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The Biological and Chemical Variability of Yacon

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This paper focuses on the biological and chemical variability of four yacon (*Smallanthus sonchifolius*) accessions cultivated under field conditions. Significant variations in tuber shape, weight, content of oligofructans, as well as in leaf isozymes, phenolics, and relative DNA contents were found. Accessions 6 and 88 were the most productive (up to 3.01 and 3.74 kg/plant); accession 48 was the most balanced from the yield aspect in three vegetative periods. A significantly higher content of β-(2→1) oligofructans was noted in accessions 48 and 88 as compared to 6 and 60. No difference in sucrose, glucose, and fructose level was observed. Only accession 6 exhibited separate acid phosphatase and esterase isoforms. Accessions 6 and 60 had the highest content of phenolics, and accession 88 had the lowest relative DNA content. Large yacon intraspecific variation may be useful in future detailed research as a good background for breeding, growing, and utilization in industrial processing.

KEYWORDS: ***Smallanthus sonchifolius*; germplasm; variability; tubers; leaves; morphology; yield; isozymes; phenolics; saccharides; relative DNA content; agronomic suitability; growing; industrial processing**

INTRODUCTION

Yacon (*Smallanthus sonchifolius* [Poepp. & Endl.] H. Robinson, Asteraceae) is a native Andean plant cultivated for its tubers, which are commonly used as a food in South America. Traditionally, yacon tubers and dried leaves are recommended to people suffering from diabetes, various digestive, and/or renal disorders (*1*). The tubers contain antioxidants (*2*−*5*), fructose, glucose, sucrose, and β-(2→1)fructooligosaccharides (inulin type oligofructans) (*6*−*8*) and thus may be prospective prebiotics as they are fermented by beneficial species of gut bacteria (*9*). They are also used as a source of natural sweeteners and syrups suitable for persons suffering from digestive problems (*10*, *11*).

Until the end of the 1980s, with the exception of Peru and Japan, the scientific community paid only scant attention to this plant species. The crop was introduced for cultivation in the Czech Republic in 1993 (*1*). Preliminary results show that this plant can be successfully cultivated outside its natural climate (*12*). A collection of 25 yacon genotypes has been maintained as a genetic resource for new crop development in the Potato Research Institute, Ltd. (Havlíčkův Brod, Czech Republic) because its popularity has increased due to reports of its antidiabetic, prebiotic, and immunomodulative features (*1*, *12*). In germplasm management, several techniques including morphological markers, isozymes, and molecular markers have been used for crops evaluation, including yacon (*13*, *14*).

Phenolic compounds, mainly chlorogenic (caffeoyl-quinic) acid and other caffeic acid derivatives (*2*−*5*, *15*, *16*), have been identified in yacon tubers. The flavonoids centaureidin and sacuranetin have been found in the leaves of a related species (*Smallanthus fruticosus*) (*17*). We have already reported (*2*, *18*, *19*) on the presence of large amounts of phenolic compounds in extracts from yacon leaves and tubers, mainly chlorogenic, protocatechuic, ferulic, rosmarinic, gallic, gentisic, and caffeic acids and their derivatives. Evidence has also emerged about its antioxidant activity (*20*), effects on oxidative damage and glucose metabolism in rat hepatocytes, and insulin-like effects of yacon leaf extracts in rat hepatoma Fao cells (*19*, *21*).

Morphological variability, isozyme polymorphism, and variation in nuclear DNA content in 25 yacon accessions have also recently been evaluated (*12*−*14*, *22*). On the basis of previous results (*12*, *13*), four genotypes were selected in order to evaluate

