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Uptake and uptake kinetics of nitrate, ammonium and glycine by pakchoi seedlings (*Brassica Campestris* L. *ssp. Chinensis* L. *Makino*)

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ABSTRACT

Plants not only absorb inorganic nitrogen (N), but also have the ability to absorb intact amino acids. Studies about pakchoi N uptake (*Brassica Campestris* L. *ssp. Chinensis* L. *Makino*) mainly focused on nitrate (NO_3^-) uptake and its accumulation in plant edible portion. This study investigated pakchoi seedlings uptake and uptake kinetics of NO_3^-, ammonium (NH_4^+) and glycine (Gly) using a mixed equimolar concentrations of the three N forms, and examined passive and active uptake ratios of the three N forms under sterile hydroponics.

Our results revealed that pakchoi N uptake was positively related to its substrate N concentration ($R = 0.89–0.99$, $p < 0.11$), while N uptake efficiency showed a negative relation with the N concentration ($R = -0.64–-0.77$, $p < 0.36$). At the N concentration from 25 to 1500 μmol L^{-1}, pakchoi took up significantly more NO_3^- than NH_4^+, but no significant difference was detected between uptakes of Gly and NO_3^- except at 250 μmol L^{-1}. At 7500 μmol L^{-1}, Gly uptake and uptake rate were significantly more than those for NO_3^- and NH_4^+. Regression analysis showed uptake rates for NO_3^-, NH_4^+ and Gly were well fitted to the Michaelis–Menten kinetics, and their affinity constant (K_m) was in the range of 177–2000 μmol L^{-1} and maximal velocity (V_{max}) 18.2–46.8 μmol $g^{-1}{}_{DW}$ h^{-1}. Pakchoi uptake for NO_3^-, NH_4^+ and Gly was dominated by active uptake. Gly active uptake at low concentration indicated plants have the ability to uptake amino acids that is relevant to field condition. However, passive uptake should not be overlooked in future studies, especially at high N concentration.

1. Introduction

Traditionally, terrestrial N cycle asserted that soil organic N must be transformed into inorganic N (NO_3^- and NH_4^+) by soil microorganisms prior to becoming available to plant roots (Warren and Adams, 2007) and N mineralization has been viewed as a critical step in plant N uptake. Recent studies have demonstrated that plants have the ability to take up intact amino acids, thereby bypassing the microbial mineralization step (Näsholm et al., 2002; Persson and Näsholm, 2008; Thornton, 2001). Plant amino acid uptake was mediated by a range of different transporters, and both high- and low-affinity transporters have been identified using Arabidopsis thaliana as a model plant (Williams and Miller, 2001). In some N limited ecosystems with low temperature (or pH) and slow N mineralization rate, amino acid uptake even constituted a high proportion of plant total N economy (Jones and Kielland, 2002; Näsholm et al., 1998; Schimel and Chapin, 1996). So it has been accepted that plants are able to absorb and assimilate amino acids as well as NO_3^- and NH_4^+ as their main N sources.

Plants possess various mechanisms that adapt N uptake to the spatial and temporal N availability changes (Hodge et al., 2003). McKane et al. (2002) reported that plant preference for a given N form was likely related to the most abundant N form in soil where plant grows. This preferential uptake could reduce N uptake competition and promote coexistence of species. Besides, some species have their particular absorbing preferences for the different N forms, such as rice (*Oryza sativa* L.) for NH_4^+ and pakchoi (*Brassica Campestris* L. *ssp. Chinensis* L. *Makino*) for NO_3^-. Pakchoi is one

