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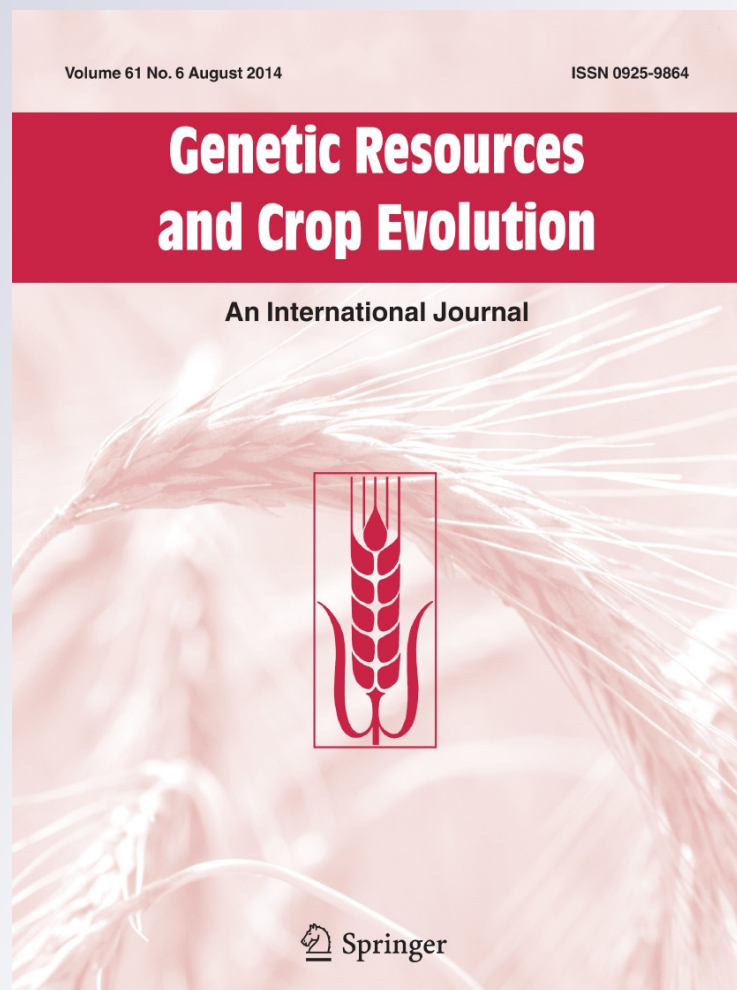
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# Variability in sesquiterpene lactones from the leaves of yacon (*Smallanthus sonchifolius*) accessions of different geographic origin

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**Abstract** The sesquiterpene lactones (STLs) content of glandular trichomes from the leaves of twenty-five yacon (*Smallanthus sonchifolius*, Asteraceae) accessions, obtained along a latitudinal gradient from Ecuador to northwest Argentina, was characterized by gas chromatography/mass spectroscopy (GC/MS). While accessions from Ecuador, Bolivia and Argentina proved to be very chemoconsistent, significant variation was found in quantitative composition of STLs from accessions in central Peru, the probable region of origin for the species.

**Keywords** Chemical variability · Glandular trichomes · *Smallanthus sonchifolius* · Yacon

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## Introduction

*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson (Heliantheae, Asteraceae), “yacon”, is a South American crop cultivated for its edible tuberous roots for more than a thousand years in the Andes slopes from Ecuador to NW Argentina. The name yacon derives from the Quechua word “yakku”, adjective meaning watery or insipid (Grau and Rea 1997). Traditionally consumed raw, as a fruit, the large tuberous roots are similar to sweet potatoes in appearance, with a rather sweet taste due to an abundance of short chain fructooligosaccharides (FOS, inulin type) and carbohydrates such as fructose, glucose, sucrose, but lacking starch (Lachman et al. 2003; Coll Aráoz et al. 2014).

Yacon has awakened worldwide interest due to numerous dietary and medicinal properties. Its unique carbohydrate composition makes it an ideal food for people with obesity and diabetes since FOS have very low nutritive value and promote the growth of intestinal beneficial bacterial species (Pedreschi et al. 2003). Roots are also rich in phenolic compounds such as caffeic, chlorogenic and ferulic acids and their derivatives (Simonovska et al. 2003).