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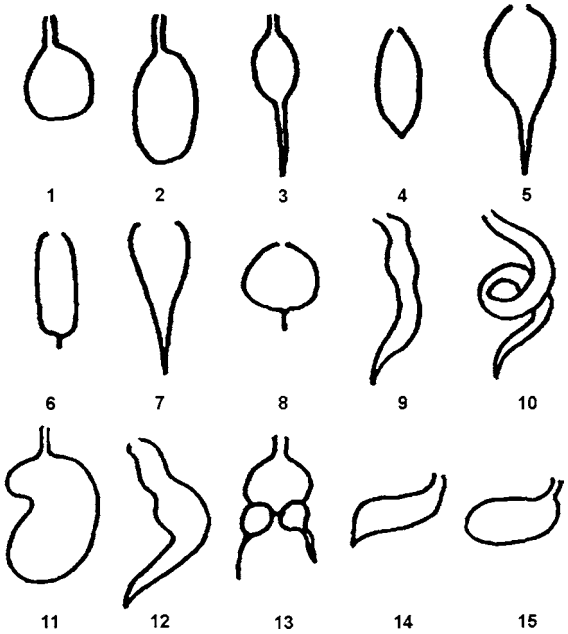

Figure 1. Categories of yacon morphotypes (*23*).

relationships between this variation and variability in nutritionally important components in yacon leaves and tubers. The aim of the present work was to investigate the variability of morphotypes, weight, and saccharide content in the tubers, isozyme polymorphism, and content of phenolics in the leaves from four different yacon clones with the aim of finding the most suitable genotype(s) for industrial processing.

MATERIALS AND METHODS

Plant Material and Growing Experiments. The sample of studied plant material included four yacon (*S. sonchifolius* [Poepp. & Endl.] H. Robinson) accessions (6, 48, 60, and 88) imported from New Zealand (the primary center of origin was Ecuador). Plants were cultivated and evaluated under experimental field conditions in the Haná region at Olomouc-Holice (altitude: 210 m; mean day temperature: 2001, 16.3 °C; 2002, 16.4 °C; and 2003, 16.7 °C; precipitation during cultivation period: 2001, 271.4 mm; 2002, 388 mm; and 2003, 293.9 mm) from May to October 2001−2003. The yacon was planted in furrows with five plants per accession at a spacing of 70 cm × 70 cm. Conventional agronomic technologies were used for cultivation (*13*, *14*). Plants were treated against insects with Decis and Karate. Young true leaves from each plant were harvested 3 months after planting and pooled for each accession. Tubers with caudices were harvested 6 months after planting.

Morphological Characterization. In 2001−2003, the aboveground parts of the plants were observed (fortnightly) during the vegetative period (*13*). For the underground yacon portion, the tubers were weighted. The tuber shape was characterized following morphological descriptors for yacon (*23*); see **Figure 1**.

Chemicals. Acetonitrile, methanol for liquid chromatography (LC; gradient grade), and H_3PO_4 (trace select purity) were purchased from Merck (Darmstadt, Germany); sucrose, glucose, fructose, diphenylamine, aniline, PVP-40 (polyvinylpropylpyrrolidone), and DAPI (4′-6-diamidino-2-phenylindole) were purchased from Sigma Chemicals (St. Louis, MO); acetone p.a., sodium dihydrogenphosphate (trace select purity), and gallic, protocatechuic, caffeic, chlorogenic acids, and NST were purchased from Fluka Chemie (Buchs, Switzerland), and Folin−Ciocalteau reagent was purchased from Jan Kulich (Hradec Králové, Czech Republic); oxalic acid was purchased from Alkaloid (Skopje, Macedonia). All other chemicals including solvents were of analytical grade from Pliva-Lachema (Czech Republic). Ultrapure purified water (Watrex International, United States) was used.

Extraction Procedures. *Extraction of the Leaves for Isozyme Analysis.* One portion of the samples was homogenized by grinding one volume of leaf material (1 g) in three volumes of extraction buffer (0.1 M Tris-HCl, pH 8.0, 78 mM 2-mercaptoethanol, 26 mM sodium pyrosulfite, 11 mM sodium salt ascorbic acid, and 4% PVP-40) (*24*) with a little sea sand and sucrose. The crude extract was centrifuged at 20800*g* for 10 min (4 °C). The supernatant was divided into three aliquots (60 μL per tube) and stored at −80 °C prior to isozyme analysis (extracts I).

