301) were higher than those of cultivars with the low nitrogenutilization (Liangshu97 and Chuanyu56) (Figure 1). At the seedling, tuber-bulking, and mature stages, the average root vigor values of cultivars with the high nitrogenutilization were 115, 53, and 18%, respectively, higher than those of cultivars with the low nitrogen-utilization.

Variations and Kinetic Characteristics of Ammonium Nitrogen Absorption





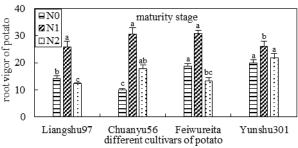


Figure 1. Root vigor of potato cultivars with different nitrogen efficiencies. Note: N0, N1, N2 indicate nitrogen contents of 0, 0.420, and 0.84 mg L^{-1} , respectively. Different superscript letters indicate significant differences at P < 0.05 (n= 4), data represent Mean±Standard Deviation (SD) of 3 replicates.

An increase in the concentration of ammonium nitrogen led to an increase in the amount and rate of ammonium nitrogen uptake by all 4 cultivars (Figure 2). The rate of increase gradually diminished, and the rate of absorption was asymptotic with the concentration of ammonium nitrogen (Figure 2). The rate of ammonium nitrogen uptake differed significantly between the high and low nitrogen-efficient cultivars. The uptake rates of the low nitrogenutilization cultivars (Liangshu97 Chuanyu56) were markedly lower than those of the high nitrogen-utilization cultivars (Feiwureita and Yunshu301); moreover, the differences between cultivars with the low nitrogen-utilization were smaller than those between cultivars with the high nitrogen-

utilization. For all 4 cultivars, the differences in uptake rates low ammonium nitrogen concentrations were larger than markedly those high ammonium nitrogen concentrations. Moreover, at nitrogen concentrations of 0.05 mmol L⁻¹ and 2.00 mmol·L⁻¹, the average uptake rates of cultivars with the high nitrogen-utilization were 87 and 38% higher than those of cultivars with the low nitrogenutilization. As the concentration ammonium nitrogen was increased, the difference in uptake rate between the high and low nitrogen-utilization cultivars was gradually reduced, indicating that cultivars with high nitrogen-utilization are better adapted to ammonium nitrogen absorption under low-nitrogen conditions.

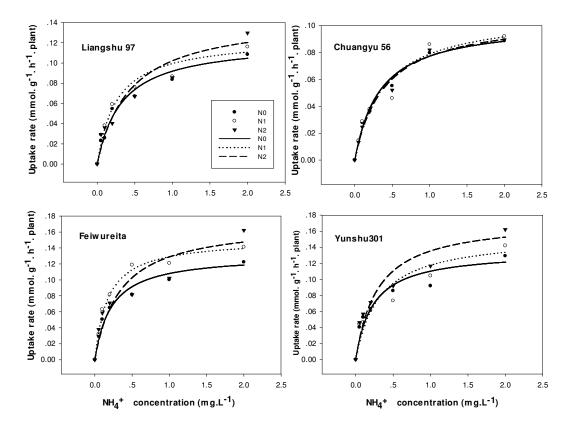


Figure 2. Ammonium nitrogen uptake rates of potato cultivars with different nitrogen efficiencies. Note that the figures of cultivar Chuanyu56 and Liangshu97 use the same vertical scale, while the scale used for cultivar Yunshu301 is the same as cultivar Feiwureita.



The rate of ammonium nitrogen uptake was further influenced by the basic nutritional supply. The rates of uptake were higher in the N1 and N2 treatments than in the N0 treatment. For all 4 cultivars and all 6 ammonium nitrogen concentrations, the average uptake rates in the N1 and N2 treatments were 11 and 10% higher, respectively, than that in the N0 treatment. Thus, high ammonium nitrogen uptake rates are associated with high root vigor.