Yacon leaves are consumed as an infusion with reputed hypoglycemic activity (Aybar et al. 2001). Apparently this is a contemporary use with no records in early mentions of yacon in the Andean cultures. Enhydrin (10), a melampolide sesquiterpene

lactone (STL), along with several caffeic acid derivatives, have been identified as the active principles responsible for the hypoglycemic activity (Genta et al. 2010; Schorr et al. 2007; Hwang et al. 1996). STLs are characteristic metabolites of *Smalanthus* spp. (De Pedro et al. 2003) which are produced in the capitate glandular trichomes of the foliar surface (Schorr and Da Costa 2005; Mercado et al. 2010). Evidence suggests that glandular trichomes are fully established and their secretory activity is concluded at early stages of leaf development. STLs and other exudates are accumulated underneath the cuticle and released only when glandular trichomes are mechanically disrupted (Mercado et al. 2012). In nature, STLs play roles as deterrents to herbivores, toxic to pathogens or serve as allelopathic compounds (Chou and Mullin 1993; Takasugi and Masuda 1996; Cis et al. 2006). Among STLs, there are numerous compounds with commercial and medicinal interest (Picman 1986; Marles et al. 1995; Chadwick et al. 2013).

Besides from enhydrin, the main STL from *S. sonchifolius* leaves, fifteen melampolide STL were previously isolated from the aerial parts of *S. sonchifolius* (Mercado et al. 2010). Smaditerpenic acids (Mercado et al. 2010), kaurenoic acid and other related diterpenes (Kakuta et al. 1992) were also found to be constituents of glandular trichomes exudates. Yacon leaves also present high content of phenolic compounds, mainly chlorogenic and caffeic acids and their derivatives (Simonovska et al. 2003), and evidence has emerged about their antioxidant activity (Valentová et al. 2005).

Yacon is a polyploid species that has been classified as semidomesticated (Dempewolf et al. 2008). Being propagated by rhizome sections, it seems likely that continued vegetative propagation and selection for root yield may have impaired flowering and fruit set. Although yacon is a clonal crop, some phenotypical and genetical variation has been reported (Milella et al. 2005; Mansilla et al. 2006; Valentová et al. 2006; Lebeda et al. 2011; Svobodová et al. 2013).

This work was aimed to explore the STL chemical variability by GC/MS of twenty-five yacon accessions, obtained from different locations distributed along a latitudinal gradient from Ecuador to the northwest of Argentina.

## Materials and methods

### Plant material

Twenty-five *S. sonchifolius* accessions were analysed. Leaves from twenty accessions were obtained from material cultivated at Centro Internacional de la Papa (CIP, Huancayo, Junín, Peru). Also five accessions belonging to the Universidad Nacional de Tucumán (UNT) collection, cultivated at Centro Universitario Horco Molle, Tucumán, were analysed. The regions of origin of all the plant materials are listed in Table 1. Accessions codes correspond to those designated by CIP germplasm ordering system and UNT ordering system. Plants were planted, according to Seminario et al. (2003), in experimental plots in August 2006. The middle leaves were harvested on March 2007. All plant material was dried at room temperature in the shade. Voucher specimens of the UNT accessions are deposited in the herbarium of Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina. CIP accessions are maintained in a germplasm collection in Lima, Peru.

### Extraction and STL analysis

Secretions of the glandular trichomes of the foliar surface were extracted from 4 g of leaves from at least five plants from each accession, following the procedure described by Schorr and Da Costa (2005) with modifications. Whole air-dried leaves were soaked individually in 250 mL of  $\text{CHCl}_3$  for 20 s at room temperature with a continuous and gentle swinging motion. The extracts obtained were filtered through a filter paper and the solvent evaporated under vacuum to yield crude residues which were dissolved in MeOH at 50 °C (2.5 mL per 100 mg of residue) to facilitate dissolution. After cooling, a 30 % of distilled water (0.8 mL per 100 mg of residue) was added dropwise to precipitate waxes. The hydromethanolic filtrates were evaporated at reduced pressure to yield dewaxed extracts containing STLs and diterpenes. The residues were analyzed by GC/MS using a 5973 Hewlett-Packard selective mass detector (quadrupole) coupled to a Hewlett-Packard 6890 GC fitted with an Elite-5MS Perkin-Elmer column (5 % phenylmethylsiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness). The following conditions were employed to

