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Investigation of Total Polyphenol Content, Antioxidant Activity, and Carbohydrate-hydrolyzing Enzyme Inhibition of Yacon Leaves among Four Domestic Cultivars and a Peru Line for over Four Years

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Abstract

Yacon, an Andean crop, was historically introduced into Japan for local consumption of its tuberous roots as a sweet vegetable. We recently examined the multi-functional effects of yacon herbal tea to establish the aerial part as a health food material. The current study aimed to investigate whether any functional differences exist among the yacon leaves from four domestic cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11) maintained for several years in the Aso area of Kyushu. The total polyphenol content (TPC) and antioxidant activity of cultivar SY237 exceeded those of SY11, but the values for individual cultivars were inconsistent over the tested years. In carbohydrate-hydrolyzing enzyme inhibition assays, there was a large variation in all cultivars among the tested years, with three cultivars including SY237 partially demonstrating α-glucosidase inhibition comparable to or stronger than that of SY11. Based on multivariate analysis with functional data from over four years, the characteristic positioning of these yacon cultivars was systematically visualized. As a result, we found that SY237 could be a candidate cultivar that is partially superior to SY11, although the yearly variance should be considered.

Discipline: Food
Additional key words: α-amylase, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) cation radical, α-glucosidase, 1,1-diphenyl-2-picrylhydrazyl radical, *Smallanthus sonchifolius*

Introduction

Yacon (*Smallanthus sonchifolius*) is an Andean crop that was historically introduced into Oceania, Europe, and several countries in Asia, including Japan (Ojansivu et al. 2011, Gurung et al. 2018). Four different yacon cultivars—'*Sarada otome*' (SY201) (Sugiura et al. 2007), '*Andesu no yuki*' (SY206) (Fujino et al. 2008), '*Sarada okame*' (SY217) (Fujino et al. 2008), and '*Andesu no otome*' (SY237) (Sugiura et al. 2014)—are currently registered with the Ministry of Agriculture, Forestry and Fisheries as the official varieties in Japan. One Peru A line (SY11) was initially introduced from Peru via New Zealand, and then spread and became a line selected in Japan for breeding and cultivation studies (Sugiura 2016, Sugiura & Yano 2016). For instance, SY11 has been used as a parental line of the new cultivar SY237, and also used as a control line for a cultivation study (Sugiura & Yano 2016). The tuberous roots of this plant have been consumed as a sweet vegetable, whereas most of the aerial part becomes harvest residue (Kabata et al. 2006), though a portion of the leaves can be processed for local consumption as an herbal tea. In terms of functional benefits, yacon leaves have exhibited hypoglycemic effects in rat models (Aybar et al. 2001, Baroni et al. 2016); and also possess antioxidant (Valentova et al. 2005, Ueda et al. 2019a), antifungal (Lin et al. 2003), and anticancer (Bai et al. 2017, Mendoza et al. 2017) activities.

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With regard to yacon leaves, we have previously reported that the commercially available yacon herbal tea in Japan from cultivar SY201 exhibits several functional effects, including antioxidant activity, especially in synthetic free radical and O_2^- radical scavenging assays (Sugahara et al. 2015), the inhibition of carbohydrate-hydrolyzing enzymes, such as α-glucosidase and α-amylase (Ueda et al. 2017a), and anti-inflammatory activity (Ueda et al. 2017b). Using the yacon leaves from cultivar SY206, we also demonstrated that heat processing can potentiate the antioxidant capacity by increasing the active phenolic content, such as caffeic acid (Ueda et al. 2019b). Therefore, we were interested in elucidating any functional differences among domestic yacon cultivars to support yacon leaves as a favorable material for manufacturing food products beneficial to health.

In earlier research on yacon cultivars, the tuberous roots of SY201 and SY217 harvested in Ibaraki, Japan over two years (November 2008 and 2009) may possess higher polyphenol content than those harvested from SY206 (Takenaka et al. 2011). We reported the phenolic parameters and antioxidant capacities of the leaves of cultivars SY201 (Sugahara et al. 2015) and SY206 (Ueda et al. 2019b). However, it has not been systematically assessed whether any functional differences exist among yacon leaves from various domestic cultivars grown over several years.

In this study, we investigated whether any functional differences exist in yacon leaves from these different cultivars (SY201, SY206, SY217, and SY237) plus a Peru A line (SY11) maintained in the Aso area of Kyushu for several years, especially in terms of total polyphenol content (TPC), antioxidant activity, and carbohydrate-hydrolyzing enzyme inhibition. Moreover, the data obtained were subjected to multivariate analysis to systematically visualize the characteristic positioning of these yacon cultivars.

Materials and methods

1. Materials

Four domestic yacon cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11) originally separated for propagation from the Western Region Agricultural Research Center, National Agriculture and Food Research Organization (Shikoku Research Station, Kagawa, Japan) have been maintained at an institutional test field, located in the Aso area of Kyushu and owned by Tokai University's School of Agriculture (32°53′29.1″N, 130°59′43.5″E, 486 m above sea level, Minamiaso, Kumamoto, Japan). The fresh seed tubers (15 g - 20 g) were planted in mid-March at 50-cm intervals in plastic film mulched rows set 100 cm apart. The fertilizer used for the planted tubers comprised 10 kg of N and P_2O_5 and 20 kg of K per 10 a (1,000 m^2). At least 80 individual plants from four yacon cultivars and SY11 were planted by random block design for 10 a, with a total of 910 plants including other lines being maintained. In this study, one or two leaves of SY201, SY206, SY217, SY237, and SY11 from almost maximum plant height were randomly sampled at the second position from the top of at least five different plants in early-to-mid November (Nov. 11, 2013, Nov. 6, 2015, Nov. 7, 2016 and Nov. 10, 2017). These leaves were collected in the season before being affected by frost, when SY206 and SY237 were flowering. As the tuberous roots were harvested in November in the test area, the leaves were collected during the same period. These leaves were stored at −20°C and then lyophilized. 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt for preparing its cation radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Folin–Ciocalteu phenol reagent were purchased from Nacalai Tesque Inc. (Kyoto, Japan). *p*-Nitrophenyl α-$_D$-glucopyranoside was obtained from Fujifilm Wako Pure Chemical Co. (Osaka, Japan). α-Amylase from porcine pancreas Type VI-B, *p*-nitrophenyl α-$_D$-maltoside, and Trolox were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). α-Glucosidase from yeast was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Acarbose was obtained from LKT Laboratories, Inc. (St. Paul, MN, USA). All other chemicals were of the highest grade commercially available.

