It Was Found That Amino Sugar Nitrogen Was a New Source of Energy for Plant

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Abstract
Amino sugar nitrogen (ASN) is one of the most important nitrogen sources for plants. Whether ASN such as glucosamine can be absorbed directly by plants has remained unclear, because the technology for investigating its mechanism of absorption has been lacking. Using Positron Emission Tomography (PET) with \textsuperscript{18}F-fluorodeoxyglucose (\textsuperscript{18}F-FDG), we observed that ASN was absorbed directly by tomatoes and rice and this absorption was significantly influenced by the carbon to nitrogen ratio (C/N) and the pH value of the growth substrate solution of tomatoes and rice. Absorption and transportation of ASN was measured at C/N ratios of 10 or 25 at pH 7.0 or 8.0 in tomato and at pH 5.5 or 6.5 in rice. A C/N ratio of 10 resulted in higher ASN absorption and transportation than a C/N ratio of 25 in both tomatoes and rice. At C/N ratio of 10, tomatoes absorbed more ASN at pH 8.0 than at pH 7.0. In rice, absorption was more at pH 5.5 than 6.5. In a word, a lower C/N ratio yielded a higher ASN absorption and transportation for both tomatoes and rice. Tomatoes showed a slight preference for alkaline conditions, whereas rice preferred acidic conditions.

Keywords: amino sugar nitrogen, positron emission tomography, C/N, tomato, rice

1. Introduction
In 1965, Bremner popularized the study of organic nitrogen by showing, using acid digestion, that the organic nitrogen that forms in soil can be classified into amino acid nitrogen, amino sugar nitrogen (ASN), hydrolysable ammonium nitrogen, hydrolysable unidentified nitrogen and non-hydrolysable organic nitrogen (Bremner, 1965). Some researchers have demonstrated that plants can absorb not only mineralized nitrogen but also organic nitrogen such as no acid nitrogen (Schobert & Komor, 1987; Schiller et al., 1998), for instance glycine, leucine and alanine (Zhang & Sun, 1984; Falkengren et al., 2000; Persson & Näsholm, 2003; Reeve et al., 2008). However, whether ASN, a refractory organic compound, can be directly used by plants remains unknown.

ASN comprises 5-10% of the total nitrogen in soil, it provides energy for plant growth and improves soil structure (Stevenson, 1982). It mainly exists in the chitinous substance of fungus and insect, 11 kinds of ASN have been detected by now. The ASN base in soil is glucosamine, which makes up approximately 59-63% of the total ASN, depending on the soil type (Table 1) (Bremner, 1967; Y. M. Liu & X. H. Liu, 2010). The dynamic process of ASN absorption and transportation into plants is difficult to observe using conventional technology.
Table 1. ASN distribution in three different soils

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Glucosamine (mg·kg⁻¹) (%)</th>
<th>Galactosamine (mg·kg⁻¹) (%)</th>
<th>Epichitosamine (mg·kg⁻¹) (%)</th>
<th>Muramic acid (mg·kg⁻¹) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black soil</td>
<td>617.5 56.32</td>
<td>316.0 28.82</td>
<td>29.3 2.67</td>
<td>77.0 7.02</td>
</tr>
<tr>
<td>Brown soil</td>
<td>644.3 63.22</td>
<td>269.7 26.46</td>
<td>28.2 2.77</td>
<td>70.4 6.91</td>
</tr>
<tr>
<td>Red soil</td>
<td>463.8 59.23</td>
<td>141.5 18.07</td>
<td>20.1 2.57</td>
<td>78.5 10.02</td>
</tr>
</tbody>
</table>