Extraction of the Leaves for Phenolic Content Analysis. The second portion of the leaves was dried at room temperature until constant weight was achieved. The dried leaves (18.15 ± 1.24 g) were extracted using a Soxhlet extractor with methanol (500 mL, 72 h). After evaporation of methanol, the extract was dispersed in water (300 mL) and chlorophyll was removed by extraction with petroleum ether (3 × 150 mL). The aqueous layer was then acidified and extracted by ethyl acetate (5 × 150 mL). The extract yield after evaporation of the solvent was 0.42 ± 0.01 g. Extracts were stored at −20 °C prior to phenolic content analysis (extracts II).

Extraction of the Tubers. Tubers were frozen (−20 °C) immediately after harvest. The frozen tubers were cut into smaller parts and homogenized in a homogenizer (MR300CA, Multiquick 300 combi, Braun, Spain) for 30 s. Ten grams of yacon tubers was transferred into a 100 mL volumetric flask and filled with water. The extraction mixtures were ultrasonicated for 15 min and filtered. Three milliliters of the filtrates was pipetted into 10 mL volumetric flasks and filled with methanol.

Acid Hydrolysis of the Tubers. Five milliliters of the diluted filtrate (150 mg) was heated with 1 mL of 5% oxalic acid (1 g/20 mL) for 30 min in a water bath. The cooled solution was transferred into a 25 mL volumetric flask and filled with methanol at room temperature.

Analyses. *Isozyme Analysis.* Leaf extracts I were thawed immediately before loading on polyacrylamide gel (8.16% separation gel 13.5 cm × 10 cm, 4% concentration gel). Electrophoresis was run at 35 mA, 390 V for 2 h, on an Adjustable Height Dual Gel Electrophoresis Unit (Sigma) with an ESP 601 power supply (Amersham Pharmacia Biotech). Polyacrylamide gel electrophoresis was performed for 16 enzyme systems: alcohol dehydrogenase (ADH), acid phosphatase (ACP), diaphorase (DIA), esterase (EST), glutamate-oxalacetate transaminase (GOT), phosphoglucomutase (PGM), glutamate dehydrogenase (GDH), glucose-6-phosphate isomerase (GPI), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), NADH dehydrogenase (NADH DH), superoxide dismutase (SOD), shikimate dehydrogenase (SHDH), and 6-phosphogluconate dehydrogenase (PGD) (*14*), following the methods of Vallejos (*24*).

Phenolic Content Analysis. Total phenolics in the leaf extracts II were determined using Folin−Ciocalteau reagent (*25*). The tested fractions in distilled water (25 μL, 1 mg/mL) were mixed with 500 μL of the reagent (previously diluted 10-fold with water) and maintained at room temperature for 5 min; 500 μL of sodium bicarbonate (75 g/L) was added to the mixture. After 90 min at 30 °C, absorbance was measured at 725 nm. Results were expressed as gallic acid equivalents.

HPLC. Solid samples of yacon extracts were dissolved in methanol. Working solutions of the extracts (∼1 mg/mL) were filtered through a nylon filter (0.2 μm) prior to analysis. Working standard solutions (∼1 mg/mL) of chlorogenic, protocatechuic, and caffeic acids (dissolved in methanol and further diluted by the mobile phase) were freshly prepared every day. The high-performance liquid chromatography (HPLC) system consisted of an ESA isocratic pump (model 582) with a pulse damper, a manual injector (Rheodyne, Cotati, CA) equipped with a 20 μL loop, and an ESA coulometric detector Coulochem III with a dual electrode standard analytical cell (model 5010A) combined with a guard cell (model 5020) in front of the injector. All fittings, ferules, and tubings were of PEEK. The HPLC column—Purospher Star RP-18e (5 μm), 125 mm × 4 mm i.d., with a guard column (the same sorbent, 4 mm × 4 mm i.d.) (all from Merck)—was thermostated at 25.0 °C during the analysis using a Techlab K5 (Techlab GmbH, Erkerode, Germany) thermostatic HPLC oven. The chromatographic station Clarity (DataApex, Prague, Czech Republic) was used for simultaneous dual-channel chromatogram recording and handling.