2. Preparation of leaf extracts

Extracts of yacon leaves were prepared using 50% methanol as previously described (Ueda et al. 2019b). Briefly, 0.781 g of lyophilized, crushed, and pooled leaves were soaked in 10 mL of methanol at 4°C overnight. The leaves in methanol were continuously kept at 4°C for another day with an additional 10 mL of MilliQ water. The mixture was filtered and lyophilized to obtain the extract, which was reconstituted in 50% ethanol at 20 mg/mL for further analysis.

3. Determination of total polyphenol content

TPC was determined as previously reported (Singleton & Rossi 1965) with minor modifications (Sugahara et al. 2015). Briefly, the mixture containing the extract and tenfold diluted Folin–Ciocalteu phenol reagent solution was incubated with 10% sodium carbonate solution for 10 min. at room temperature (20°C-25°C). The absorbance of the mixture was

measured at 600 nm using a grating microplate reader (SH-1000Lab, Corona Electric, Ibaraki, Japan).

4. Measurement of antioxidant activity

$ABTS^+$ radical scavenging activity was measured as previously described (Thaipong et al. 2006). Briefly, the $ABTS^+$ solution was prepared by mixing an equal amount of 7.4 mM ABTS and 2.6 mM potassium peroxodisulfate solution for 15 h. The mixture was incubated on a rotator in the dark at room temperature. The reaction was started by adding an ABTS-working solution to the extract. After 2 h of incubation in the dark at room temperature, the absorbance of the solution was measured at 734 nm.

DPPH radical scavenging activity was measured as previously described (Blois 1958). The reaction was initiated by adding 0.5 mM DPPH dissolved in ethanol into the assay mixture containing the extract, 70% ethanol, and 0.1 M sodium acetate buffer (pH 5.5). The mixture was incubated for 30 min. at room temperature. The absorbance of the solution was measured at 517 nm.

5. Measurement of carbohydrate-hydrolyzing enzyme inhibition

α-Amylase inhibition was measured as previously described (Sama et al. 2012) with a minor modification (Ueda et al. 2017a). The enzymatic reaction was initiated by adding 130 U/mL α-amylase into the assay mixture containing the extract (final conc. 1.00 mg/mL), 1% sodium chloride, 0.1 M phosphate buffer (pH 6.7), MilliQ water, and 6.5 mM *p*-nitrophenyl α-$_D$-maltoside. The mixture was incubated for 0 min. and 180 min. at 37°C, and the absorbance of the solution was measured at 400 nm. Varying concentrations of acarbose were used as the standard inhibitor for preparing a calibration curve (the calculated half maximal inhibitory concentration (IC_{50}); 0.0213 ± 0.0025 mg/mL)). The inhibition activity (%) of the leaf extracts (32.6% to 56.8% at 1.00 mg/mL) and extraction yields from dry weight (D.W.) of the leaves were first determined. Thereafter, the inhibiting activity was expressed as "mmol acarbose equivalent (AE)/g D.W. of the leaves."

α-Glucosidase inhibition was measured as previously described (Matsui et al. 1996) with a minor modification (Ueda et al. 2017a). The enzymatic reaction was initiated by adding 0.887 mM *p*-nitrophenyl α-$_D$-glucopyranoside into the assay mixture containing the extract (final conc. 1.00, 2.00, and 3.00 mg/mL) and 0.08 U/mL α-glucosidase. The mixture was incubated for 15 min. at 37°C, and the absorbance of the solution was measured at 400 nm. Varying concentrations of acarbose were used as the standard inhibitor (IC_{50}; 0.496 ± 0.032 mg/mL). The IC_{50} values (mg/mL) or inhibition activity (%) of the leaf extracts at the maximum concentration tested (35.5% to 51.2% at 3.00 mg/mL), and extraction yields from D.W. of the leaves were first determined. Thereafter, the inhibiting activity was expressed as "mmol AE/g D.W. of the leaves."

6. Measurement of yielded weight of the aerial part and tuberous roots of yacon

The weight of aerial parts of the ten yacon plants was measured using a digital scale immediately after cutting at the border of the ground. The fresh weight of the tuberous roots was then measured after soil removal. Individual tuberous roots at 50 g or more were used to determine the yielded weight, and roots less than 50 g were treated as waste. Data were expressed as the mean value from the ten plants.

7. Statistical analysis

The data are expressed as the mean ± standard deviation (SD) of three or four independent experiments. The data in part were analyzed using a statistical add-on software program (Statcel 4, OMS Co., Saitama, Japan) for Excel 2016 (Microsoft Co., Redmond, WA, USA). A post-hoc Tukey–Kramer test was conducted for multiple comparisons, and differences at $P < 0.05$ were considered significant. For multivariate analysis, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were conducted using the Excel-Solver add-on program, and data were normalized to mean 0 and variate 1. HCA was determined by the Euclidean distance method.

Results and discussion

1. TPC in yacon leaves among four domestic cultivars and a Peru A line (SY11)

We first determined TPC in the yacon leaves among four different cultivars (SY201, SY206, SY217, and SY237) and a Peru A line (SY11). The amount of TPC in 1 g D.W. of the leaves was expressed as the chlorogenic acid equivalent (CAE) value. Among the four cultivars plus SY11 collected in 2013, the amount of TPC in 1 g D.W. of leaves from SY237 (57.0 mg) was significantly higher than that from SY201 (45.9 mg), SY206 (33.8 mg), SY217 (47.6 mg), and SY11 (44.9 mg), with a 1.27-fold difference from SY11 as the control line (Table 1). Among samples collected in 2015, 2016 and 2017, the highest values were obtained in SY201 (58.2 mg), SY217 (43.8 mg), and SY237 (73.4 mg), respectively. Only SY237 had more TPC than SY11 in the four different years, although the values for individual cultivars were inconsistent throughout the tested years. As the major phenolic

