A Hydroponic Culture for Detection of Theanine from Roots of Tea Plant (*Camellia sinensis*)

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Summary

The beneficial effects of green tea are well documented. However, most research has reported the effects of green tea brewed solely from leaves or leaf extracts. We focused on tea roots and developed a hydroponic system to explore the effect on roots that biosynthesize one of the rarest functional amino acids, theanine. The level of theanine in tea roots was much higher than in leaves, which was analyzed using HPLC. Moreover, a higher level of theanine was detected in white rootlets than in lignified roots. Thus, tea roots cultured hydroponically in a controlled environment might be considered a natural drug containing theanine, which could lead to synergistic effects with other ingredients of the root. This novel medicinal material from the roots demonstrates a significant medical function for tea that extends beyond its leaves.

Introduction

Theanine (γ-ethylamide-L-glutamic acid), one of the rarest amino acids and an ingredient of green tea (also found in *Camellia* genus*, C. assamica, C. taliensis, C. irrawadiensis C. furfuracea*), and has not been found in any other plant and has only been found in one inedible mushroom (Isemura, *et al*., 2002).

Recently, the biosynthesis of theanine in two species belonging to the genus *Schima* (*S.wallichii and S. mertensiana*) was also investigated (Wei-Wei, *et al*., 2010). The current research has shown that theanine has psychoactive properties, because it is readily absorbed and permeates the blood-brain barrier to function in the brain (Yokogoshi, e*t al.*, 2002), leading to reduced mental and physical stress, improved cognition, and boosting of mood in a manner that is synergistic to caffeine (Juneja, *et al*., 1999; Kimura, *et al*.,2007; Haskell, *et al*., 2008; Park *et al*., 2011). Thus, tea leaves containing theanine, which can exhibit preventive or ameliorating effects on brain dysfunction, have begun to attract attention in our aging and stressed society. Though theanine is synthesized from glutamic acid and ethylamine by γ-L-glutamylethylamide ligase in the roots, and accumulates in leaves through stems (Konishi and Takahashi, 1969), the roots have not been extensively studied. Detailed quantitative analysis of roots cultivated in soil is complicated by the presence of a lignified taproot with very fine lateral roots that are intricately shaped. In addition, it appears that lignified taproots contain less theanine than leaves. We therefore employed a modified hydroponic culture system to examine whether the roots of tea plants could be used as a potential source of theanine. We analyzed the root theanine content and assessed the potential application of tea roots as a medicine for improving human physiological function.

Materials and methods

Hydroponic Culture of Tea Plants

in soil until roots were established for approximately 1-2 months in order to conveniently obtain young plants with roots. The plants with fresh roots were moved to plastic pots and cultured in a nutrient solution with continuous aeration under controlled conditions in a Biotron incubator (Nihonika, Japan) (Saito and Ikeda, 2012). Day/night temperatures were kept at 25/18°C, photosynthetic photon flux density (PPFD) at the plants was 40.0 μmol m^{-2} s^{-1} during the 12 h day period, and the relative humidity was about 60%. The nutrient solution was changed once a week. The roots were shaded and cultured for several months to supply

materials for this experiment.

Determination of Theanine

To determine the concentration of theanine, actively growing white roots were washed with distilled water, dried in a drying oven at 50°C overnight, homogenized with three times the volume of 3% sulfosalicylic acid solution using an ultrasonic homogenizer, and then centrifuged at 12,000 g for 10 min. The concentration of the amino acids in the filtered supernatant was analyzed using an L-8500 automatic amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan).

Results and discussion

We employed hydroponics to allow quantitation of the content of theanine in the roots of tea. Fig. 1A shows the appearance of a representative plant cultured hydroponically for one month after transplanting from soil, and then the plants were grown for six months to obtain a large amount of fine white roots. The yield of roots of the tea plant produced depends entirely on the growth (data not shown).

Tea roots cultivated hydroponically were ideally suited for the analysis and biosynthesis of theanine; the white rootlets contained 12 g theanine per 100 g dry weight of roots, a value three times higher than that of lignified taproots cultivated hydroponically (Table 1); for comparison, the typical theanine content of leaves from plants cultivated in soil is about 1-2 g/100 g.

Table 1. Concentration of theanine produced by different cultivation systems

Sample	Cultivation	Conc.(g/100g)[a]
Leaves	Soil	1.30 ± 0.61[b]
Leaves	Hydroponics	1.45 ± 0.26
Lignified taproots	Hydroponics	3.33 ± 1.15
Fine white roots	Hydroponics	9.8 ± 1.75

[a] *Values represent the means ± SEM (n=3).*

[b] *Max amount when plants were shaded and cultivated.*

The various biosynthesized substances obtained by hydroponic cultivation (e.g. saccharides, flavonoids) were present at lower amounts than in plants cultivated in soil due to the effect of PPFD on photosynthesis in leaves (data not shown). In the presence of sunshine or other light, theanine is converted to other compounds, such as catechins, so high PPFD inhibits the accumulation of theanine in leaves (Konishi and Takahashi, 1969; Saito and Ikeda, 2012). In addition, only a trace amount of theanine was detected in roots cultivated in soil, indicating that roots cultivated in soil are not a suitable source of theanine. However, hydroponically cultivated tea roots may contain higher amounts of theanine.

Generally, high-quality green tea is cultured in the shade so that it will accumulate theanine, which has a pleasant flavor; shade inhibits the decomposition of theanine. However, this procedure leads to only 2% theanine in dried leaves, which is inefficient for collection of theanine and is not industrially practical. Accordingly, a chemical means of synthesis was developed as a method for industrial production of theanine in large quantities (Kimura and Miura, 1971). However, the yield of this organic synthesis is low, and the operation is complicated by the need for separation and purification of theanine from a mixture of unreacted materials and by-products. In addition, recently a synthetic method of theanine using bacteria was developed, which has now become an important source of theanine (Kawagishi and Sugiyama,1992). However, the product obtained by this method is not the genuine theanine from Camellia genus. In this study, our findings suggested that hydroponic culture could be employed as an alternative method to obtain large amounts of theanine, albeit not in high purity. However, tea roots may offer a new type of drug based not only on the function of theanine but also possible synergy with other tea root components, which might offer benefits as a Chinese herbal medicine.

Consequently, hydroponics makes it possible to control environmental conditions during growth of tea

plants. We have already succeeded in rooting cuttings of tea plants in a nutrient solution only. Therefore, it is likely that this approach to cultivation will facilitate the extraction of theanine from the roots.

Recent demand for theanine has increased due to its use as a food additive for enhancing flavor and as a supplement for supporting human health, especially mental health (Yokogoshi, *et al*., 2002). Indeed, we propose that the roots of tea plants, which contain high amounts of theanine, may attenuate brain dysfunction. Further study using animals will likely reveal the effects of tea roots on the brain and other organs.

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