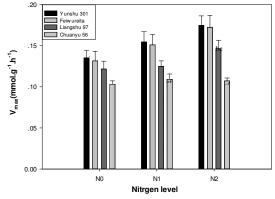
Our data analysis revealed that the rates of ammonium nitrogen uptake by all 4 cultivars corresponded with the Michaelis-Menten equation. The $K_{\rm m}$ and $V_{\rm max}$ values did not differ greatly between the 4 cultivars (Figure 3). Compared with cultivars with the low nitrogen-efficient, cultivars with the high nitrogen-utilization had slightly lower $K_{\rm m}$ values, and slightly higher V_{max} values. For all 3 nitrogen treatments, the average $K_{\rm m}$ value of cultivars with the high nitrogenutilization was 26% lower than that of cultivars with the low nitrogen-utilization, while the average $V_{\rm max}$ value was 29% higher than that of the low nitrogenutilization cultivars. Data indicated that cultivars with high nitrogen-utilization had a higher affinity and greater maximum absorption potential for ammonium nitrogen than do cultivars with low nitrogenutilization.

The $K_{\rm m}$ and $V_{\rm max}$ of the cultivars varied

according to the nitrogen supply level. An increasing level of basic nutrition led to a rise in the $K_{\rm m}$ and $V_{\rm max}$ values, indicating reduced affinity, but increased maximum absorption potential for ammonium nitrogen. In comparison with cultivars with low nitrogen-utilization, cultivars with high nitrogen-utilization showed larger increases in $K_{\rm m}$ and $V_{\rm max}$ values. For cultivars with the high nitrogen-utilization, the average $K_{\rm m}$ values in the N1 and N2 treatments were 9 and 29% higher, respectively, than that in the N0 treatment. Meanwhile, for cultivars with the low nitrogen-utilization, the average $K_{\rm m}$ values in the N1 and N2 treatments were 2% lower and 28% higher, respectively, than that in the N0 treatment. These data further indicate that high nitrogen-utilization cultivars have a higher affinity for ammonium nitrogen than do low nitrogen-utilization cultivars.

Variations and Kinetic Characteristics of Nitrate Nitrogen Absorption

Similar to ammonium nitrogen, an increase in the concentration of nitrate nitrogen led to an increase in the amount and rate of nitrate nitrogen uptake by all the 4 cultivars. The rate of nitrate nitrogen uptake differed among cultivars, and was highest in cultivars with the high nitrogen-utilization,



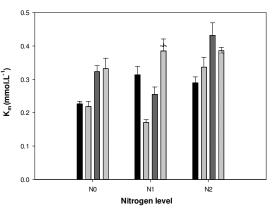


Figure 3. Kinetic parameters of ammonium nitrogen uptake in potato cultivars with different nitrogen efficiencies. Different superscript letters indicate significant differences at P < 0.05 (n= 12); data represent the Mean±Standard Deviation (SD) of 3 replicates.

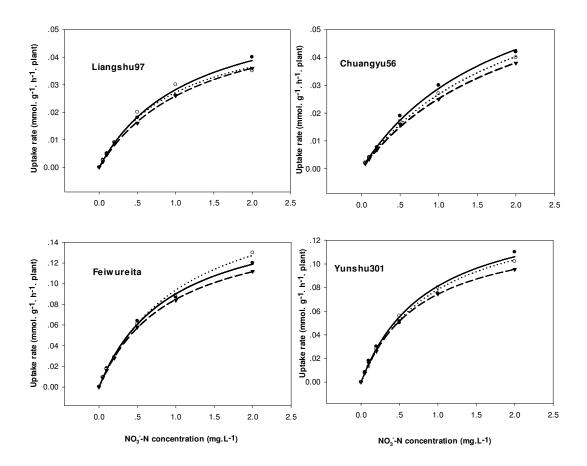


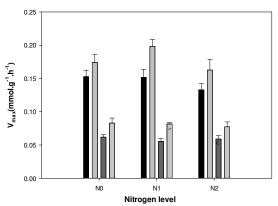
Figure 4. Nitrate nitrogen uptake rates of potato cultivars with different nitrogen efficiencies. Note that the figure of cultivars Chuanyu56 and Liangshu97 use the same vertical scale, while the scale used for cultivar Yunshu301 is the same as cultivar Feiwureita.

especially at low concentrations of nitrate nitrogen (Figure 4).