Table 1. Total polyphenol content in yacon leaves among four different domestic cultivars and a Peru A line collected in four different years*

Years	SY201	SY206	SY217	SY237	SY11
	TPC (mg CAE / g D.W.)**				
2013	$45.9 \pm 2.6^{a,A}$ (1.02)	$33.8 \pm 1.8^{b,A}$ (0.75)	$47.6 \pm 1.7^{a,A}$ (1.06)	$57.0 \pm 1.3^{c,A}$ (1.27)	$44.9 \pm 3.4^{a,A}$ (1.00)
2015	$58.2 \pm 3.1^{a,B}$ (1.74)	$36.9 \pm 1.3^{bc,A}$ (1.10)	$24.5 \pm 1.6^{d,B}$ (0.73)	$40.7 \pm 1.6^{b,B}$ (1.22)	$33.4 \pm 1.5^{c,B}$ (1.00)
2016	$34.0 \pm 1.8^{a,C}$ (1.39)	$28.4 \pm 2.2^{b,B}$ (1.16)	$43.8 \pm 0.8^{c,C}$ (1.80)	$34.4 \pm 0.8^{a,C}$ (1.41)	$24.4 \pm 1.0^{d,C}$ (1.00)
2017	$9.68 \pm 0.33^{a,D}$ (0.32)	$52.1 \pm 2.1^{b,C}$ (1.74)	$29.8 \pm 1.0^{c,D}$ (0.99)	$73.4 \pm 1.9^{d,D}$ (2.45)	$30.0 \pm 0.7^{e,B}$ (1.00)

* Yacon leaves from four different cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11) collected in November of four different years (2013, 2015, 2016 and 2017) were used in this study. Several leaves from different plants were sampled at the second position from the top of plants, and then lyophilized, crushed, and pooled for investigation (see the Materials and methods section).

** Data shown represent mean ± SD from four independent experiments. Tukey–Kramer's test was conducted for multiple comparisons; values not sharing a common superscript (lowercase or uppercase letter) are considered significantly different at $P < 0.05$ among individual cultivars or individual years, respectively. Data in parentheses indicate the relative amounts of TPC in individual cultivars vs. SY11 (1.00) in the same year. TPC: total polyphenol content, CAE: chlorogenic acid equivalent, D.W.: dry weight of sample

compounds, chlorogenic acid, caffeic acid, and ferulic acid are present in the yacon leaves (Valentova et al. 2005, 2006). We previously reported that chlorogenic acid, caffeic acid, and proanthocyanidin are the phenolic constituents present in the leaves of SY206 (Ueda et al. 2019b). A preliminary qualitative HPLC analysis confirmed the appearance of gallic acid-, (-)- epicatechin-, and rutin-like peaks in the leaves of SY206 (data not shown), which have been reported to be present in yacon leaves (de Andrade et al. 2014, Russo et al. 2015a, Marchyshyn et al. 2017). Another report investigated the levels of sesquiterpene lactones in several yacon cultivars maintained in Japan (Kitai et al. 2015), although the phenolic differences among those cultivars have yet to be fully elucidated. It is thus necessary to further investigate the levels of representative phenolic phytochemicals present in different yacon cultivars registered in Japan.

2. Antioxidant activity of yacon leaves among four domestic cultivars and SY11

In the $ABTS^+$ radical scavenging assay, we first determined the half maximal effective concentration (EC_{50}) value (μg/mL) using different concentrations of the leaf extract (see Supplementary Table 1). Results indicated that the leaf extracts possessed the scavenging effects with 20.6 μg/mL to 82.4 μg/mL of the EC_{50} value. The lowest EC_{50} value of the leaf extract (showing strong activity) among the yacon cultivars and SY11 in 2013, 2015, 2016 and 2017 was 20.7 μg/mL (SY237), 20.6 μg/mL (SY201), 29.4 μg/mL (SY237), and 21.2 μg/mL (SY237), respectively. In contrast, 82.4 μg/mL of the highest EC_{50} value was obtained from SY201 in 2017. In this experimental setting, varying concentrations of trolox demonstrated activity with EC_{50}; 8.59 ± 0.15 μg/mL. Using these activity data of the leaf extract and extraction yields from D.W. of the leaves, the radical scavenging activity was further calculated and expressed as the trolox equivalent antioxidant capacity (TEAC) value per gram D.W. of the leaves, wherein trolox was used as a representative antioxidant (Sugahara et al. 2015). In the $ABTS^+$ radical scavenging assay with the samples from 2013, the TEAC values in 1 g D.W. of the leaves from SY201 (268 μmol) and SY237 (280 μmol), respectively, were comparable to and higher than those from SY11 (253 μmol), and significantly higher than those from SY206 (191 μmol) and SY217 (240 μmol) (Table 2). Among the samples collected in 2015, 2016 and 2017, the highest values were obtained in SY201 (322 μmol), SY217 (264 μmol), and SY237 (358 μmol), respectively.

In the DPPH radical scavenging assay, we first determined the EC_{50} value (μg/mL) using different concentrations of the leaf extract (see Supplementary Table 1). Results indicated that the leaf extracts possessed the scavenging effects with 25.6 μg/mL to 128 μg/mL of the EC_{50} value. The lowest EC_{50} value of the leaf extract among the yacon cultivars and SY11 in 2013, 2015, 2016 and 2017 was 25.6 μg/mL (SY237), 30.2 μg/mL (SY201

Supplementary Table 1. Antioxidant activity of extracts prepared from yacon leaves among four different domestic cultivars and a Peru A line collected in four different years*

