Seasonal Variation in the Yield and Polyphenol Content of Sweet Potato (Ipomoea batatas L.) Foliage

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Despite increasing demand for sweet potato foliage, which is rich in functional components, efficient methods to maximize yield are still needed. In this study, cultivation tests for sweet potato (line Kyukei05303-3) were conducted over three consecutive years at a greenhouse to characterize seasonal changes in the foliage yield (leaves and stem-petioles), as well as the polyphenol content. The sweet potato foliage was harvested from May to November every week, and the average leaf yield was $855.3 \text{ g·m}^{-2} \cdot \text{year}^{-1}$ on a dry weight (DW) basis. The yield and polyphenol content of the leaves were negatively correlated. The yield increased from spring to summer but decreased after mid-August. In contrast, the polyphenol content was highest in May, lower during the summer (June to August), and increased again after September. The average polyphenol content in the leaves was $6.9 \text{ g·100 g}^{-1}$ DW and the total annual polyphenol yield was $59.0 \text{ g·m}^{-2}$. The major component of polyphenols was caffeoylquinic acids. The seasonal changes in caffeoylquinic acids were highly correlated with the changes in total polyphenols. The polyphenol content was significantly correlated with air temperature, but not with sunshine duration, suggesting that air temperature is an important determinant of the polyphenol content during cultivation. These results provide a basis for the rapid cultivation of sweet potato for foliage production.

Key Words: caffeoylquinic acid, culture condition, polyphenol contents, sweet potato foliage yield.

Introduction

In Japan, the foliage (leaves, petioles, and stems) of sweet potato (Ipomoea batatas L.) is not as widely utilized as its storage roots, which are commonly consumed or used as raw materials for various purposes such as food processing, ‘shochu’ (sweet potato liquor) production, starch production, and pigment production. In contrast, sweet potato foliage is popular as a leafy vegetable in some countries in East Asia, Southeast Asia, and Africa, given its high nutritional value (Antia et al., 2006; Ishida et al., 2000). Therefore, in Japan, Suioh, a sweet potato variety with a pleasant taste, has been bred (Ishiguro et al., 2004). Sweet potato foliage is rich in functional components such as luteins and polyphenols, as compared to other commercial vegetables (Ishiguro and Yoshimoto, 2006). The major components of polyphenols in the foliage tissue of sweet potato are caffeic acid (CA) and caffeoylquinic acids (CQAs), including chlorogenic acids (ChA), 3,4-di-O-caffeoylquinic acid (3,4-DCQA), 3,5-di-O-caffeoylquinic acid (3,5-DCQA), 4,5-di-O-caffeoylquinic acid (4,5-DCQA), and 3,4,5-tri-O-caffeoylquinic acid (3,4,5-TCQA). These components have various health-promoting functions, such as antioxidative activities (Islam et al., 2002), anti-mutagenic activities (Yoshimoto et al., 2002), and the suppression of cancer cell growth (Kurata et al., 2007). CA and CQAs were observed in all 1,389 sweet potato genotypes investigated by Islam et al. (2002). Shimada et al. (2010) developed an efficient method for the extraction of polyphenols from sweet potato foliage. Using this method as a key technology, industries in Japan have started the large-scale extraction of polyphenols from sweet potato. The demand for sweet potato foliage as a functional food material is increasing in Japan. Accordingly, our main research goals are to improve mass cultivation techniques for industrial use and maximize yield. Sweet potato is a vegetatively propagated crop, grown using stem cuttings prepared from the mature sprouts of the storage roots. In Japan, farmers plant the storage roots in nursery beds placed in plastic greenhouses from January to March and prepare stem cut-
tions from April to June for transplantation to the field. The remaining ground parts of the cut sprouts in the nursery beds regenerate immediately, enabling multiple preparations of stem cuttings during the transplantation period. Normally, when cut sprouts are transplanted to the field, the nursery beds are closed. A system in which sweet potato nursery beds are used continuously by repeating the harvesting and regeneration cycle has the potential for high yield and space-saving foliage production. However, seasonal changes in the yield or polyphenol content of foliage have not been evaluated using such a cultivation system, and nor have the environmental factors contributing to these changes. In this study, the sweet potato line Kyukei05303-3, characterized by a higher polyphenol content than Suioh, was used to clarify seasonal variation; we repeated the harvesting and regeneration of sweet potato foliage in nursery beds and analyzed seasonal changes in the yield and polyphenol content, as well as the environmental factors affecting these changes.