**Table 1** *Smallanthus sonchifolius* accessions codes and sites of origin

Code	Country of origin	Region, locality, state	masl	Latitude	Longitude
CIP 205002	Perú	Cajamarca, Celendín, Sucre	2,600	6°55'S	78°9'W
CIP 205004	Perú	Lima, Yauyos, Tintín	3,200	12°17'S	75°48'W
CIP 205005	Perú	Cajamarca, Cajamarca, Can-can	2,600	–	–
CIP 205006	Perú	Cajamarca, Hualgayoc, Bambamarca	2,500	6°40'S	78°30'W
CIP 205007	Perú	Cajamarca, Celendín, José Gálvez	2,600	6°55'S	78° 7'W
CIP 205008	Perú	Cajamarca, Celendín, José Gálvez	2,600	–	–
CIP 205011	Perú	Ancash, Yungay, Cochayó	3,800	9°13'S	77°43'W
CIP 205013	Perú	Ancash, Carhuaz, Arwaypampa	2,700	9°17'S	77°38'W
CIP 205017	Perú	Ayacucho, Vilcashuamán, Chili Cruz	3,000	13°41'S	73°58'W
CIP 205018	Perú	Ancash, Bolognesi, Chiquián	–	8°21'S	78° 3'W
CIP 205019	Perú	Ancash, Bolognesi, La Florida	2,900	–	–
CIP 205021	Perú	Cajamarca, Hualgayoc, Bambamarca, Frutillo	2,600	6°41'S	75°31'W
CIP 205022	Perú	Cajamarca, Chota, San Juan Pampa	2,300	6°39'S	75°26'W
CIP 205023	Perú	Cajamarca, Cajamarca, Chota	2,900	6°33'S	75°39'W
CIP 205028	Perú	Cajamarca, Cajabamba, Machacuay	2,810	7°38'S	78°2'W
CIP 205029	Perú	Apurímac, Andahuaylas, Roncopata	3,180	13°39'S	73°21'W
CIP 205049	Perú	Junín, Concepción, Mariscal Castilla	2,900	11°36'S	75°05'W
CIP DPA006	Perú	Pasco	–	10°26'S	75°9'W
CIP DPA001	Perú	Huanuco	–	9°56'S	76°14'W
CIP 205033	Perú	Junín, Huancayo, Chupuro	3,177	12°09'S	75°14'W
UNT LIEY 97-1	Bolivia	Erquis, Tarija	1,860	21°28'S	64°50'W
UNT LIEY 97-2	Ecuador	Cultivated in New Zealand	–	–	–
UNT LIEY 97-3	Argentina	Condado, Salta	1,860	22°13'S	64°37'W
UNT LIEY 06-4	Argentina	Barcena—Jujuy	1,893	23°59'S	65°27'W
UNT LIEY 06-5	Argentina	Los Yacones—Salta	1,570	24°40'S	65°30'W

Accessions codes correspond to those designated by CIP germplasm ordering system and UNT ordering system

analyze STLs: injector, GC–MS interphase, ion source and selective mass detector temperatures were maintained at 220, 280, 230 and 150 °C, respectively; ionization energy, 70 eV; injection size: 1 µL (split mode 80:1); carrier gas, helium at a flow rate of 1.2 mL min<sup>-1</sup>. The oven was programmed as follows: from 180 to 300 °C at 2 °C min<sup>-1</sup> and then held at 300 °C for 10 min. In order to be injected, the samples were dissolved in methylene chloride using 25 µL of solvent per mg of STL mixture. Percentages are reported as the means of at least 3 runs and were calculated from the TIC (Total Ion Chromatogram) by the computer.

STLs were identified by their GC retention times and their mass spectra in comparison with pure

samples previously isolated in our laboratory (Mercado et al. 2010).

#### Statistical analysis

Cluster analysis was used to classify and group the 25 accessions of *S. sonchifolius* according to their glandular trichomes exudates composition. STL were used to build a matrix of nine variables and 25 samples, considering the presence and amount of STL in each accession. The evaluation of similarity coefficient and cluster analysis was carried out with the Multivariate Statistical Package V. 3.1 (MSVP) Copyright © 1985–2006 Kovach Computing Services.