	SY201	SY206	SY217	SY237	SY11	Trolox
	EC_{50} value (μg extract / mL) in $ABTS^+$ radical scavenging assay**					EC_{50} (μg / mL)
2013	22.2 ± 1.3	30.9 ± 0.2	26.2 ± 0.5	20.7 ± 0.4	22.2 ± 0.5	8.59 ± 0.15
2015	20.6 ± 0.3	27.2 ± 0.4	42.9 ± 0.7	24.2 ± 0.5	34.7 ± 1.0	
2016	38.9 ± 1.0	42.0 ± 0.6	32.2 ± 0.2	29.4 ± 0.2	37.5 ± 0.2	
2017	82.4 ± 0.8	26.7 ± 0.6	41.7 ± 0.6	21.2 ± 0.4	41.5 ± 0.3	
	EC_{50} value (μg extract / mL) in DPPH radical scavenging assay**					EC_{50} (μg / mL)
2013	31.8 ± 0.7	32.7 ± 0.2	29.1 ± 0.3	25.6 ± 0.2	26.8 ± 0.2	7.32 ± 0.08
2015	30.2 ± 0.8	39.7 ± 0.3	78.4 ± 1.1	30.2 ± 0.7	61.8 ± 1.0	
2016	55.1 ± 0.3	53.4 ± 0.3	52.0 ± 1.8	52.6 ± 0.8	68.6 ± 0.6	
2017	128 ± 5.0	54.0 ± 1.2	58.6 ± 0.7	45.4 ± 1.3	54.3 ± 0.3	

* Yacon leaves from four different cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11) collected in November of four different years (2013, 2015, 2016 and 2017) were used in this study. Several leaves from different plants were sampled at the second position from the top of plants, and then lyophilized, crushed, and pooled. The leaves were extracted with 50% methanol, then the supernatant was lyophilized, and reconstituted in 50% ethanol for investigation (see the Materials and methods section).

** Data shown represent mean ± SD from four independent experiments. D.W.: dry weight of sample, $ABTS^+$: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) cation, DPPH: 1,1-diphenyl-2-picrylhydrazyl, EC_{50}: half maximal effective concentration

Table 2. Antioxidant activity of yacon leaves among four different domestic cultivars and a Peru A line collected in four different years*

	SY201	SY206	SY217	SY237	SY11
	TEAC value (μmol TE / g D.W.) in $ABTS^+$ radical scavenging assay**				
2013	$268 \pm 17^{ab,A}$ (1.06)	$191 \pm 1^{c,A}$ (0.75)	$240 \pm 5^{d,A}$ (0.95)	$280 \pm 5^{a,A}$ (1.11)	$253 \pm 5^{bd,A}$ (1.00)
2015	$322 \pm 5^{a,B}$ (1.35)	$245 \pm 4^{bc,B}$ (1.03)	$199 \pm 3^{d,B}$ (0.84)	$250 \pm 5^{b,B}$ (1.05)	$238 \pm 7^{c,B}$ (1.00)
2016	$196 \pm 5^{ab,C}$ (1.03)	$185 \pm 3^{c,A}$ (0.97)	$264 \pm 1^{d,C}$ (1.38)	$200 \pm 1^{a,C}$ (1.05)	$191 \pm 1^{bc,C}$ (1.00)
2017	$68.7 \pm 0.7^{a,D}$ (0.42)	$301 \pm 7^{b,C}$ (1.86)	$171 \pm 3^{c,D}$ (1.06)	$358 \pm 7^{d,D}$ (2.21)	$162 \pm 1^{c,D}$ (1.00)
	TEAC value (μmol TE / g D.W.) in DPPH radical scavenging assay**				
2013	$159 \pm 3^{a,A}$ (0.89)	$154 \pm 1^{b,A}$ (0.86)	$184 \pm 2^{c,A}$ (1.03)	$193 \pm 2^{d,A}$ (1.08)	$179 \pm 1^{e,A}$ (1.00)
2015	$187 \pm 5^{a,B}$ (1.65)	$143 \pm 1^{b,B}$ (1.27)	$92.9 \pm 1.3^{c,B}$ (0.82)	$171 \pm 4^{d,B}$ (1.51)	$113 \pm 2^{e,B}$ (1.00)
2016	$118 \pm 1^{a,C}$ (1.33)	$124 \pm 1^{b,C}$ (1.40)	$139 \pm 5^{c,C}$ (1.57)	$95.1 \pm 1.4^{d,C}$ (1.07)	$88.7 \pm 0.8^{e,C}$ (1.00)
2017	$37.9 \pm 1.4^{a,D}$ (0.36)	$127 \pm 3^{b,C}$ (1.21)	$104 \pm 1^{c,D}$ (0.99)	$142 \pm 4^{d,D}$ (1.35)	$105 \pm 1^{c,D}$ (1.00)

* Yacon leaves from four different cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11) collected in November of four different years (2013, 2015, 2016 and 2017) were used in this study. Several leaves from different plants were sampled at the second position from the top of plants, and then lyophilized, crushed, and pooled for investigation (see the Materials and methods section).

** Data shown represent mean ± SD from four independent experiments. Tukey–Kramer's test was conducted for multiple comparisons; values not sharing a common superscript (lowercase or uppercase letter) are considered significantly different at $P < 0.05$ among individual cultivars or individual years, respectively. Data in parentheses indicate the relative TEAC values for individual cultivars vs. SY11 (1.00) in the same year. TEAC: trolox equivalent antioxidant capacity, TE: trolox equivalent, D.W.: dry weight of sample, $ABTS^+$: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) cation, DPPH: 1,1-diphenyl-2-picrylhydrazyl

and SY237), 52.0 μg/mL (SY217), and 45.4 μg/mL (SY237), respectively. In contrast, 128 μg/mL of the highest EC_{50} value was obtained from SY201 in 2017. In this experimental setting, varying concentrations of trolox demonstrated activity with EC_{50}; 7.32 ± 0.08 μg/mL. Using these activity data of the leaf extract and extraction yields from D.W. of the leaves, the radical scavenging activity was further calculated and expressed as the TEAC value per gram D.W. of the leaves, wherein trolox was used as a representative antioxidant (Sugahara et al. 2015). In the DPPH radical scavenging assay with the samples in 2013, 2015, 2016 and 2017, the highest TEAC values were observed for SY237 (193 μmol), SY201 (187 μmol), SY217 (139 μmol), and SY237 (142 μmol), respectively. The characteristic order of results present in both radical scavenging assays was similar to the results obtained for TPC. In this study, we used the pooled leaf samples after lyophilization for assays. Thus, it was thought that there was a trend of showing varying antioxidant activities of yacon leaves in different years (see Table 2), possibly in line with their different amounts of polyphenols (see Table 1). Similarly, only SY237 had TEAC values higher than those for SY11 in the four different years, although the values for individual cultivars were inconsistent throughout the tested years. Created from the parent strain SY11, SY237 possesses a unique character with deep reddish and purple color in the skin of tuberous roots as well as its stem and leaf parts (Sugiura et al. 2014, Sugiura & Yano 2016). It will be interesting to clarify whether the plant pigments specific to SY237 such as anthocyanins, in comparison with SY11, may contribute to better antioxidant activities. Our previous reports demonstrated antioxidant activity in free radical assays and a granulocytic cellular assay using the yacon leaves of SY201 (Sugahara et al. 2015) and SY206 (Ueda et al. 2019b). Further investigation of other antioxidant and cellular systems may shed light on the functional differences among these cultivars.