PET is a non-invasive imaging technology that is used to observe and probe biological processes in vivo in medicine (Lee et al., 2011). There is a structural similarity between FDG and glucosamine (structure shown in Figure 1) (Yang et al., 2004). At position 2 of the sugar, FDG and glucosamine have the fluorine and amino group, respectively. Similar to FDG, the cellular uptake of glucosamine is by a glucose transporter process (Yang et al., 2003). For example, PET with ^18F-FDG as a substitute for glucose or glucosamine has been used for clinical diagnosis by oncologists (Weissleder, 2006). It was developed in the 1990s to study the transfer activities within living plants, using positron-emitting isotopes. With this technology, dynamic real-time imaging of the isotope in plants can be carried out. Studies using PET technology and the positron-emitting isotope markers $^{13}$NH$_3$, $^{13}$NO$_3$, H$_2$^18F and $^{13}$CO$_2$ have been described (Ohtake et al., 2001; Nakanishi et al., 2001; Ryuichi et al., 2008). In 2008, Hattori et al. (2008) e first to demonstrate that a planar positron imaging system (PPIS) could also be used in plants non-invasively to detect the distribution of photosynthetic products substituted with $^{18}$F-FDG. In 2012, Ferri et al. (2012) demonstrated that $^{18}$F-FDG was a good metabolite for PPIS studies to assess the stress resistance of plants under various conditions. In the current study, $^{18}$F-FDG was used as a radioactive tracer to explore the uptake of ASN by plants using PET.

![Chemical structures of F-FDG (A) and glucosamine (B).](image)

Figure 1. Chemical structures of $^{18}$F-FDG (A) and glucosamine (B). $^{18}$F-FDG is molecular formula is C$_6$H$_{11}$F$_{18}$O$_5$, and its molecular weight is 182. The half-life of $^{18}$F-FDG is 109.77 min.

2. Materials and Methods

2.1 The Synthesis of 2-$^{18}$F-β-D-deoxyglucose ($^{18}$F-FDG)

Anhydrous acetonitrile, Amino polyether K$_{2,2,2}$/K$_2$CO$_3$: USA Aldrich company; Sep-Pak C18/Al$_2$O$_3$ column: USA Waters company; IC-H column: USA Alltech company; USP alcohol: USA Milliper company; NaOH, HCl: Beijing Chemical Works.

HM-20S accelerator: JAP Sumitomo; $^{18}$F-FDG automatic synthesis module: Pat (Beijing) technology co., LTD.

$^{18}$F-FDG was produced with a domestic $^{18}$F-FDG automatic synthesis module. First, after capture by QMA, the $^{18}$F-products produced by the Sumitomo HM-20S accelerator were placed into a reaction tube after the K$_{2,2,2}$/K$_2$CO$_3$ acetonitrile solution was eluted. The azotropic water was evaporated and then dried again after the addition of 2 ml of acetonitrile. One milliliter of anhydrous acetonitrile solution containing 20 mg of 3-fluorine mannose was then placed into reaction tubes and heated for 5 min at 83 °C after which the acetonitrile was removed and 14 ml of water was added. The mixture was placed into a Sep-Pak C18 chromatography column, where the intermediate persisted in the column, which was washed twice with 10 ml of water. Then, 1 ml of 2 M
NaOH was added to the column, and the sample was hydrolyzed for 2 min at room temperature. Next, the hydrolysis reaction mixture was purified using an IC-H column, a Sep-Pak C18 column (which was activated with 5 ml of alcohol before compounding), and an Al₂O₃ column. The final product was obtained after the column was washed with 10 ml of water, and the product was then passed through a sterile membrane.

2.2 Plant Materials and Growth Conditions

Tomatoes (dicotyledons) and rice (monocotyledons) were selected as the crops. The tomato strain ZHONGZA NO.9 was supplied by the Institute of Vegetables and Flowers Chinese Academy of Agricultural Sciences, and the rice strain JIJING NO.88 was supplied by the Institute of Crop Science Chinese Academy of Agricultural Sciences. The seeds were sterilized with 10% H₂O₂, washed with distilled water and germinated at room temperature on paper towels soaked with distilled water. After germination, the plantlets were transferred to a nursery site with vermiculite. After 5 days, the plants were transferred to substrate solution in a greenhouse under natural light. The substrate solution for tomato plants was 3.75 mM Ca(NO₃)₂·4H₂O, 1 mM MgSO₄·7H₂O, 1.5 mM KH₂PO₄, 0.25 mM (NH₄)₂SO₄, 1.25 mM K₂SO₄, 3 mM KNO₃, 46 μM H₃BO₃, 12.6 μM MnSO₄·H₂O, 0.77 μM ZnSO₄·7H₂O, 0.32 μM CuSO₄·5H₂O, 0.02 μM (NH₄)₆Mo₇O₂₄·4H₂O and 0.05 μM Fe-EDTA. The substrate solution for rice plants was 1 mM (NH₄)₂SO₄, 0.3 mM NaH₂PO₄, 0.7 mM K₂SO₄, 2.0 mM CaCl₂, 0.5 mM MgSO₄, 10 μM H₃BO₃, 0.5 μM MnSO₄, 0.2 μM CuSO₄, 0.5 μM ZnSO₄, 0.01 μM (NH₄)₆Mo₇O₂₄ and 0.1 mM Fe-EDTA (Kiyomiya et al., 2001). For the different treatments, pH was adjusted using 0.1 M HCl and NaOH and the C/N ratio was adjusted using glucose.