Materials and Methods

Plant materials

Sweet potato plants (line Kyukei05303-3) were cultivated in a plastic greenhouse (40 m long, 5 m wide, and 3.2 m high, covered with a 0.1-mm-thick plastic sheet) at Kyushu Okinawa Agricultural Research Center, NARO (Miyakonojo, Miyazaki, Japan) from 2010 to 2012. The air in the greenhouse was ventilated by forced air. This ventilation was started at 35°C by using a thermostat. Stem cuttings (Fig. 1A) with 2 to 3 nodes were planted to achieve 53.3 cuttings·m$^{-2}$ (15×15 cm) in 2010 (planted on April 28) and 2011 (planted on April 15), and 66.7 cuttings·m$^{-2}$ (15×10 cm) in 2012 (planted on April 2), in a 1.5-m-wide nursery bed in the greenhouse. Stem cuttings were used instead of seed tubers to avoid variation in planting density, which may result from variation in the number of sprouts per seed tuber. One month after transplantation, the foliage of plants with more than 7 nodes was harvested once a week from 9 am to 10 am. Seven or more seedlings were harvested every week for uniform sampling. The day of the first harvest was considered 0 days after cutting (0 DAC). In Figure 1B, images obtained at 0, 7, and 14 DAC are shown. The harvested plants were washed with tap water, and the leaf blades (hereafter ‘leaf samples’) were separated from the other parts containing the stems and petioles (hereafter ‘stem-petiole samples’) (Fig. 1C). As shown in Table 1, the foliage was harvested from May 26 to October 22 (22 weeks) in 2010, from May 13 to November 11 (27 weeks) in 2011, and from May 11 to November 30 (30 weeks) in 2012. In 2012, it was iterated three times, but 2010 and 2011 were not iterated. The stem-petiole samples were collected in 2011 and 2012 only. After measuring the fresh weight (in 2011 and 2012 only), the samples were immediately freeze-dried. After measuring the dry weight of the leaves, the samples were milled into a fine powder and stored at −30°C until the polyphenol content was measured.

Chemicals

CA was purchased from Wako Pure Chemical Industries (Osaka, Japan). ChA was obtained from

| Year | Sampling period (Number of times) | Annual yield | Average yield per sampling | Average polyphenol content | Annual polyphenol yield |
|------|----------------------------------|--------------|---------------------------|---------------------------|-------------------------|
|      |                                  | Dry weight (g·m$^{-2}$·year$^{-1}$) | Leaf | Stem-petiole | Leaf | Stem-petiole | Leaf | Stem-petiole | Leaf | Stem-petiole |
| 2010 | 5/26–10/22 (22)                  | 740.3        | —                      | 33.7                      | —                      | 6.5              | —          | 48.4              |
| 2011 | 5/13–11/11 (27)                  | 854.2        | 606.9                  | 31.6                      | 22.5                  | 7.8              | 3.5        | 66.5              | 21.4          |
| 2012 | 5/11–11/30 (30)                  | 971.5        | 732.3                  | 32.4                      | 24.4                  | 6.4              | 2.7        | 62.3              | 20.0          |
| Average |                                  | 855.3±94.4  | 669.6±62.7            | 32.6±0.87                | 23.5±0.95            | 6.9±0.64        | 3.1±0.40   | 59.1±7.73        | 20.7±0.70    |
Sigma Chemicals (St. Louis, MO, USA). 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, and 3,4,5-triCQA were extracted and purified (> 97%) from sweet potato leaves (Kurata et al., 2011).

**Polyphenol extraction and quantitative analysis**

Fifty milligrams of freeze-dried powder from sweet potato leaves were added to 5 mL of 80% ethanol (v/v) and boiled for 5 min. After centrifugation at 3000 rpm for 15 min, the supernatant was collected and used to measure the total polyphenol content and CQAs. The total polyphenol content of the extract was measured by a partially modified Folin–Ciocalteu method (Coseteng and Lee, 1987) and calculated as ChA equivalents (equiv.) using ChA as the standard substance.

Figure 2 shows chromatograms of CA and CQAs measured by high-performance liquid chromatography (HPLC) following the methods of Okuno et al. (2010). Briefly, the extract was filtered through a polytetrafluoroethylene membrane (0.20 μm; ADVANTEC, Tokyo, Japan), a 5-μL portion of the filtrate was injected into the HPLC system and it was eluted as described below. The HPLC system consisted of a DUG-12A degasser, an SIL-10A XL auto-injector, a CTO-10A column oven, an SPD-M10A UV-VIS detector, an SCL-10A VP system controller, and two LC-10AD pumps (Shimadzu Co., Kyoto, Japan). The system was controlled by the LC solution (version 1.24 SP1) workstation (Shimadzu). A YMC-Pack ODS-AM (75 mm × 4.6 mm i.d., 3 μm particles; YMC Co., Ltd., Tokyo, Japan) was used, and the temperature of the column oven was set to 40°C. The CA and CQA derivatives were detected by absorbance at 326 nm. The mobile phase consisted of water containing 0.2% (v/v) formic acid (A) and acetonitrile (B). Elution was performed with a linear gradient of B as follows: 2% to 19% from 0 to 8 min, 19% to 52% from 8.01 to 16 min, 100% isocratic from 16.01 to 20 min, 100% to 2% from 20 to 20.01 min, and 2% isocratic from 20.01 to 26 min. The flow rate of B was 1 mL·min⁻¹. The obtained polyphenols were then compared with the authentic reagents.