3. Carbohydrate-hydrolyzing enzyme inhibition of yacon leaves among four domestic cultivars and SY11

In the α-amylase inhibition assay, we first determined the inhibition activity (%) of the leaf extracts at 1.00 mg/mL of a single concentration (see Supplementary Table 2). Results indicated that the leaf extracts possessed 32.6% to 56.8% of the inhibition activity among individual samples. Due to these activities closer to 50%, experiments were not conducted with higher concentrations. In this experimental setting, varying concentrations of acarbose demonstrated activity with IC_{50}; 0.0213 ± 0.0025 mg/mL. Using these activity data of the leaf extract and extraction yields from D.W. of the leaves, enzyme inhibition was further calculated and

Supplementary Table 2. Carbohydrate-hydrolyzing enzyme inhibition of extracts prepared from yacon leaves among four different domestic cultivars and a Peru A line collected in four different years*

	SY201	SY206	SY217	SY237	SY11	Acarbose
	Activity (%) at 1.00 mg leaf extract / mL in α-amylase inhibition assay**					IC_{50} (mg / mL)
2013	45.3 ± 1.3	39.5 ± 1.2	37.0 ± 1.8	40.3 ± 3.0	42.2 ± 0.5	0.0213 ± 0.0025
2015	48.3 ± 2.6	42.1 ± 0.4	32.6 ± 4.1	48.6 ± 1.4	36.4 ± 2.4	
2016	53.4 ± 4.0	50.7 ± 2.3	52.5 ± 1.5	56.8 ± 1.8	53.0 ± 2.8	
2017	40.6 ± 2.5	52.4 ± 1.9	50.2 ± 2.4	50.5 ± 3.1	52.3 ± 2.0	
	IC_{50} value (mg leaf extract / mL) in α-glucosidase inhibition assay**					IC_{50} (mg / mL)
2013	2.69 ± 0.08	> 3.00 (Max 49.8±2.0%)	2.69 ± 0.07	2.73 ± 0.13	2.75 ± 0.06	0.496 ± 0.032
2015	> 3.00 (Max 51.2±1.0%)	> 3.00 (Max 39.5±1.4%)	> 3.00 (Max 35.5±0.4%)	> 3.00 (Max 43.0±2.1%)	> 3.00 (Max 35.7±2.0%)	
2016	2.80 ± 0.04	2.74 ± 0.05	> 3.00 (Max 45.4±1.1%)	1.72 ± 0.06	> 3.00 (Max 42.9±1.1%)	
2017	2.77 ± 0.02	> 3.00 (Max 48.2±1.0%)	2.49 ± 0.04	2.14 ± 0.17	> 3.00 (Max 46.7±1.0%)	

* Yacon leaves from four different cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11) collected in November of four different years (2013, 2015, 2016 and 2017) were used in this study. Several leaves from different plants were sampled at the second position from the top of plants, and then lyophilized, crushed, and pooled. The leaves were extracted with 50% methanol, then the supernatant was lyophilized and reconstituted in 50% ethanol for investigation (see the Materials and methods section).

** Data shown represent mean ± SD from three (in α-amylase assay) or four independent experiments (in α-glucosidase assay). Data in parentheses indicate activity (%) obtained at 3.00 mg/mL of the maximum concentration. AEIC: acarbose equivalent inhibition capacity, AE: acarbose equivalent, D.W.: dry weight of sample, IC_{50}: half maximal inhibitory concentration

expressed as the acarbose equivalent inhibition capacity (AEIC) value per gram D.W. of the leaves; acarbose has been used as a representative inhibitor/drug for the management of type-2 diabetes (Chiasson et al. 2002). In the α-amylase inhibition assay with the samples from 2013, the AEIC value in 1 g D.W. of the leaves from SY201 (5.82 μmol) was slightly higher than those in the other cultivars, although there was almost no difference among these values in 2015 (Table 3). Among the samples collected in 2016 and 2017, the highest values were observed in SY217 (10.5 μmol) and SY206 (9.89 μmol), respectively.

In the α-glucosidase inhibition assay, we first determined the IC_{50} value (mg/mL) using different concentrations of the leaf extract or inhibition activity (%) at the maximum concentration tested (see Supplementary Table 2). Results indicated that the leaf extracts possessed inhibitory effects with 1.72 mg/mL to 2.80 mg/mL of IC_{50}, or 35.5% to 51.2% of the maximum inhibition at the 3.00 mg/mL concentration. In 2016 and 2017, the leaf extract of SY237 showed the lowest IC_{50} at 1.72 mg/mL and 2.14 mg/mL, respectively, in comparison with other cultivars plus SY11. In this experimental setting, varying concentrations of acarbose demonstrated activity with IC_{50}; 0.496 ± 0.032 mg/mL. Using these activity data of the leaf extract and extraction yields from D.W. of the leaves, enzyme inhibition was further calculated and expressed as the AEIC value per gram D.W. of the leaves. In the α-glucosidase inhibition assay with the samples from 2013, the AEIC value in 1 g D.W. of the leaves from SY206 (56.1 μmol) was slightly higher than those in the other cultivars. Among samples collected in 2015, 2016 and 2017, the highest values were observed in SY201 (65.0 μmol), SY237 (76.6 μmol), and SY237 (79.9 μmol), respectively. These four cultivars had variable enzyme inhibition during the tested years, which was comparable to or stronger than that of SY11 as a control line, except for SY201 in 2017. Further analytical studies are needed to delineate the levels of active constituents among different cultivars. Specific derivatives of caffeoylquinic acid present in yacon leaves may contribute to α-amylase (Narita & Inouye 2009,