2.3 Accumulation of Root Radioactivity

Tomato and rice seedlings were placed in Petri dishes containing normal substrate solution (without ¹⁸F-FDG), and only the root could access the substrate solution. Next, 0.5 mCi ¹⁸F-FDG was injected into the substrate solution, and 3 seedlings were removed from each dish at 0 min, 1 min, 5 min, 10 min, 20 min, and 30 min, flushed with deionized water and dried. The root was cut for radioactivity measurements with a well-type detector. The data were corrected using 109.77 min as the ¹⁸F half-life and according to the same count time.

2.4 PET Imaging

We injected 2.425 mCi ¹⁸F-FDG into every treated substrate solution. Five hours after injection, the plants were washed with deionized water and fixed on the Explore Vista Micro PET System for imaging. The data were automatically corrected using 109.77 min as the ¹⁸F half-life. We used image analysis software (VISTA-CT Visualization & Analysis) to quantify the signal intensity in specific regions of interest. Subsequently, we separated the seedlings into their respective roots, stems and leaves to assess the radioactivity using the well-type detector. The data were corrected using 109.77 min as the ¹⁸F half-life and the same count time. Lastly, we quantified the radioactive contributions from the root, stem and leaf equaled the total plant radioactivity.

3. Results

3.1 ASN Absorption in Roots

To identify whether ASN is directly absorbed by plants, the specific activity of the roots of tomatoes and rice that had been dipped in ¹⁸F-FDG substrate solution was assayed. The specific activity of tomato roots showed an approximately linear increase with time from application of the tracer, within the first 30 min (Figure 2A). Maximal specific activity was not investigated. Specific activity also increased in rice roots, albeit at a different rate, with a slow increase in activity from 0 min to 20 min followed by a rapid increase from 20 min to 30 min (Figure 2B). It has been previously established that the passive absorption rate decreases as the osmotic pressure decreases. However, a reduction in the absorption rate was not observed in the current study; rather, an increased absorption rate from 20 min to 30 min in rice was observed, which suggests that rice absorbs ASN via an initiative-absorption-process. This discovery demonstrates that ASN is a nutritional source of plant organic nitrogen. Moreover, the rice roots displayed a greater amount of radioactivity than the tomato roots throughout the process, which is likely because rice, a monocotyledon, possesses more fibrils, and its roots therefore display a larger contact area with the substrate solution.
Figure 2. Time course of accumulation of $^{18}$F-FDG in the roots of tomatoes (A) and rice (B). Each plant experiment was repeated at least three times to confirm the reproducibility of the results. Error bars are the SD

3.2 PET Imaging

Absorption and transportation of ASN are influenced by the pH and C/N ratio of plant growing environment. To assess the effects of pH and C/N ratio on ASN absorption and transportation in tomatoes and rice, roots were dipped in $^{18}$F-FDG substrate solution. A total of 8 distinct treatments at different C/N ratio and pH values were tested. A C/N ratio of 10 or 25 was tested for each plant at either pH 7.0 or 8.0 in tomato or pH 5.5 or 6.5 in rice (Table 2) according to pH and C/N of soil. $^{18}$F radioactivity images (Figure 3) showed that $^{18}$F-FDG entered both tomato and rice plants. In tomato at either pH 7.0 or 8.0, the stem image at a C/N ratio of 10 was clearer (the signal intensity was greater) than at a C/N ratio of 25, and the translocation was higher. At either C/N ratio, the stem image at pH 8.0 was clearer than at pH 7.0, and the translocation was higher (the $^{18}$F-FDG signal had travelled higher in the plant) (Figure 3A). These results demonstrated that, of the treatments tested, a pH of 8.0 and a C/N ratio of 10 promoted the greatest ASN absorption and transportation in tomatoes. In rice, the images were clearer and the translocation was higher at a C/N ratio of 10 for both pH conditions. The stem images were clearer, and the translocation was higher at pH 5.5 than at pH 6.5 for both C/N ratios (Figure 3B). Therefore, a pH of 5.5 and a C/N ratio of 10 yielded the strongest ASN absorption and transportation in rice. The discrimination center (DC) is the note of rice root and stem, it was observed in rice, where there was an accumulation of $^{18}$F-FDG. This was consistent with previous studies that demonstrated that the DC could accumulate and sort nutrients (Hattori et al., 2008; A. Sigel & H. Sigel, 1998; Nakanishi et al., 1999; Kiyomiya et al., 2001). The tomato and rice gray values (the specific activity detected in tomato and rice obtained by PET system) indicated the radioactivity, which also supported the results that a pH of 8.0 and a C/N ratio of 10 promoted the strongest ASN absorption and transportation in tomatoes (Table 3).