**Statistical analysis**

The air temperature outside the greenhouse and sunshine duration were obtained from weather data recorded by the Miyakonojo Observation Station, which forms part of the Automated Meteorological Data Acquisition System (AMeDAS) of the Japan Meteorological Agency.

Correlations between the weather data, dry weight, and polyphenol content were calculated, and the significance of the results was analyzed using the t-test.

**Results**

**Sweet potato foliage yield**

In our cultivation system, the regeneration of sweet potato foliage was quite fast and foliage could be harvested many times within a short period (Fig. 1B). The regeneration rate increased as the temperature increased in the summer and decreased as the temperature fell in the autumn. The total yields of the leaf samples per unit area (in terms of dry weight (DW)) were 740.3 g DW·m⁻² in 2010, 854.2 g DW·m⁻² in 2011, and 971.5 g DW·m⁻² in 2012. The average yield of the leaf samples per harvest was 32.6 ± 0.87 g DW·m⁻², suggesting an approximate variation of 3% between sampling. The average polyphenol content in the leaf samples was 6.9 g·100 g⁻¹ DW, more than twice that in the stem-petiole samples. The average annual yield of polyphenols from the leaf samples was 59.1 ± 7.73 g·m⁻² (Table 1).

**Seasonal changes in sweet potato foliage yield**

Figure 3 shows the seasonal changes in the foliage yield in 2012 on a DW basis. The highest and lowest values of leaf samples were 48.3 g DW·m⁻² and 19.3 g DW·m⁻² observed on August 3 and November 15, respectively. The yield of the stem-petiole samples was 51.3% to 90.2% that of the leaf yield. The leaf yield was unstable in May and November and stably obtained in the summer (June to September). Similar seasonal changes in leaf yields were also observed in 2010 and 2011 (Supplementary data 1), and high values were observed around August.

**Seasonal changes in total polyphenol and CQAs content**

Figure 4 shows the seasonal changes in the total polyphenol and CQAs content in leaf samples in 2012. After recording the highest value of 10.29 g ChA equiv·100 g⁻¹ DW on May 11, the total polyphenol content decreased and reached the lowest value of 4.91 g ChA equiv·100 g⁻¹ DW on August 10. The content increased again thereafter, reaching 7.98 g ChA equiv·100 g⁻¹ DW on November 30. The average CQAs content was 4.01 ± 0.74 g·100 g⁻¹ DW. The correlation coefficient between total polyphenol and amount of CQAs content in the seasonal variation was r = 0.935 (P < 0.01) and showed the same behavior. The seasonal change in CQAs was highly cor-
related with the change in total polyphenols; the highest value was recorded on May 11 (6.56 g·100 g$^{-1}$ DW) and the lowest value was observed on July 20 (2.92 g·100 g$^{-1}$ DW), followed by an increase to 4.97 g·100 g$^{-1}$ DW on November 30. Similar results were obtained in 2010 and 2011, with the total polyphenol and CQAs being higher in the spring and autumn and lower in the summer (Supplementary data 2).

Among the CQAs, 3,5-DCQA was the most abundant, accounting for 52.6% to 72.6% of the total CQAs. ChA and 4,5-DCQA accounted for 11.5% to 37.2% and 4.4% to 14.0% of the total CQAs, respectively. The total content of CQAs appeared to decrease between June and September, and the average value of total CQAs per month decreased significantly ($P < 0.05$) in July, August, and September compared to May. Conversely, the 3,5-DCQA with the highest content among the total CQAs increased in June, and the average values in May, September, October, and November compared to the June level decreased significantly ($P < 0.05$). The second highest content ChA of total CQAs decreased in July, while mean values in May, October and November significantly increased ($P < 0.05$). A negative correlation $r = -0.857$ ($P < 0.01$) was observed for the content of 3,5-DCQA and ChA in the seasonal variation (Supplementary data 3).

**Relationship between the dry matter yield and polyphenol content**

Because the total polyphenol and CQAs content were higher in the spring and autumn when the dry matter yield of the leaf samples was low, the leaf yield and polyphenol content were expected to be negatively correlated. As shown in Figure 5, statistically significant negative correlations were observed in 2011 and 2012. In 2010, the correlation was not significant; it is possible that the first sampling data were outliers.