Table 3. Carbohydrate-hydrolyzing enzyme inhibition of yacon leaves among four different domestic cultivars and a Peru A line collected in four different years*

	SY201	SY206	SY217	SY237	SY11
	AEIC value (μmol AE / g D.W.) in α-amylase inhibition assay**				
2013	$5.82 \pm 0.19^{a,AC}$ (1.15)	$4.89 \pm 0.19^{b,A}$ (0.97)	$4.79 \pm 0.30^{b,A}$ (0.95)	$4.93 \pm 0.46^{b,A}$ (0.97)	$5.06 \pm 0.08^{b,A}$ (1.00)
2015	$7.04 \pm 0.45^{a,A}$ (1.14)	$6.00 \pm 0.06^{ab,B}$ (0.97)	$5.55 \pm 0.93^{b,A}$ (0.90)	$6.48 \pm 0.22^{ab,B}$ (1.05)	$6.17 \pm 0.53^{ab,B}$ (1.00)
2016	$9.58 \pm 0.79^{ab,B}$ (1.08)	$9.23 \pm 0.46^{abc,C}$ (1.04)	$10.5 \pm 0.3^{b,B}$ (1.18)	$7.90 \pm 0.26^{cd,C}$ (0.89)	$8.90 \pm 0.52^{ad,C}$ (1.00)
2017	$5.27 \pm 0.36^{a,C}$ (0.64)	$9.89 \pm 0.38^{b,C}$ (1.20)	$8.39 \pm 0.44^{c,C}$ (1.01)	$8.98 \pm 0.59^{bc,C}$ (1.09)	$8.27 \pm 0.35^{c,C}$ (1.00)
	AEIC value (μmol AE / g D.W.) in α-glucosidase inhibition assay**				
2013	$49.5 \pm 1.4^{a,A}$ (1.08)	$56.1 \pm 2.8^{b,A}$ (1.22)	$52.3 \pm 1.4^{ab,A}$ (1.14)	$47.7 \pm 2.2^{a,A}$ (1.04)	$45.9 \pm 0.9^{a,A}$ (1.00)
2015	$65.0 \pm 1.6^{a,B}$ (1.40)	$46.6 \pm 2.2^{b,B}$ (1.00)	$45.2 \pm 1.5^{b,B}$ (0.97)	$47.1 \pm 2.3^{b,A}$ (1.01)	$46.5 \pm 3.6^{b,A}$ (1.00)
2016	$61.1 \pm 0.9^{a,C}$ (1.27)	$63.5 \pm 1.0^{a,C}$ (1.31)	$62.2 \pm 1.4^{a,C}$ (1.29)	$76.6 \pm 2.8^{b,B}$ (1.59)	$48.3 \pm 4.4^{c,A}$ (1.00)
2017	$45.8 \pm 0.2^{a,D}$ (0.78)	$70.1 \pm 3.0^{b,D}$ (1.20)	$64.2 \pm 1.0^{bc,D}$ (1.10)	$79.9 \pm 6.7^{d,B}$ (1.37)	$58.4 \pm 3.8^{c,B}$ (1.00)

* Yacon leaves from four different cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11) collected in November of four different years (2013, 2015, 2016 and 2017) were used in this study. Several leaves from different plants were sampled at the second position from the top of plants, and then lyophilized, crushed, and pooled for investigation (see the Materials and methods section).

** Data shown represent mean ± SD from three (in α-amylase assay) or four independent experiments (in α-glucosidase assay). Tukey–Kramer's test was conducted for multiple comparisons; values not sharing a common superscript (lowercase or uppercase letter) are considered significantly different at $P < 0.05$ among individual cultivars or individual years, respectively. Data in parentheses indicate the relative AEIC values for individual cultivars vs. SY11 (1.00) in the same year. AEIC: acarbose equivalent inhibition capacity, AE: acarbose equivalent, D.W.: dry weight of sample

Oboh et al. 2015) and α-glucosidase inhibition (Terada et al. 2003, 2006). Sesquiterpenic lactone enhydrin and smallanthaditerpenic acids in yacon leaves may be other candidate constituents (Honoré et al. 2015). Interestingly, the AEIC values (45.2-79.9 μmol) of domestic yacon leaves obtained in this α-glucosidase inhibition assay were higher than the reported data (0.5-31.6 μmol) tested with 27 traditional medicinal herbs (Feng et al. 2013).

4. Multivariate analysis of the data of yacon leaves among four domestic cultivars and SY11

We aimed to characterize the functional profiles of the leaves of domestic yacon cultivars and SY11 cultivated in Japan, and consequently applied multivariate analysis, which is suitable for visualizing the positioning of individual values. Based on the correlation matrix, the experimental data were normalized, and principal component analysis (PCA) was conducted. In individual cultivars, the averaged data from four years were prepared and subjected to PCA. The first two principal components (PCs) could describe 97.7% of the initial data variability (PC1 75.6% and PC2 22.1%, respectively) (Fig. 1 (A)). The loadings of PC1 may be due to strong positive correlations with TPC, antioxidant activity, and α-glucosidase inhibition, whereas those of PC2 appear to be correlated with α-amylase inhibition. Hierarchical cluster analysis (HCA) delineated the presence of three independent clusters resulting from the strong correlation and higher similarity (defined as a closer distance) in the obtained dendrogram (Fig. 1 (B)). Based on their position in PCA, SY201 and SY11, SY217 and SY206, and SY237 alone were categorized into independent clusters (see Fig. 1 (A)), according to the dendrogram (see Fig. 1 (B)). SY11 and SY201, which have relatively lower TPC, lower antioxidant activity, and lower α-glucosidase inhibition were plotted at the lower position of the PC1 axis, while SY237, which possesses relatively higher values for all parameters, appeared at higher positions. Previous reports from other countries have documented that there may be several correlations between different yacon landraces with phenolic parameters and *in vitro* biological activities (Russo et al. 2015b), and those of phenolics with different yacon origins (Lachman et al. 2007). We recently demonstrated that TPC in the yacon leaves of SY206 can be influenced by seasonal differences and years (Ueda et al. 2019b). It is important to elucidate whether yearly differences may interfere with the characteristic positioning of yacon cultivars. Additional PCA was conducted using whole data from individual years to visualize their scattered plots (Fig. 1 (C)). Notably, SY11 was placed in a small narrow circle located closer to the merged center of the x- and y-axes, while other cultivars were in larger circles. Subsequently, their scattered data plots were visualized in circles for each year's data (Fig. 1 (D)). Interestingly, the data from 2013 and 2015 were located at a lower position on the y-axis, while the data from 2016 and 2017 were located at higher positions, wherein the major differences among cultivars were observed in 2015 and 2017.

