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Short Article

Are baby sprouts eating the proteins in the mother sweet potato?

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Abstract

Sweet potatoes sometimes sprout the purple color of stems with several small leaves in the house pantry. In the present study, we investigated the trophic hierarchy between a mother sweet potato and its baby sprouts grown without any light in a dark house pantry, based on stable nitrogen isotopic composition (δ^{15}N, ‰ vs. AIR) of glutamic acid and phenylalanine. The isotope data reveal that glutamic acid has a significant ^{15}N-enrichment (by 6.9‰) from the mother sweet potato to its baby sprout while phenylalanine has a little ^{15}N-enrichment (by 0.6‰) between them. Interestingly, the isotopic heterogeneity found within the sweet potato is very similar to the isotopic discrimination generally found in the combination between plants and herbivores during grazing food webs (ca. 8.0‰ for glutamic acid and ca. 0.4‰ for phenylalanine). These results suggest that the proteins in the mother sweet potatoes are major resources for not only proteins in their baby sprouts but also growth energy in the sprouting, when they are grown heterotrophically without any light.

Key words: plants, heterotrophy, nitrogen isotopic composition, amino acids, trophic hierarchy

1. Introduction

Sweet potatoes are stored without any light in a dark place to overwinter and ready to sprout in the spring. In spring, some small baby sprouts are found on the mother sweet potatoes, when it will be time to bed down the roots. However, these sprouts can grow for more than 5 months until the early autumn without any light in a dark place, and finally appear the purple colored long stems (>15−20 cm) with several small leaves on the mother sweet potatoes. For this phenomenon, we have a very simple question on the trophic hierarchy between mother sweet potatoes and their sprouts, whether the latters are ecologically one trophic level higher than the formers.

This question may seem to be no or little relationship with geochemistry, but it is very closely related to biogeochemical studies investigating the effects of catabolism in algal and vascular plants as well as heterotrophic organisms (e.g., Sessions, 2006; Zhang et al., 2009; Aoyagi et al, 2013). Plants can convert CO_2, H_2O, and light energy into organic materials (e.g., glucose) and O_2 during photosynthesis (i.e., anabolism), which is much larger than respiration (i.e., catabolism) that converts the organic materials and O_2 into CO_2, H_2O and life energy (or growth energy), when plants are growing with light. On the other hand, plants can produce organic materials even in catabolic stages without any light, which may appear totally different signals in the molecular and isotopic compositions from anabolic stages (e.g., Sessions, 2006). To clarify the effect of catabolism in plants is therefore an essential issue to reduce uncertainty on the molecular and isotope proxies used in organic geochemical studies. After harvest, the catabolism only occurs in sweet potatoes, because of no input of photosynthates from leaves. It is simply expected that sweet potatoes can employ their stocked organic materials (e.g., sugars, proteins, and/or lipids) as energy resources to survive and sprout in the catabolic stage. Thus, to answer the simple question on the trophic hierarchy within sweet potatoes allows better understanding not only plant physiology to survive and sprout, but also its associated changes in the molecular and isotopic compositions of organic materials in catabolic stages.

Stable nitrogen isotopic composition (δ^{15}N, ‰ vs. AIR) of a couple of amino acids, glutamic acid ($\delta^{15}N_{Glu}$) and phenylalanine ($\delta^{15}N_{Phe}$), has recently been employed as a potential powerful tool to evaluate the trophic linkage and energy flow among plant and animal species in the network of ecosystems (e.g., Chikaraishi

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et al., 2007; McCarthy et al. 2007; Popp et al. 2007). This tool has been constructed based on the isotopic discrimination associated with the amino acid metabolisms: glutamic acid shows significant ^{15}N-enrichment by 8.0±1.1‰ from resource to consumer species in the grazing food web, whereas phenylalanine shows a little ^{15}N-enrichment by 0.4±0.4‰ (Chikaraishi et al., 2010). Thus, a comparison of the δ^{15}N values between these two amino acids from a single organism corresponds to the trophic position of the organism in the ecological food web (e.g., Chikaraishi et al., 2009), which defined by the following equation (1):

$$TP = [(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + \beta)/7.6] + 1 \quad (1)$$

where the TP represents the estimated trophic position and the β represents a fix number related to the difference between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values in algal (−3.4±0.9‰) and vascular plants (+8.4±1.6‰) (Chikaraishi et al., 2010). The standard deviation (1σ) of accuracy in the TP value was estimated to be 0.17 units for terrestrial organisms (Chikaraishi et al., 2011).