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of the main leafy vegetables grown in China and tends to accumulate high levels of NO_3^- in its edible portion (Chen et al., 2005). High NO_3^--N fertilizer is the main cause of high NO_3^- accumulation in vegetable leaves (Chen et al., 2004), which correspondingly increase the risk of nasopharyngeal and esophageal cancers (Eichholzer and Gutzwiller, 1998). In contrast, amino acid composition and their contents are important quality measures for vegetables (Yu et al., 2005). Numerous studies have demonstrated that partial replacement of NO_3^- by amino acids in the nutrient solution significantly increased pakchoi quality by reducing NO_3^- content and improving vegetable nutrition (Inal and Tarakcioglu, 2001; Wang et al., 2008). NO_3^-/NH_4^+ ratios also affected NO_3^- concentration in cucumber (Kotsiras et al., 2002). However, of the studies to date, little is known about pakchoi uptake of amino acids and its uptake kinetics parameters.

During the past 20 years, with the ^{13}C and ^{15}N double-labeled technology, root uptake rate for amino acids, NO_3^- and NH_4^+ have been studied using excised or intact roots in hydroponics or field experiments (Kielland et al., 2006; Persson et al., 2006; Thornton and Robinson, 2005; Warren and Adams, 2007). However, using excised roots excavated from soil was unable to differentiate the uptake and conversion of NH_4^+ or amino acids in rhizosphere microorganisms from root cell (Neumann and Römheld, 2000), because the C and N balance of root was irreversibly altered. The injection method does not ensure heterogeneous distribution of the labeled N, thus causing significant error due to the "hotspots" (Augustine and Frank, 2001; Farrar et al., 2003). In addition, the appearance of ^{15}N in plant might also derive from the mineralization of intact amino acids in unsterile experiment (McKane et al., 2002). These various factors have affected the reliability of the existing methodology. The concentration of individual free amino acids in bulk soil solution is in the region of 0.01–10 μmol L^{-1}, but its concentration in plant and animal cells is in the region of 1–10 mmol L^{-1} (Jones and Darrah, 1994; Jones et al., 2005). Therefore, it can be expected that high concentrations of amino acids will exist at least transiently in soil after cell death. However, many experiments only adopted a narrow range of amino acid concentrations (mostly below 2000 μmol L^{-1}, Warren, 2009).

So we investigated the uptake and uptake kinetics of pakchoi seedlings for NO_3^-, NH_4^+ and Gly using solution containing equimolar of the three N forms at the concentrations of 25, 250, 1500 and 7500 μmol L^{-1}. The equimolar of NO_3^-, NH_4^+ and Gly were used for the purpose of investigating the preferential uptake of different N forms and their interaction effects (Persson et al., 2006). Plant seedlings, solution and cultivation environment were completely sterilized to ensure that amino acids could not be decomposed by microorganism. The objectives of this study were to (1) investigate how the N concentrations and N forms affect pakchoi preferential uptake and uptake kinetics for NO_3^-, NH_4^+ and Gly, (2) determine the roles of passive and active uptakes for NO_3^-, NH_4^+ and Gly at the different N concentrations.

2. Materials and methods

2.1. Plant material and culture

The experiment was conducted in October 2012 at the Plant Organic Nutritional Laboratory, Zhejiang University. Seeds of pakchoi (*B. Campestris* L. *ssp*. *Chinensis* L. *Makino*) were obtained from the Zhejiang Provincial Academy of Agricultural Sciences, China. The cultivar was Zhebai 6. Pakchoi seeds were sterilized by the method described in Wu et al. (2005), and then placed in the sterile Petri dish covered with parafilm for germination. Three days later, the germinated seeds were transplanted to a new sterile Petri dish with 0.5% agar (NA) substrate at a density of approximately 20 seeds per dish.