It is important to consider any possible relationships between the results obtained above and the genetic/breeding character of the individual yacon cultivars. SY11 is one of the Peru A lines, initially introduced and spread in Japan due to its stable productivity (Sugiura 2016). Therefore, SY11 was used as a parental line for providing novel domestic cultivars. Due to the frequent occurrence of cracks in the tuberous roots in SY11, SY201 has been breed and selected from another Peru A line closer to SY11 and a line from Bolivia to possess a character with a lesser incidence of cracks (Sugiura et al. 2007). SY206 and SY217 have been bred and selected from the lines with closer phenotypes originated from the International Potato Center; resulting in SY206 showing character with stable and high yield, better storability, and white color in the internal edible roots, with SY217 showing that with high yield, high sugar content, and clear orange color in the internal tuberous roots (Fujino et al. 2008). SY237 has a genetic background of SY201 and SY11, and this cultivar possesses unique character including higher yield with less cracking, a deep reddish and purple skin color, and better growth even at the aerial part (Sugiura et al. 2014, Sugiura & Yano 2016). The functional similarity and differences among the individual cultivars are not clear in this study, although multivariate analysis with the averaged functional data from the four different years may be able to partly demonstrate the potential contribution of genetic background of these yacon cultivars as shown in Figure 1 (A) and (B). More work is needed to clarify this point with genotyping approaches as previously reported (Lachman et al. 2007).

Table 4 compiles the yielded weights, as fresh weight (F.W.), of yacon plants with the aerial part and tuberous roots. SY206 and SY237 had higher weights than the others, indicating their greater growth and production of higher yields throughout all tested years. The yields of the tuberous roots of individual plants in 2015 and 2017 were much higher than those in the other years excepting for SY201 in 2017, implying that the cultivating environment was better for the growth of yacon plants. Presumably, the normal temperature during the summer to autumn season observed in 2015 and 2017 at the tested area (JMA 2020) may have resulted in improved growth as well as large differences in functional parameters

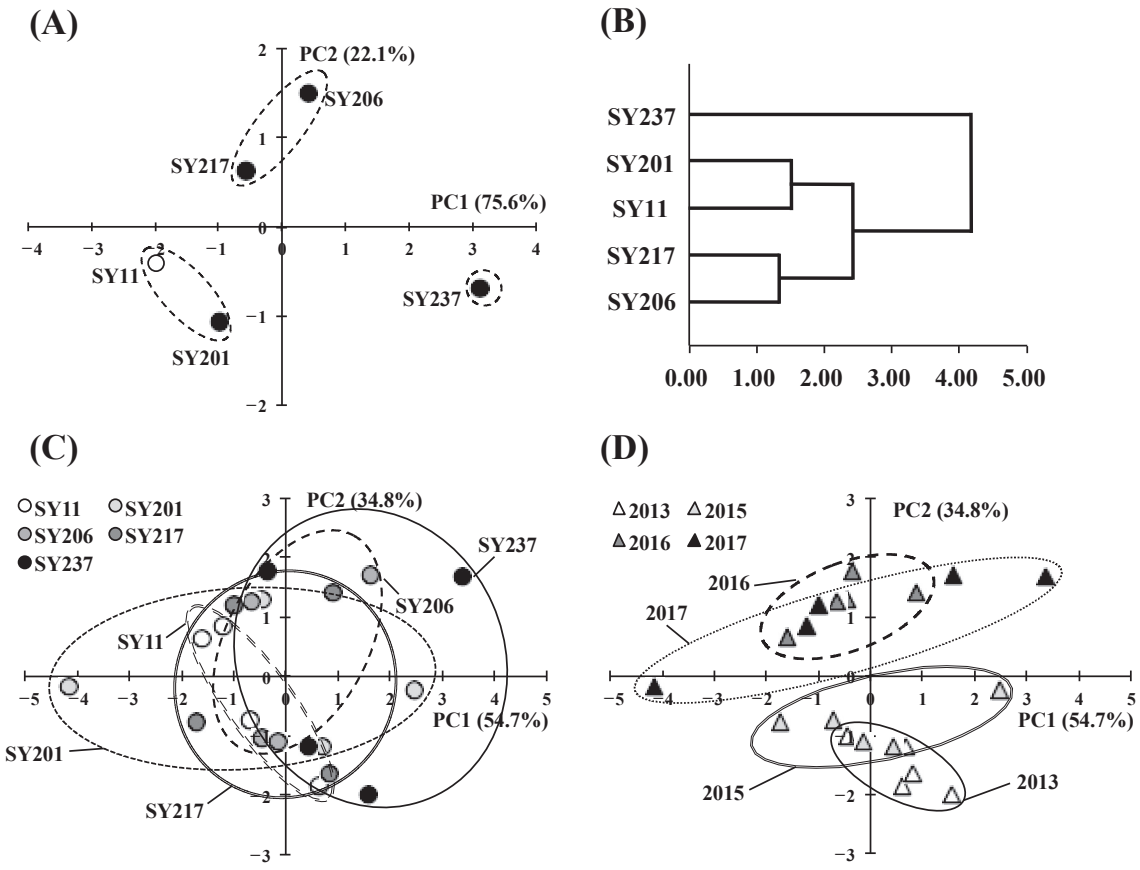

Fig. 1. Multivariate analysis of the data obtained for total polyphenol content, antioxidant activity, and carbohydrate-hydrolyzing enzyme inhibition of yacon leaves among four different cultivars and a Peru A line collected in four different years