The rate of nitrate nitrogen uptake was further influenced by the basic nutritional supply. In contrast to ammonium nitrogen, the rates of nitrate nitrogen uptake were highest in the N0 treatment and lowest in the N2 treatment. Therefore, the higher the basic nutritional level, the lower the rate of nitrate nitrogen uptake, especially concentrations of nitrate nitrogen. Moreover, for all the 4 cultivars and all 6 nitrate nitrogen concentrations, the average uptake rates in the N0 and N1 treatments were 8 and 7% higher, respectively, than that of the N2 treatment. The observed variation in the pattern of results for ammonium nitrogen and nitrate nitrogen uptake may be caused by different absorption and assimilation mechanisms for nitrate nitrogen and ammonium nitrogen.

The kinetic parameters of nitrate nitrogen uptake varied considerably among the cultivars (Figure 5). In comparison with cultivars with the low nitrogen-utilization, cultivars with the high nitrogen-utilization showed much higher V_{max} values, but lower $K_{\rm m}$ values. The variation in kinetic parameters according to the basic nutrition supply was similar to the observations for ammonium nitrogen. The $K_{\rm m}$ and $V_{\rm max}$ values for nitrate nitrogen uptake were significantly positively correlated with those for ammonium nitrogen (correlation coefficients 0.5740 and 0.5812, P< 0.05, n=





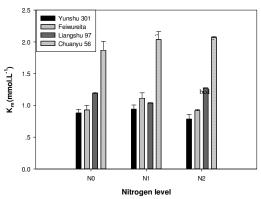


Figure 5. Kinetic parameters of nitrate nitrogen uptake in potato cultivars of different nitrogen efficiencies. Different superscript letters indicate significant differences at P< 0.05 (n= 12); data represent the Mean±Standard Deviation (SD) of 3 replicates.

12). However, a number of differences were observed.

Firstly, the $K_{\rm m}$ value for nitrate nitrogen uptake was higher than that for ammonium nitrogen uptake. For the high nitrogen-utilization cultivars, For two cultivars withhigh and low rate of nitrogen utilization, the average Km values for absorbing NO3-is 258% and 348% higher than that of NH4+respectively, than those of ammonium nitrogen. Thus, potato plants (particularly low nitrogen-utilization cultivars) have a higher affinity for ammonium nitrogen than that for nitrate nitrogen.

Secondly, for cultivars with low nitrogenutilization, the $V_{\rm max}$ value cultivars with for nitrate nitrogen uptake was lower than that for ammonium nitrogen uptake. Meanwhile, for cultivars with high nitrogen-utilization, the $V_{\rm max}$ value for nitrate nitrogen uptake was higher than that for ammonium nitrogen in the N0 and N1 treatments, but lower than that for ammonium nitrogen in the N2 treatment.

Thirdly, within the investigated range of nitrate and ammonium nitrogen concentrations (0-2.00 mmol L⁻¹), all cultivars showed a lower increase in the rate of nitrate nitrogen uptake than in the rate of ammonium nitrogen uptake. For all the 4 cultivars, all 3 nitrogen treatments, and an ionic concentration of 2.00 mmol L⁻¹, the average rate of ammonium nitrogen uptake

reached 96% of the $V_{\rm max}$ value, while the average rate of nitrate nitrogen uptake reached only 61% of the $V_{\rm max}$ value. For cultivars with the high nitrogen-utilization, when the average rate of nitrogen uptake for the 3 nitrogen treatments reached 80% of the $V_{\rm max}$ value, the concentrations of ammonium nitrogen and nitrate nitrogen were 1.07 and 3.41 mmol L⁻¹, respectively. Meanwhile, for cultivars with the low nitrogen-utilization, the concentrations of ammonium nitrogen and nitrate nitrogen were 1.38 and 8.96 mmol L⁻¹, respectively. For all the 4 cultivars, when the average rate of nitrogen uptake reached 80% of the $V_{\rm max}$ value, the concentration of nitrate nitrogen was 4.05 times higher than that of ammonium nitrogen.

Finally, the $V_{\rm max}$ values for nitrate nitrogen uptake of plants in the N2 treatment were lower than those of plants in the N0 treatment.