Table 2. Tomato and rice treatments

<table>
<thead>
<tr>
<th>Plants</th>
<th>Treatment</th>
<th>pH</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomatoes</td>
<td>pH7.0+C/N10</td>
<td>7.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>pH 7.0+C/N25</td>
<td>7.0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>pH8.0+C/N10</td>
<td>8.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>pH8.0+C/N25</td>
<td>8.0</td>
<td>25</td>
</tr>
<tr>
<td>Rice</td>
<td>pH 6.5+C/N10</td>
<td>6.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>pH 6.5+C/N25</td>
<td>6.5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>pH 5.5+C/N10</td>
<td>5.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>pH 5.5+C/N25</td>
<td>5.5</td>
<td>25</td>
</tr>
</tbody>
</table>
Table 3. Incorporation of $^{18}$F-FDG in tomato and rice seedlings

<table>
<thead>
<tr>
<th>Plants</th>
<th>Treatment</th>
<th>Mean (Value/pixel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomatoes</td>
<td>pH7.0+C/N10</td>
<td>1171.92</td>
</tr>
<tr>
<td></td>
<td>pH7.0+C/N25</td>
<td>731.70</td>
</tr>
<tr>
<td></td>
<td>pH8.0+C/N10</td>
<td>4671.14</td>
</tr>
<tr>
<td></td>
<td>pH8.0+C/N25</td>
<td>1430.73</td>
</tr>
<tr>
<td>Rice</td>
<td>pH6.5+C/N10</td>
<td>5490.06</td>
</tr>
<tr>
<td></td>
<td>pH6.5+C/N25</td>
<td>4292.78</td>
</tr>
<tr>
<td></td>
<td>pH5.5+C/N10</td>
<td>5506.08</td>
</tr>
<tr>
<td></td>
<td>pH5.5+C/N25</td>
<td>2153.52</td>
</tr>
</tbody>
</table>

Figure 3. Translocation of $^{18}$F-FDG in tomatoes and rice treated. (A) and (C) are representative gross images of the tomato and rice plant, respectively, which were measured using the PET system. The lower root half was immersed in the substrate solution containing $^{18}$F-FDG under different pH and C/N ratio conditions. PET images of tomatoes (B) and rice (D) were obtained 5 hours later after $^{18}$F-FDG was injected into the substrate solution.

3.3 Effects of pH and C/N Ratio

Radioactivity present in roots, stems and leaves following the various pH and C/N ratio treatments were measured by a detector after analyzing $^{18}$F-FDG images obtained using PET. In tomato, the radioactivity of roots, stems and
leaves decreased gradually from bottom to top; the results are displayed in decreasing order of transportation (Figure 4A): pH8.0+C/N10 treatment > pH7.0+C/N10 treatment > pH8.0+C/N25 treatment > pH7.0+C/N25 treatment. The root radioactivity at the pH7.0+C/N10 and pH8.0+C/N10 treatments was similar and significantly higher than at the pH8.0+C/N25 and pH7.0+C/N25 treatments. These results demonstrated that the lower C/N ratio treatment promoted the absorption of the amino sugar by tomato roots. A significant difference between the stem and leaf radioactivity were also observed, which followed the same pattern of treatment preference.