The mobile phase [20 mM sodium dihydrogen phosphate pH 3.0/acetonitrile (85/15, v/v)] was filtered through a 0.2 μm filter and

Table 1. Basic Shapes of Root Tubers Recorded in Yacon Accessions in the Years 2001–2003

accession no./year	2001	2002	2003
6	14	5, 12	4, 12
48	14	14	1, 4, 5, 11, 12
60	5	4, 12, 14	5, 12
88	4, 12	4, 8, 11, 12, 14	5, 12

Table 2. Means of Yacon Root Tubers Production (kg/plant) in the Years 2001–2003

accession no./year	2001	2002	2003
6	3.01	1.75	1.082
48	2.83	3.73	2.016
60	2.71	1.90	1.342
88	3.74	1.83	1.79

degassed under vacuum prior to use. The flow rate was 0.8 mL/min. The Coulochem III settings were applied as follows: guard cell potential, +280 mV vs Pd; first and second cell working potential, +60 and +250 mV vs Pd, respectively; and current range, 20 μA/V. All analyses were carried out in triplicate.

Saccharide Analysis. Native and hydrolyzed yacon tubers were analyzed for sugar content by high-performance thin-layer chromatography (HPTLC). One to four microliters of the extract solutions was applied on HPTLC plates as the test solutions. A mixed solution of glucose, fructose, and sucrose (2.0 mg/L each) in MeOH/H_2O (8:2, v/v) was used as the standard. One to six microliters (0.2−1.2 μg) of the standard mix solution was applied to the HPTLC plates. TLC was performed on 10 cm × 20 cm silica gel 60 HPTLC plates (Merck, Merck Art. 1.05641). Plates were pretreated by development in chloroform−methanol (1:1, v/v) to the top and dried at 110 °C for 30 min before use. All standards and samples were applied by means of Camag Automatic TLC Sampler 4 (Camag, Muttenz, Switzerland). Plates were developed twice using acetonitrile/NST (0.7 g/L): water 17:3 v/v in horizontal chamber (sandwich configuration). After the second development, the dry plate was dipped into the DAP detection reagent (20 g/L diphenylamine, 2% aniline, and 10% H_3PO_4 in acetone) in the Camag chromatogram immersion device II for 4 s, dried with a hairdryer, and placed on a heating plate at 110 °C for 10 min. Documentation of TLC plates was performed by Camag Video Documentation System, coupled to a Reprostar 3 transilluminator and a frame grabber system equipped with a 3 × 1/2 in. CCD camera (model HV-C20, Hitachi Denshi, Japan). The Video Documentation System was operated using VideoStore 2 V2.30 software. Densitometric evaluation was performed at 560 nm using Camag TLC Scanner 3.

Relative DNA Content Measurement. Relative nuclear DNA content estimation was conducted by using a PAS flow cytometer (Partec GmbH, Germany). Approximately 20 mg of fresh leaf tissue was chopped in 500 μL of OTTO I buffer (*26*). After the addition of 1000 μL of OTTO II buffer (*26*) containing DAPI stock solution, the suspension of isolated nuclei was filtered through a nylon mesh (40 μm pore size) and examined. Pea (*Pisum sativum*) cv. Ctirad (2C DNA 9.09 pg, PI) was used as an internal standard for analysis of yacon germplasm set.

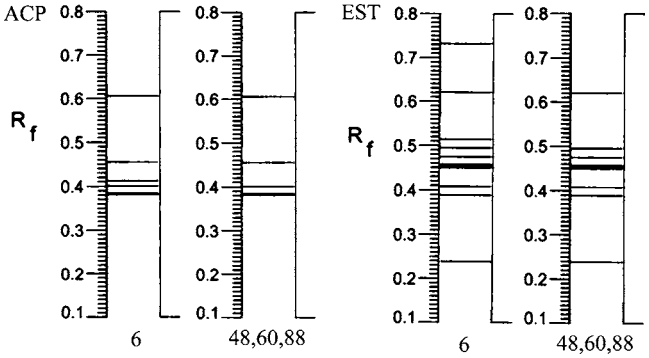

Figure 2. Isozyme (ACP, EST) spectra in leaf extracts from various yacon accessions.