When the main root length of seedlings was approximately 1.5 cm, aseptic seedlings were transplanted into centrifugal tubes containing 50 ml 0.5% (w/w) sterile agar (NA). The centrifugal tubes have internal radius 1.3 cm, total depth 11 cm. Each tube was covered with a plastic cap, which had a 0.5 cm diameter hole drilled in the center for seedling to grow out of the tube. After seedlings had completely grown out of the holes, the 0.5% agar inside the tubes was replaced with aseptic nutrient solution containing 6.25 mmol L^{-1} N (NO_3^- 2.08, NH_4^+ 2.08 and Gly 2.08 mmol L^{-1}, respectively), 1.0 mmol L^{-1} KH_2PO_4, 1.0 mmol L^{-1} K_2SO_4, 0.7 mmol L^{-1} $MgSO_4$, 2.0 mmol L^{-1} $CaCl_2$, 0.00005 mmol L^{-1} $NaMoO_4$-$2H_2O$, 0.0002 mmol L^{-1} $CuSO_4 \cdot 5H_2O$, 0.0005 mmol L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.004 mmol L^{-1} H_3BO_3, 0.005 mmol L^{-1} $MnCl_2$, 0.0025 mmol L^{-1} Na_2EDTA and 0.009 mmol L^{-1} $FeSO_4 \cdot 7H_2O$, which is one half of nutrient strength of the complete cultivation solution (1/2-nutrient solution). Then the holes in the center of the caps were sealed with Nan Da 704 silicone rubber. Five days later, the ½-nutrient solution was replaced by the complete cultivation solution, which contains double nutrient concentrations of the ½-nutrient solution.

The air quality in Plant Organic Nutritional Laboratory achieved a sterility of 100-grade (USA Federal Standard, 209D) under the effects of aseptic laminar cover. The numbers of air particles (>0.5 μm) are less than 100 per cubic foot, which satisfies the requirement for sterile cultivation. N-free solution was sterilized by steam under high pressure at 121 °C for 1 h. N nutrient solution was sterilized by passing through a 0.22 μm cellulose filter (Millipore, PES Membrane, Ireland). The solution pH was adjusted to 6.5 with sodium hydroxide. During cultivation, the nutrient solution was replaced every three days. To test the contamination, the replaced nutrient solution was immediately inoculated to the sterile agar (NA) substrate. If microbial growth was identified in the inoculated agar plates, the corresponding cultivation tube was discarded. The sterile seedlings were grown under mean temperature 30 °C (day) and 25 °C (night), relative humidity 60%, maximum photosynthetic photon flux density 300 μmol m^{-2} s^{-1}, and photoperiod 12 h. During the cultivation, the seedlings were watered with sterile water as needed.

2.2. Experiment design

After 21 days of cultivation with unlabeled nutrient solution (seven leaves stage), the aseptic seedlings were cultivated with the ^{15}N labeled solution to determine pakchoi preferential uptake for NO_3^-, NH_4^+ and Gly under the sterile environment.

Experiment 1: Pakchoi preferential uptake for the different N forms was determined using the 50.24 atom% $^{15}NO_3^-$, 50.17 atom% $^{15}NH_4^+$, 50.11 atom% ^{15}N-Gly, obtained from Shanghai Research Institute of Chemical Industry, China. Pakchoi seedlings were first cultivated in the deionized water for 4 h to create a nutrient starvation condition. Then the pakchoi seedlings were simultaneously supplied with the equimolar N of NO_3^-, NH_4^+ and Gly solutions, with total N concentrations of 25, 250, 1500 and 7500 μmol L^{-1}, respectively. For each N concentration, only one N form was labeled with the ^{15}N resulting in 16 different treatments (3 N forms × 4 concentrations, plus four control treatments with unlabeled N sources). Cultivation solution also contained 10 mg L^{-1} ampicillin to control the microbial growth and 100 μmol L^{-1} Ca^{2+} for plant cell membrane stability (Warren, 2009). Each treatment had 12 replications.

Experiment 2: The protonphore Carbonyl Cyanide *m*-Chlorophenylhydrazone (CCCP) was used to separate passive and active uptake by pakchoi seedlings (Persson and Näsholm, 2002). Since CCCP inhibits plant root active uptake, the appearance of ^{15}N in plant treated with CCCP is the result of passive uptake.