The nitrogen isotope analysis of amino acids is thus a potential approach to access the trophic hierarchy within sweet potatoes. In the present study, we (1) determined the isotopic composition of glutamic acid and phenylalanine in a mother sweet potato and its baby sprouts grown in a dark house pantry, (2) revealed the trophic hierarchy within a sweet potato, and (3) discussed its catabolism associated with the sprouting in sweet potatoes.

2. Samples and methods

A sweet potato (ca. 30 cm long, 10 cm maximum diameter) was collected from a farm in Yugawara (35º08'N, 139º07'E) in October 2013, washed down by tap water, and stored in a paper bag in a dark house pantry. A number of purple colored sprouts (ca. 10−20 cm) was found on the sweet potato in August 2014. A couple of pieces of the baby sprout stems (ca. 2.0 cm length from the middle of sprouts) and mother sweet potato (ca. 0.25 cm^3 from the middle of the potatoes) were collected at that moment and stored at −20°C until analysis.

These samples were prepared for stable nitrogen isotope analysis of glutamic acid and phenylalanine, after HCl hydrolysis and *N*-pivaloyl/isopropyl (Pv/iPr) derivatization, according to the procedure in Chikaraishi et al. (2009). The isotopic composition was determined by gas chromatography/isotope ratio mass spectrometry (GC/IRMS) using a 6890N GC (Agilent Technologies) instrument coupled to a Delta^{plus}XP IRMS instrument through combustion (950ºC) and reduction (550ºC) furnaces *via* a GC-C/TC III interface (Thermo Fisher Scientific). The isotopic composition was expressed relative to atmospheric nitrogen (δ^{15}N, ‰ vs. AIR) on a scale normalized to the known δ^{15}N values of nine isotopic reference amino acids (from –25.9‰ to +45.6‰, Indiana University and SI science co., Sato et al., 2014). The accuracy and precision for the isotope measurements of the reference amino acids were 0.0‰ (mean of Δ) and 0.4‰ (mean of 1σ), respectively. The TP values were calculated using equation (1) with 8.4‰ for the β value.

3. Results and discussion

3.1. Stable nitrogen isotopic composition of amino acids and estimated trophic position

The $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values were −0.4‰ and +5.2‰, respectively, for the mother sweet potato and +6.5‰ and +5.8‰, respectively, for its baby sprout (Fig. 1). Thus, a significant heterogeneity in the $\delta^{15}N_{Glu}$ value was found between mother sweet potato and its baby sprout within a single sweet potato, whereas a little heterogeneity was found in the $\delta^{15}N_{Phe}$ value between them. These isotope data lead to a significant difference in the TP value between the mother sweet potato (1.4) and its baby sprout (2.2), as the latter has 0.8 unit higher trophic position than the former.

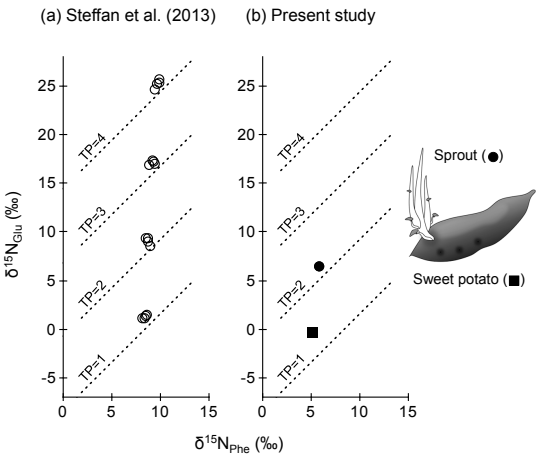

Fig. 1. Cross-plots of the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values for (a) plants, caterpillars, and carnivorous insects in a controlled feeding experiment in Steffan et al. (2013) and (b) the mother sweet potato and its sprout in the present study. Dash lines indicate the trophic isocline for TPs 1−4 with slope of 1.0 created based on the equation (1).

3.2. Trophic hierarchy of mother sweet potatoes and their baby sprouts

In general, the grazing food web starts from primary producers such as algae and plants, and they are eaten by herbivores and omnivores. Then herbivores and omnivores are eaten by carnivores and finally by predators at the top of the food web pyramid. It has been designed that the TP values of organisms estimated based on the equation (1) with the observed $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values can correspond to the defined, actual trophic position of organisms within 0.17 units as an error (1σ) of accuracy for terrestrial food webs (Chikaraishi et al., 2011). Moreover it has been proved by several investigations using controlled feeding experiments and well-characterized wild species (e.g., Steffan et al., 2013; Bradley et al., 2014; Downs et al., 2014). On cross-plots for the $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values (Fig. 1), resource and consumer species should be arrayed in an almost vertical column within a narrow range on the $\delta^{15}N_{Phe}$ values of the resource species and with 8.0‰ interval of the $\delta^{15}N_{Glu}$ values for each integer-based number of the trophic position, if the consumers feed on only the resource species (Chikaraishi et al., 2014).