Statistical Analysis. Statistical analysis was performed using Statistica statistical software. Analysis of variance, Scheffe's, and LSD tests were employed to analyze the variation in mean relative DNA content.

RESULTS

Morphological and Yield Assessment. Results of the morphological assessment of underground parts of yacon are summarized in **Tables 1** and **2**. Shapes 1, 4, 5, 8, 11, 12, and 14 were present in the four accessions during the three vegetative periods. In 2001 and 2002, shape 14 was the most frequent; however, the type was not recorded in 2003, when the most frequent shape 12 occurred in all genotypes studied. In addition, in 2003, shape 5 was observed by accessions 48, 60, and 88. On the other hand, shapes 1, 8, and 11 were recorded in a minority. With respect to yacon tuber production, the plants were most productive in 2001 and 2002 (**Table 2**).

Isozyme Analysis. Four yacon accessions were screened with 16 enzyme-staining systems (see Materials and Methods). Only ACP and EST showed a relatively high degree of polymorphism (**Figure 2**). The remaining 14 systems were monomorphic. Among all staining systems, 55 bands (isoforms) were observed.

Phenolic Content. The total phenolic content in extracts from young yacon leaves was evaluated using Folin−Ciocalteau phenolic reagent. Moreover, the content of protocatechuic, chlorogenic, and caffeic acids was determined by HPLC coupled with coulometric detection. Chlorogenic acid was prevalent in the extracts, followed by caffeic acid. Accessions 6 and 60 had the highest content of phenolic compounds (**Table 3**).

Saccharide Analysis. On the basis of the results from phenolic content analysis, the sample was divided into two groups: I (accessions 6 and 60) and II (48 and 88). The content of glucose, fructose, and sucrose was determined by HPTLC in extracts from native and hydrolyzed tubers from the two groups. Significantly higher contents of β-(2→1) oligofructans were noted in group II as compared to group I. No difference in sucrose, glucose, and fructose content was observed between the two groups (**Table 4**).

Table 3. Phenolic Content in Young Yacon Leaves from Various Accessions

accession no.	phenolic content (mg/g of dried leaves)				
	protocatechuic acid	chlorogenic acid	caffeic acid	total phenolic content[a]	group/significance of differences[b]
6	0.011	0.160	0.044	2.44 ± 0.09	A
48	0.006	0.037	0.062	1.51 ± 0.13	B
60	0.011	0.092	0.097	2.33 ± 0.17	A, C
88	0.008	0.098	0.044	1.94 ± 0.11	C

[a] Results were expressed as means ± SD; $n = 9$. [b] Genotypes marked with the same letter are not significantly different ($p < 0.01$).

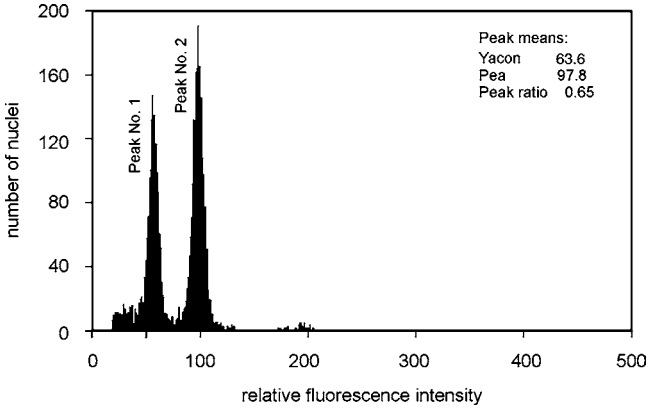

Figure 3. Histogram of relative fluorescence intensity obtained after simultaneous flow cytometric analysis of DAPI-stained yacon nuclei and nuclei of pea. *Pisum sativum* cv. Ctirad (2C DNA 9.09 pg, PI) was used as an internal reference standard. Peak 1, yacon, relative 2C DNA = 5.91; peak 2, pea.