DISCUSION

Root vigor is an indicator of activity and life ability for the plant root system, and it is one of the main indices to measure the root function, which directly affects the nutritional status and the yield level of plant. Previous studies on rice (Cheng *et al.*, 2007), rape seed (Liu *et al.*, 2008), corn (Sen

et al., 2012; Wang et al., 2011), and other crops have indicated that root vigor is closely related to nitrogen uptake. The present study demonstrates that the root vigor differs significantly between different potato cultivars. In particular, cultivars with high nitrogen-utilization showed higher root vigor than did cultivars with low nitrogenutilization, especially at the seedling stage. These results are in accordance with previous findings, namely, that the root systems of cultivars with high nitrogenutilization do much better in absorbing nitrogen, especially at the seedling stage (Sun et al., 2006). At the early growth stage, an increase in the nitrogen supply leads to enhanced root vigor. By contrast, at the tuber-bulking and mature stages, root vigor is initially enhanced but then suppressed. Our findings indicate the importance of a balanced nitrogen supply, and also the requirement for more nitrogen at the seedling stage than during the later growth stages. Therefore, in potato production, the application of topdressing in addition to adequate amounts of base fertilizer is essential.

The nutrient uptake rate of crop root the Michaelis-Menten obeys equation; however, the parameters vary among crops and between genotypes(He et al., 1999; Zhang et al., 2002). In the present study, the significant differences in the of kinetic characteristics ammonium nitrogen and nitrate nitrogen uptake between different potato varieties were demonstrated. Similar to other crops (Sun et al., 2006; Yee and X, 1992; Zhao et al., 2006), the $K_{\rm m}$ values for were lower than those for low nitrogen-efficient cultivars, whereas the V_{max} values showed the opposite trend. This may be associated with high root vigor, which was significantly positively correlated with $V_{
m max}$ (the correlation coefficients of ammonium nitrogen and nitrate nitrogen at the seedling stage were 0.6869 and 0.6448 [n= 12], respectively). Thus, high root vigor, low $K_{\rm m}$ values, and high $V_{\rm max}$ values probably form the pivotal physiological basis for efficient nitrogen usage by potato varieties.

The response to different forms of nitrogen varies among crops and cultivars. Some plants preferentially utilize ammonium nitrogen, while others preferentially utilize nitrate nitrogen (Dai et al., 1998; Tian and Li, 2000). In comparison with nitrate nitrogen, ammonium nitrogen tends to inhibit potato growth (Cao and W.Tibbitts, 1993). However, in the present study, the rate of ammonium nitrogen uptake rate by potato root systems was more rapid than that of nitrate nitrogen; this may be due to different absorption and assimilation mechanisms of these forms of nitrogen. During the process of nitrate nitrogen uptake, nitrate is first converted ammonium nitrogen and then assimilated, with the dissipation of energy. Nitrate reductase is a type of inducible enzyme. Furthermore, the absorption of ammonium nitrogen and nitrate nitrogen is pHdependent. At pH 7.0, the amount of ammonium nitrogen uptake is the highest, whereas at pH 5.0, the amount of nitrate nitrogen uptake is highest (Cao et al., 1994). The present study was conducted at a pH of 6.5, therefore, ammonium nitrogen uptake was promoted.

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توانایی رشد ریشه و ویژگی های سینتیکی و کار آیی مصرف نیتروژن در کولتیوارهای سیب زمینی (Solanum tuberosum L.)

س. ل. ژنگ، ه. چنگ، پ. ه. لي، و ج. س. يوان

چکیده

در پژوهش حاضر، توانایی رشد ریشه و ویژگی های سینتیکی جذب نیتروژن در کولتیوارهای سیب زمینی به نام های کاشت در شن و کشت در محلول غذایی بررسی شد. چهار کولتیوار سیب زمینی به نام های دامت المناسب المناسب



جذب کم) و میانگین مقادیر Kmبرای جذب نیتروژن نیتراتی به ترتیب ۲۵۸٪ و ۲۴۸٪ بیشتر از نیتروژن آمونیومی بود، (۳) در کولتیوارهای داری نرخ جذب کم ، مقدار Vmaxدر مورد نیتروژن نیتراتی کمتر از نیتروژن آمونیومی بود، و (۴) در کولیتوارهای دارای نرخ بالای جذب نیتروژن، مقدار Vmaxبرای نیتروژن نیتراتی بیشتر از نیتروژن آمونیومی بود. از این قرار، این پژوهش مبنایی برای بهنژادی کولتیوارها برای جذب نیتروژن بالا و در نتیجه کارآیی بهتر کود دهی نیتروژن فراهم آورده است.