**Table 2** Relative percentage of STL found in the accessions of *S. sonchifolius*

Accession	1	2	3	4 + 5	6	7	8	9	10	Others
<i>Chemotype enhydrin (subtype uvedalin)<sup>#</sup></i>										
CIP 205002	2.3	6.9	5.0	2.0	19.6	4.5	8.3	6.6	43.56	0.3
CIP 205006	2.4	4.6	5.2	2.9	14.3	4.7	9.0	6.9	50.0	–
CIP 205007	2.6	5.6	4.1	4.2	13.5	3.2	10.6	6.8	49.5	–
CIP 205008	2.5	5.9	4.2	3.4	15.5	3.3	10.4	5.9	48.9	–
CIP 205017	2.5	4.6	5.8	3.1	15.0	6.1	8.1	5.6	49.3	–
CIP 205021	2.1	6.5	4.5	4.5	16.0	3.2	8.9	5.9	48.5	–
CIP 205022	2.5	4.9	4.4	3.7	14.4	3.8	9.4	5.8	51.1	–
CIP 205023	1.8	6.7	4.1	11.1	13.7	3.6	8.8	4.0	46.3	–
UNT LIEY 97-1	1.5	6.5	5.5	4.6	16.9	4.0	10.1	3.9	47.1	–
UNT LIEY 97-2	2.9	2.6	2.5	2.4	28.1	6.9	1.4	4.7	47.0	1.6
UNT LIEY 97-3	1.3	5.6	5.6	3.8	16.4	4.6	8.5	3.4	50.9	–
UNT LIEY 06-4	1.0	3.9	3.6	3.6	16.6	4.2	7.0	5.3	54.8	–
UNT LIEY 06-5	1.0	5.0	2.6	3.9	16.8	2.5	9.2	7.6	51.4	–
<i>(Subtype fluctuanin)<sup>#</sup></i>										
CIP 205005	3.8	5.8	4.4	8.4	10.8	3.1	14.2	5.3	44.3	–
CIP 205011	3.4	5.6	4.9	9.5	8.7	3.5	14.8	4.4	45.3	–
CIP 205013	4.2	5.7	4.9	9.1	8.9	3.4	14.9	4.3	44.7	–
CIP 205028	5.0	6.7	3.9	10.7	9.3	2.1	16.9	3.6	41.8	–
CIP 205029	4.7	6.7	5.5	7.9	8.9	2.9	17.6	2.8	43.0	–
<i>(Subtype uvedalin aldehyde)<sup>#</sup></i>										
CIP 205049	3.2	2.6	14.2	2.3	6.9	17.6	6.2	4.7	42.4	–
CIP DPA006	3.7	2.5	17.6	2.9	6.0	21.7	5.1	2.7	37.9	–
CIP DPA001	3.5	2.3	13.2	2.5	7.2	18.3	5.8	3.8	43.5	–
CIP 205033	3.8	3.2	12.5	2.7	9.5	14.9	6.5	3.8	43.1	–
<i>Chemotype uvedalin</i>										
CIP 205004	4.6	9.4	6.2	11.6	44.9	2.3	1.1	1.8	4.8	13.4
CIP 205018	4.5	12.5	2.0	11.8	59.1	2.5	–	–	7.7	–
CIP 205019	3.6	13	–	9.1	68.0	–	–	–	6.3	–

Relative percentage amounts calculated by TIC

<sup>#</sup> Subtypes based on the second majoritarian STL

Distance was estimated by arithmetic averages. Similarity estimates were analysed by UPGMA, and the resulting clusters were expressed as dendrograms.

## Results

Although yacon is a clonal crop, some variation in the STLs profiles of the 25 accessions was found (Table 2). Variation does not extended to differences in skeletal types; instead the chemistry of accessions is differentiated by the relative amounts of the melampolide STLs. The structures of the melampolides

are represented in Fig. 1. Enhydrin (**10**) was the main lactone component in the glandular trichomes exudates from most of the accessions. Uvedalin (**6**) was found to be the main component in three accessions from central Peru (Departments Lima and Ancash). Accessions were grouped in two chemotypes based on their majoritarian STL. Chemotype enhydrin comprehended 22 accessions divided into three subtypes uvedalin, fluctuanin and uvedalin aldehyde based on the second majoritarian STL (Table 2; Fig. 2a–c). Chemotype uvedalin was represented only by 3 accessions (collected in Departments Cajamarca and Ancash, Peru) (Table 2; Fig. 2d). Within accessions

relative abundance of STL remained constant between individual plants.