Table 4. Saccharide Content in Yacon Tubers[a]

	group I (accessions 6 and 60)	group II (accessions 48 and 88)
sucrose	5.72 ± 0.24	5.82 ± 0.14
glucose	8.94 ± 0.28	8.60 ± 0.01
fructose	25.57 ± 1.70	24.06 ± 1.00
β-(2→1) oligofructans	42.84 ± 3.61	49.13 ± 1.08[b]

[a] Results were calculated in mg per g of fresh yacon tubers and expressed as means ± SD; $n = 3$. [b] $p < 0.01$ as compared to group I.

Table 5. Means of Relative Leaf Nuclear DNA Content and Differences in Yacon Accessions

accession no.	2C DNA content		group/significance of differences[a]
	mean	variation	
6	5.96	5.94–5.98	A
48	5.97	5.86–6.06	A
60	5.99	5.92–6.05	A
88	6.01	5.87–6.12	B

[a] Genotypes marked with the same letter are not significantly different ($p < 0.05$).

Relative DNA Content Analysis. The means of relative 2C DNA amount ranged from 5.96 to 6.01. Statistical analysis (LSD test) based on relative DNA data showed that accessions 6, 48, and 60 grouped together, while accession 88 significantly differed ($p \leq 0.05$) with nuclear DNA (**Table 5** and **Figure 3**).

DISCUSSION

The first detailed studies focused on morphological and biological yield, and yacon variability showed significant differences in some characteristics (*10*, *16*, *17*). Because of large variation in morphological features of yacon root tubers and yield parameters (*16*, *17*), genotypes were chosen with respect to their stability during the three subsequent vegetative periods and in relation to the content of nutritional components. Although accessions 6 and 88 were the most productive (mean tuber yield reached 3.01 and 3.74 kg, respectively), accession 48 was the most balanced in the three vegetative periods (mean tuber yield was 2.5 kg). From an agronomical and technological viewpoint, regularity of tuber shape is the most important parameter. Relatively regular (2−8, 14, and 15) and irregular (10−13) shapes were therefore grouped together in order to achieve better clarity of presentation. Shape (morphotype) 9 remained between these two substantially different groups, neither regular nor irregular, but yet usable for growing and industrial processing. With respect to technological processing, the above-mentioned genotypes (6, 48, 60, and 88) were chosen (**Table 1**).

The studied sample of yacon genotypes can be divided into two groups according to the profile of ACP and EST isozymes spectra. The genotypes 48, 60, and 88 grouped together, while the other genotype 6 exhibited separate isoforms (**Figure 2**). As reported previously (*13*), approximately 70% of yacon accessions studied showed the same profiles in both staining systems (ACP and EST). This variation does not correspond to the morphology of root tubers (*13*, *14*). It was concluded from the 3 year period of growth and observation that the morphological parameters of root tubers can be substantially affected by environmental factors, mainly soil texture (*12*). The data on root tuber morphology showed a large variation, but on the other hand, the most common shapes 12 and 14 occurred with a relatively high frequency (**Table 1**; *12*−*14*). This indicates that the shape of root tubers must be genetically determined. The mechanism of this phenomenon remains as an important topic for future genetic and breeding research.

Phenolic compounds are the products of plant secondary metabolism, and they are valued for their antioxidant properties (*27*). Moreover, the phenolics in yacon seem to be responsible for its biological activities including radical scavenging, cytoprotective, and antihyperglycemic effects (*19*−*21*). While the total content of phenolics reflects both monomeric and polymeric phenolic compounds, HPLC coupled with coulometric detection determines only electrochemically oxidizable monomeric compounds, mainly phenolic acids with antioxidant activity (*15*, *18*). The total content of phenolics was higher than the sum of individual phenolic acids. The total content of phenolics in young yacon leaves was lower than in leaves collected at the time of tubers harvest: maximum 2.67 ± 0.30 vs 3.20 ± 0.08 (*20*) mg/g of the dried drug, respectively. The content of individual phenolic acids previously determined by HPLC with amperometric detection was also higher in mature plants (*18*). Phenolic acids determined in individual yacon genotypes are reported here for the first time. Statistical evaluation of the data revealed significant differences between the genotypes; accessions 6 and 60 had the highest content of phenolic compounds (**Table 3**). Our results revealed significant variability between yacon accessions not only in total phenolic content but also in relative content of individual phenolic acids (**Table 3**) that differ slightly in the biological activity (*27*).