Yacon leaves from four different cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11) collected in November of four different years (2013, 2015, 2016 and 2017) were used in this study. PCA (A) and HCA (B) were conducted to visualize the data obtained from the different cultivars plus SY11. Additional PCA was conducted to visualize the scattered plots for the different cultivars plus SY11 including the four different years (C) and in the four different years including the four cultivars plus SY11 (D). Circles and dotted circles in the figures denote clusters. PCA: principal component analysis, HCA: hierarchical cluster analysis

among the cultivars. However, the high temperature during the summer to autumn season observed in 2013 and 2016 might have caused lower growth with smaller functional differences. In the tested area, the average temperatures in July and August 2013 were 24.7°C and 24.9°C, respectively, and those in 2016 were 24.5°C and 24.7°C. Those temperatures were higher than 23.6°C in July and 23.9°C in August of the reference temperatures taken during 1981-2010. These two years showed a trend of relatively higher temperatures than those of the reference in September (20.7°C in 2013 and 22.1°C in 2016 vs. ref. 20.5°C) and October (16.1°C in 2013 and 17.9°C in 2016 vs. ref. 14.6°C) as well. In contrast, the temperatures observed in July to October (23.0°C, 23.6°C, 19.6°C and 13.8°C, respectively) in 2015 were lower than the reference temperatures (23.6°C, 23.9°C, 20.5°C and 14.6°C, respectively). In 2017, the temperatures observed in July and August (25.0°C and 24.9°C, respectively) were higher than the reference temperatures, but then reached the reference temperature levels in September (19.6°C) and October (13.8°C). It has been reported that SY206 and SY237 have a character to stably generate greater yield with good shapes (Fujino et al. 2008, Sugiura et al. 2014, Sugiura & Yano 2016). Our results obtained in the Aso area of Kyushu also supported this point. It was suggested that the tuberous roots of SY206 and SY237 might grow late, and thus these cultivars might not be affected by high temperature in the summer to autumn season. In a previous report, the variation of TPC in sweet potato leaves may be influenced by the cultivation environment, especially in air temperature, but not sunshine duration (Kobayashi et al.

Table 4. Yielded weight of the aerial part and tuberous roots of yacon among four different domestic cultivars and a Peru A line cultivated in four different years*

	SY201	SY206	SY217	SY237	SY11
	Aerial part (g F.W. / plant)**				
2013	981 (0.90)	761 (0.70)	938 (0.86)	1,098 (1.01)	1,087 (1.00)
2015	819 (0.85)	1,194 (1.24)	904 (0.94)	1,296 (1.34)	965 (1.00)
2016	958 (0.68)	1,252 (0.89)	947 (0.67)	1,720 (1.22)	1,410 (1.00)
2017	1,076 (0.59)	1,217 (0.67)	1,517 (0.83)	1,634 (0.89)	1,826 (1.00)
	Tuberous roots (g F.W. / plant)**				
2013	486 (1.14)	1,300 (3.06)	476 (1.12)	1,582 (3.73)	425 (1.00)
2015	776 (0.53)	2,254 (1.54)	1,477 (1.01)	1,795 (1.23)	1,460 (1.00)
2016	432 (0.61)	1,439 (2.04)	489 (0.69)	2,079 (2.95)	705 (1.00)
2017	342 (0.31)	2,537 (2.31)	1,134 (1.03)	2,435 (2.22)	1,099 (1.00)

* Yacon from four different cultivars—'*Sarada otome*' (SY201), '*Andesu no yuki*' (SY206), '*Sarada okame*' (SY217), and '*Andesu no otome*' (SY237)—and a Peru A line (SY11); the yields in November of four different years (2013, 2015, 2016 and 2017) were used in this study. F.W.: fresh weight.
** Data shown represent the mean value from ten plants. Data in parentheses indicate the relative values for individual cultivars vs. SY11 (1.00) in the same year.

2019). In this case, high TPC can be triggered by low temperature as the stressor. Nevertheless, it is still unclear why functional parameters such as TPC and the TEAC values of the leaves from SY201 in 2017 were lower than the others. Whether the lower yield of tuberous roots of SY201 may be due to its late reproductive growth in that year, thereby showing the lower phenolic parameters in the leaves, remains an open question. In general, cultivar SY201 has a character of possessing greater growth and higher yield than SY11 in several domestic test fields investigated in 1990s (Sugiura et al. 2007) and in 2007-2009 (Sugiura et al. 2014). In our investigation, SY201 conversely showed less growth and yield than SY11. Therefore, there may still be some controversy regarding whether the results of multivariate analysis using the data taken in this study can be specific to this region. As reported in other countries, multivariate analysis is a useful tool for investigating the individual distances of the targeting variables, as demonstrated in different yacon landraces and/or strains (Lachman et al. 2007, Russo et al. 2015b). The functional datasets with different years might delineate the characteristic positioning of yacon cultivars and possibly other crops with more confidence, rather than those of a single year study (see Fig. 1 (A) and (B)). Furthermore, a longer term investigation as well as a comparison with regional differences regarding the characterization of different yacon cultivars will be needed to conclude this point. Taking into consideration the growth of plants with environmental factors, it may be an interesting study to clarify if such weather conditions as temperature and rainfall can influence the functional characteristics of individual cultivars.

In conclusion, we demonstrated the functional similarities/differences among yacon leaves from four domestic cultivars and SY11 as a control line, maintained in the Aso area of Kyushu, over four years. TPC and antioxidant activity of cultivar '*Andesu no otome*' (SY237) were higher than those in SY11, although the values for individual cultivars were inconsistent throughout the tested years. In enzymatic assays, all cultivars were inhibitory with a large variation among the tested years. More importantly, multivariate analysis with functional data over four years enabled a systematic visualization of the characteristic positioning of these yacon cultivars. As a result, SY237 may be a candidate cultivar that is partially superior to SY11 in this limited data set. However, we found that the yearly variance should be partly taken into consideration. To gain greater insight into the clear functional/physiological differences among these cultivars and SY11, it will be necessary to undertake additional basic cultivation studies in pots using a growth chamber and/or a greenhouse under the control of environmental factors. Further work is also necessary to fully elucidate the phenolic parameters, chemical constituents, cellular antioxidant capacities, and *in vivo* antidiabetic effects of the leaves among yacon cultivars including SY237, and to delineate the usefulness of yacon cultivars in Japan for health benefits.

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