In the present study, the $\delta^{15}N_{Phe}$ value of the baby sprout (+5.8‰) is very close to that of the mother sweet potato (+5.2‰), while the $\delta^{15}N_{Glu}$ value of the sprout (+6.5‰) is more positive value than that of the sweet potato (−0.4‰). Thus, both baby sprout and mother sweet potato are likely arrayed in a vertical column on the $\delta^{15}N_{Phe}$ value of the sweet potato (Fig. 1b). Moreover, although the magnitude of ^{15}N-enrichment is slightly small for glutamic acid, the isotopic heterogeneity within the sweet potato (i.e., between the mother sweet potato and its baby sprout) is similar to the isotopic discrimination generally found in the combination between plants and herbivores during grazing food webs (8.0 ± 1.1‰ for glutamic acid and 0.4 ± 0.4‰ for phenylalanine, Chikaraishi et al., 2010). These results strongly suggest an interesting trophic hierarchy that the baby sprouts are approximately one trophic level higher than the mother sweet potatoes.

For the sprouting, proteins in mother sweet potatoes could be reconstructed to build proteins in baby sprouts. Simultaneously, their stocked organic materials (e.g., sugar, protein, and/or lipids) could be employed as energy resources for the reconstruction of proteins. In our knowledge, because sweet potatoes stock a large pool of starch (i.e., sugars), it may be assumed that the sugars stocked in mother sweet potatoes are available as major energy resources in sprouting, while the proteins stocked may be only converted into proteins in baby sprouts (but not into growth energy). If this assumption is correct, an absence of the change in the $\delta^{15}N$ value should be found for any amino acids between mother sweet potatoes and baby sprouts due to no isotopic deamination occurring on amino acids. However, much and less heterogeneities in the $\delta^{15}N$ value for glutamic acid and phenylalanine, respectively, within a single sweet potato (as shown in Fig. 1) suggest that the proteins in the mother sweet potatoes are major resources for not only proteins in their baby sprouts but also growth energy in the sprouting when they are grown heterotrophically without any light, as very similar to general phenomenon (i.e., catabolism) found in resource and consumer species during grazing food webs. Plants have both pathways for anabolism and catabolism, whereas heterotrophic animals have a single pathway only for catabolism. It is highly likely that the isotopic discrimination process–isotopic fractionation factor and flux on the deamination of amino acids–in the catabolism is common or very similar in both plants and animals.

The TP values of plant materials such as leaves, nuts, and sap have always been reported to be 1.0 within 0.2 as 1σ so far (e.g., Chikaraishi et al., 2011, Steffan et al., 2013). However, the TP value of the sprout in the present study is 2.2, which implies that the TP values of plant materials are potentially not always found within 1.0 ± 0.2, particularly if the respiration (i.e., catabolism) is much larger than the photosynthetic production (i.e., anabolism), as the stages of sprouting and blooming. Moreover, the TP value of the mother sweet potato in the present study is 1.4, which borders on the upper TP value of plants within 2σ. Although we have only one data point in the present study, this relatively high TP value (i.e., 1.4) for the mother sweet potato may potentially suggest that sweet potatoes employ stocked proteins as energy resources to survive for a long time in winter or to sprout in spring season.

Base on the data in the present study, we found three tentative summaries as follows:

(1) the baby sprouts are approximately one trophic level higher than the mother sweet potatoes,
(2) this is probably because the proteins in the sweet potatoes are major resources for not only proteins in the baby sprouts but also growth energy in the sprouting, when they are grown without any light, and
(3) the TP values of plant materials may somewhat increase (i.e., not always within 1.0 ± 0.2) when the respiration is much larger than the photosynthetic production, as the stages of overwintering, sprouting, and/or blooming.

3.3. Implication in isotope ecology

The number of studies using the nitrogen isotopic composition of amino acids has increased significantly during the last 5 years. Indeed, it has been employed as a potential powerful tool to understand ecosystems and

elucidate ecological issues accurately and precisely. However, the universality of the trophic isotopic discrimination of amino acids is still in the investigation stage for various taxa of organisms at this moment. In the present study, we demonstrated that magnitude of the isotopic discrimination during the catabolic stages of sweet potatoes is very similar to that generally found in heterotrophic animals (Fig. 1). If these results are commonly found in other plants, it is highly supportive of that magnitude of the trophic isotopic discrimination of amino acids is, in principal, common for the catabolic stages of both plants and animals.

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