**Relationships between the polyphenol content and environmental conditions**

No significant correlation was observed between the total polyphenol content and the average sunshine duration during the 1-week period before harvest (Fig. 6). In contrast, significant negative correlations were observed between the total polyphenol content and average temperature 1 week before harvest in all 3 years (Fig. 7).

**Discussion**

In the current study, a rapid cultivation system for sweet potato foliage production on nursery beds was used for 3 years at a greenhouse and seasonal changes in the yield (leaves and stem-petioles) and polyphenol
content of foliage were clarified. The sweet potato foliage could be harvested from May to November every week, and the average yield of leaves was 855.3 g·m\(^{-2}\)·year\(^{-1}\) on a DW basis. The yields of spinach and \textit{Brassica rapa} \textit{L.} \textit{Perviridis} (spinach mustard; komatsuna) in Japan are reported to be 1,210 g·m\(^{-2}\) and 1,660 g·m\(^{-2}\) on a fresh weight (FW) basis, respectively (MAFF, 2014). Even though these vegetables can be harvested three to four times a year, the maximum yields are approximately 4,840 g·m\(^{-2}\) and 6,640 g·m\(^{-2}\) for spinach and komatsuna, respectively. The average annual yield of sweet potato leaves obtained in this study (6,011.2 g FW·m\(^{-2}\)) is comparable to the yields of these leafy vegetables. Thus, the sweet potato leaf has the same level amount of biomass production as these leaf vegetables harvested from spring to autumn.

Both the dry matter yield and polyphenol content were higher in the leaf samples than in the stem-petiole samples. Notably, the average polyphenol content in the leaf samples was more than twice that in the stem-petiole samples in 2011 and 2012 (Table 1). Thus, leaves are a preferable source of polyphenols for industrial extraction. The total polyphenol yield was 59.1 g ChA equiv.·m\(^{-2}\), reaching 6.9% of the dried leaf weight. The planting density was 53.3 plants·m\(^{-2}\) in 2010 and 2011 and 66.7 plants·m\(^{-2}\) in 2012. The yields of the leaf and stem-petiole samples did not increase in 2012, suggesting that a higher planting density does not effectively increase the total polyphenol yield.

The yield of the leaf samples increased in summer (Fig. 3). Foliage growth is expected to be promoted by a higher temperature during the summer because sweet potato originates from the tropical Americas. In contrast, the polyphenol content was higher in the spring and autumn, but lower in the summer, showing a negative correlation with the leaf yield. This suggests that lower temperatures before harvest increase the total polyphenol content. This result supports the results of Taira et al. (2007), who investigated the polyphenol content of sweet potato leaves in the spring and summer. The CA and CQAs also showed the same pattern. CA used for the biosynthesis of ChA and CQAs is also a precursor for the biosynthesis of lignin, which is nec-
necessary for plant growth (Vanholme et al., 2010). It is possible that enhanced plant growth in the summer increased the rate of lignin biosynthesis, thus reducing the polyphenol content. Another possibility is that the sweet potato grows vigorously in the summer and the dry matter production activity is very high, but the polyphenol content is low because polyphenol synthesis cannot keep up with dry matter production. When the polyphenols of sweet potato foliage are used commercially, the polyphenol concentration becomes very important. An increase in the polyphenol content of sweet potato grows vigorously in the summer and the production of polyphenols. Clarification of the seasonal fluctuation of polyphenols. Another possibility is that the polyphenol content is low because polyphenol synthesis cannot keep up with dry matter production. When the polyphenols of sweet potato foliage are used commercially, the polyphenol concentration becomes very important. An increase in the polyphenol content of sweet potato grows vigorously in the summer and the production of polyphenols. Clarification of the seasonal fluctuation of polyphenols.

Islam et al. (2003) reported that compared to 0% shading, artificial shading at 40% and 80% decreases the phenolic content of sweet potato leaves. However, in this study, the polyphenol content was not correlated with the sunshine duration before harvest. It is possible that a lower sunlight intensity in the greenhouse compared to the outside environment reduced the effect of sunlight. We observed a negative correlation between the average air temperature and the polyphenol content. This suggests that lower temperatures before harvest increase the total polyphenol content. Additionally, CA and CQAs are synthesized by enzymatic reactions (El-Seedi et al., 2012). The synthesized CA and CQAs are used for lignin synthesis. It is possible that enhanced plant growth in a high air temperature environment increased the rate of lignin biosynthesis, thus reducing the polyphenol content. On the other hand, it is possible that the optimum temperature for the biosynthesis of CA and CQAs is lower than the average air temperature in the greenhouse. Since a greenhouse has a high heat retention effect, cooling is not very effective even if a fan ventilation system is operating. Therefore, the polyphenol content of sweet potato leaves cultivated in open land, where the temperature fluctuation is lower than in a greenhouse, may be different. Further studies are necessary to clarify the relationship between the polyphenol content and environmental conditions, especially temperature.

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