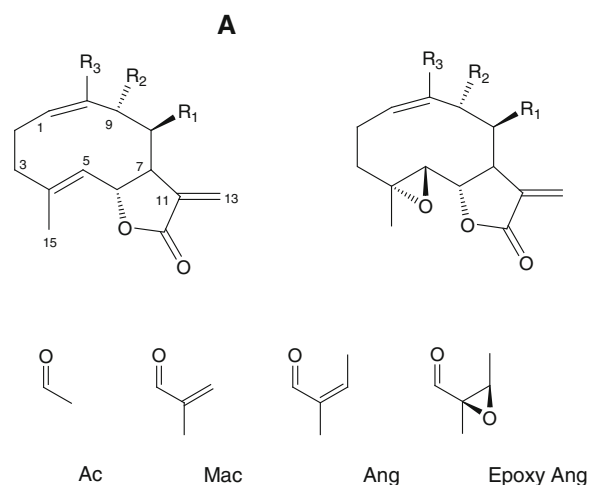
A cluster analysis was performed based on the content of STLs, the resulting dendrogram (Fig. 3) shows the relationships between the studied accessions. Chemotype uvedalin (accessions CIP 205004, 205018, 205019) was only present in the central region of Peru.

## Discussion

The results indicate a very low intraspecific variability in STL profiles of different *S. sonchifolius* accessions.

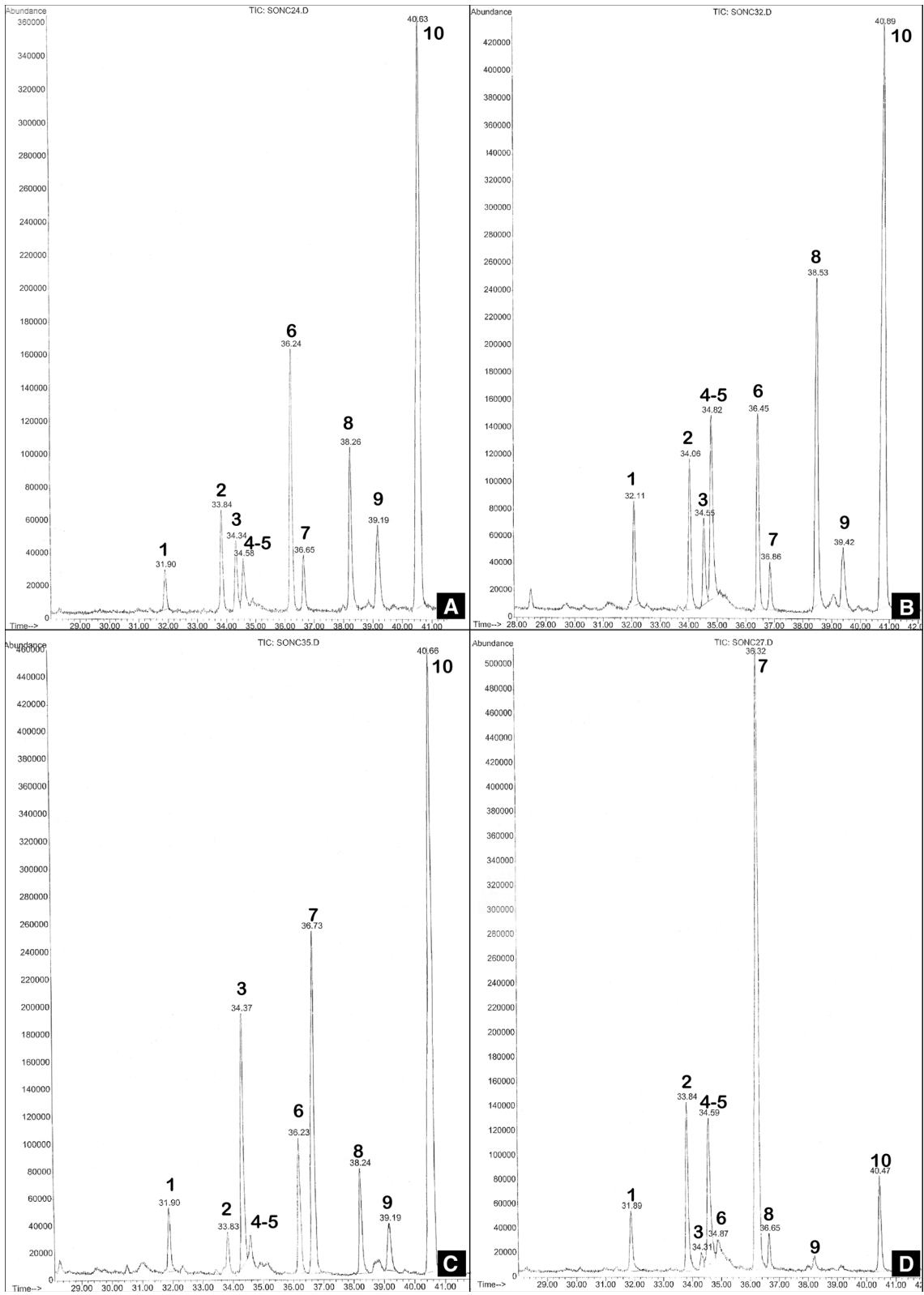
No qualitative variability was found, indicating yacon as a highly chemoconsistent taxon. All the STLs identified corresponded to melampolide type (Fig. 1). The different lactones are differentiated by the presence of a double bond or an epoxide group between C-4 and C-5, the nature of the ester moiety at C-8 and C-9, and the presence of CO<sub>2</sub>Me or an aldehyde group CHO at C-10. Sonchifolin (1) is considered a precursor for the more oxidized STLs.