The radioactivity of rice roots, stems and leaves also varied according to treatment conditions. Root radioactivity after the pH6.5+C/N10 treatment was greater than that after the other treatments. At pH 5.5, only a slight difference in radioactivity was observed between a C/N of 10 and of 25, which suggests that the effect of the C/N ratio on ASN absorption is diminished at low pH values. The stem radioactivity displayed the following decreasing order of transportation: pH5.5+C/N10 treatment > pH6.5+C/N25 treatment > pH5.5+C/N25 treatment > pH6.5+C/N10 treatment (Figure 4B). The leaf radioactivity exhibited the following decreasing order of transportation: pH5.5+C/N10 treatment > pH6.5+C/N10 treatment > pH6.5+C/N25 treatment > pH5.5+C/N25 treatment. In conclusion, the pH8.0+C/N10 and pH5.5+C/N10 treatments yielded the greatest absorption and transportation outcomes for tomatoes and rice, respectively. The individual radioactive contributions from the root, stem and leaf were equivalent to the total plant radioactivity. These experiments validated the finding that of the treatments tested, pH8.0+C/N10 and pH5.5+C/N10 yielded the greatest absorption and transportation for tomatoes and rice, respectively. The tomato root radioactivity accounted for a range of 57.24%-89.64% of the total radioactivity, as displayed in the following descending order of activity (Figure 5A): pH7.0+C/N25 treatment > pH7.0+C/N10 treatment > pH8.0+C/N25 treatment > pH8.0+C/N10 treatment. The stem and leaf contributions were 8.74%-38.20% and 1.62%-4.57%, respectively. The highest stem and leaf radioactivity was observed after the pH8.0+C/N10 treatment. These data demonstrated that ASN could be transported to the upper part of the tomatoes at an optimum pH of 8.0 and C/N ratio of 10. In rice, the root contributed up to 99.51% of the whole-plant radioactivity in the pH6.5+C/N10 treatment; only a small amount of $^{18}$F-FDG was transported to the stems and leaves. The maximum stem and leaf proportions were reached with the pH5.5+C/N10 treatment, with values of 28.56% and 8.02%, respectively (Figure 5B). These data revealed that ASN could be transported to the upper part of the rice plant at an optimum pH of 5.5 and C/N ratio of 10.
4. Discussion

The results of our study suggested that of the treatments tested, a C/N of 10 was greatest for ASN absorption and translocation. Furthermore, tomatoes displayed better ASN absorption and transportation with slightly alkaline substrates, whereas rice had the greater ASN absorption and translocation in slightly acidic substrates. Previous research has demonstrated that 18F-FDG and/or its metabolites could translocate via the phloem (Hattori et al., 2008), and the strength of the phloem transportation capacity was one of the key factors that influenced the ASN absorption and transportation.

Previous research has confirmed that neutral, acid and alkaline amino acid transporters existed at the root surface (Kinraide, 1981; Datko & Mudd, 1985; Bush, 1993; Tanner & Caspari, 1996; Fischer et al., 1998). The activities of these transporters were controlled by different environmental conditions, which were influenced by the pH as well as the sucrose and glucose content (Rentsch et al., 2007; Thornton, 2001). Some researchers have proposed that amino acid transporter expression was closely related to plant tissue and developmental stage (Liu & Bush, 2006). Similarly, transporters were necessary for plants to absorb ASN, and its activity and gene expression might be influenced by the pH and C/N ratio. To elucidate the mechanism of ASN absorption and transportation, it would be important to further explore physiological and biochemical plant indicators such as superoxide dismutase, peroxidase and MDA, the ultrastructure and protein structure of the roots, and the plant transcriptome, at the optimal pH and C/N ranges.

5. Conclusion

In order to explore whether ASN can be absorbed directly by plants and what influenced the absorption. Positron Emission Tomography (PET) with 18F-fluorodeoxyglucose (18F-FDG) was conducted in this study. We designed different C/N level (10 and 25) and pH level (5.5 and 6.5 in rice and 7.0 and 8.0 in tomato). The results suggested that ASN was absorbed directly by tomatoes and rice and this absorption was significantly influenced by the carbon to nitrogen ratio (C/N) and the pH value of the growth substrate solution of tomatoes and rice. A C/N of 10 was greatest for ASN absorption and translocation. Furthermore, tomatoes displayed better ASN absorption and translocation with slightly alkaline substrates, whereas rice had the greater ASN absorption and translocation in slightly acidic substrates.

Acknowledgements

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Reference


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