Also, β-(2→1)fructooligosaccharides play a role in the biological activity of yacon tubers. They are not digested in the upper gastrointestinal tract and are therefore fermented by gut bacteria and thus promote the growth of beneficial bacterial species from the *Lactobacillus* and *Bifidobacterium* species, which exert a range of positive physiological effects (*9*). For yacon tuber use as a functional food or food supplement for people suffering from metabolic disorders of glucose metabolism (metabolic syndrome, diabetes etc.), a low content of glucose and a high content of β-(2→1)fructooligosaccharides are favorable. HPTLC is an appropriate technique for quantitative screening of such compounds, and the experimental arrangement presented here permits a distinction between glucose, fructose, and sucrose. The total β-(2→1) fructooligosaccharides content was calculated as the difference between fructose and glucose content in hydrolyzed and native tubers, considering the amount

of fructose and glucose originating from sucrose. A significantly higher content of β-(2→1) oligofructans was noted in group II (accessions 48 and 88) as compared to group I (6 and 60). No difference in other sugars content was observed between the two groups (**Table 4**). Surprisingly, a more beneficial sugar composition was observed in the tubers from accessions having a lower content of phenolic compounds in the leaves. This fact might be important for potential leaf/tuber exploitation for prevention of chronic diseases involving oxidative stress and disorders of glucose metabolism.

Statistical analysis (LSD test) of relative DNA data showed five significantly different groups of yacon accessions (*22*). The variability observed on the level of nuclear DNA content and isozymes (EST and ACP) is rather low in contrast to a large variation in morphological characters and yield parameters (*12*). The results obtained from the analysis of relative DNA amount in yacon show that there is variability on this level. However, a clear relationship among morphological characteristics, isozyme polymorphism and variation in total phenolic, saccharide and relative DNA content, or relative content of main essential oils shown elsewhere (*28*) was not found.

In view of these results, genotype 48 seems to be the most suitable for tuber industrial processing. Over 3 years of cultivation, only 6.5% tubers had irregular shapes, tuber yield was the most balanced, and β-(2→1)oligofructan content was the highest of all of the genotypes studied. Only total leaf phenolic content was the lowest; genotypes 6 and 60 would probably be more appropriate for leaf exploitation, e.g., in medicinal teas. Taken together, for future cultivation, we recommend genotypes 6, 48, and 60 as the most appropriate from the viewpoint of industrial processing and yield parameters.

This is the first comprehensive research of the biological and chemical diversity of original Andean yacon genotypes. A large intraspecific variation in yacon (demonstrated following such an approach) may serve for future detailed research, as a good background for breeding, selection, growing, and utilization in industrial processing.

ABBREVIATIONS USED

ACP, acid phosphatase; ADH, alcohol dehydrogenase; DAPI, 4′-6-diamidino-2-phenylindole; DIA, diaphorase; EST, esterase; GDH, glutamate dehydrogenase; GOT, glutamate-oxalacetate transaminase; GPI, glucose-6-phosphate isomerase; HPTLC, high-performance thin-layer chromatography; IDH, isocitrate dehydrogenase; LAP, leucine aminopeptidase; MDH, malate dehydrogenase; ME, malic enzyme; NADH DH, NADH dehydrogenase; NST, 2-aminoethyl diphenyl borinate; PGD, 6-phosphogluconate dehydrogenase; PGM, phosphoglucomutase; PVP-40, polyvinylpropylpyrrolidone; SHDH, shikimate dehydrogenase; SOD, superoxide dismutase.

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