Cluster analysis showed very high similarity values, as expected by the long-term selection for desired agronomic traits during yacon evolution. Accessions were grouped in chemotypes based on their two



Compound	Skeleton	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	RT [min]
1 Sonchifolin	A	OAng	H	CO <sub>2</sub> Me	31.85
2 Polymatin B	A	OAng	OAc	CO <sub>2</sub> Me	33.80
3 Polymatin B aldehyde	A	OAng	OAc	CHO	34.30
4 Fluctuadin	B	OMac	OAc	CO <sub>2</sub> Me	34.53
5 Polymatin A	A	OAng	OH	CO <sub>2</sub> Me	34.53
6 Uvedalin	A	OEpoxy Ang	OAc	CO <sub>2</sub> Me	36.45
7 Uvedalin aldehyde	A	OEpoxy Ang	OAc	CHO	36.65
8 Fluctuanin	B	OAng	OAc	CO <sub>2</sub> Me	38.27
9 Polymatin C	B	OAc	OEpoxyAng	CO <sub>2</sub> Me	39.20
10 Enhydrin	B	OEpoxyAng	OAc	CO <sub>2</sub> Me	40.58

**Fig. 1** Structures of melampolides isolated from the leaves of *S. sonchifolius*. RT retention times in GC under the conditions described in STL analysis





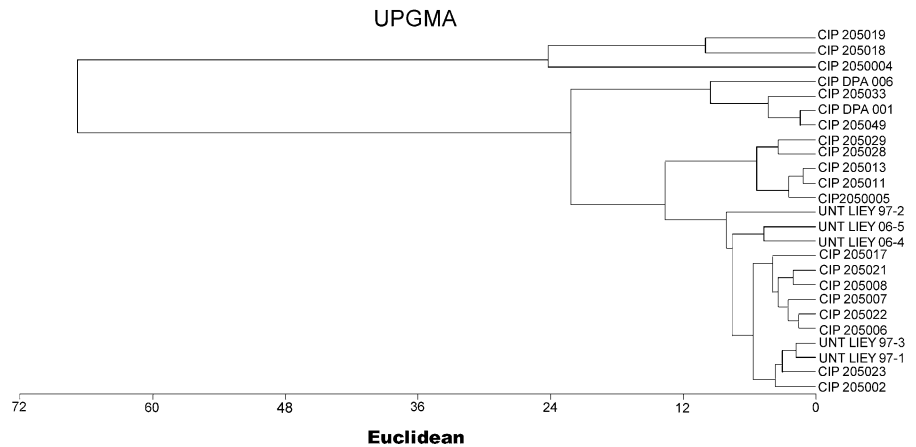
**Fig. 2** Typical GCs of *S. sonchifolius* chemotypes. Numbers indicate the STLs identified in the foliar rinse extracts, as designated in Fig. 1. **A** Chemotype enhydrin, subtype uvedalin; **B** chemotype enhydrin, subtype fluctuanin; **C** chemotype enhydrin, subtype uvedalin aldehyde and **D** chemotype uvedalin

majoritarian STLs. Peru showed the highest chemodiversity, with all the chemotypes represented in the central region of the country (Fig. 4).

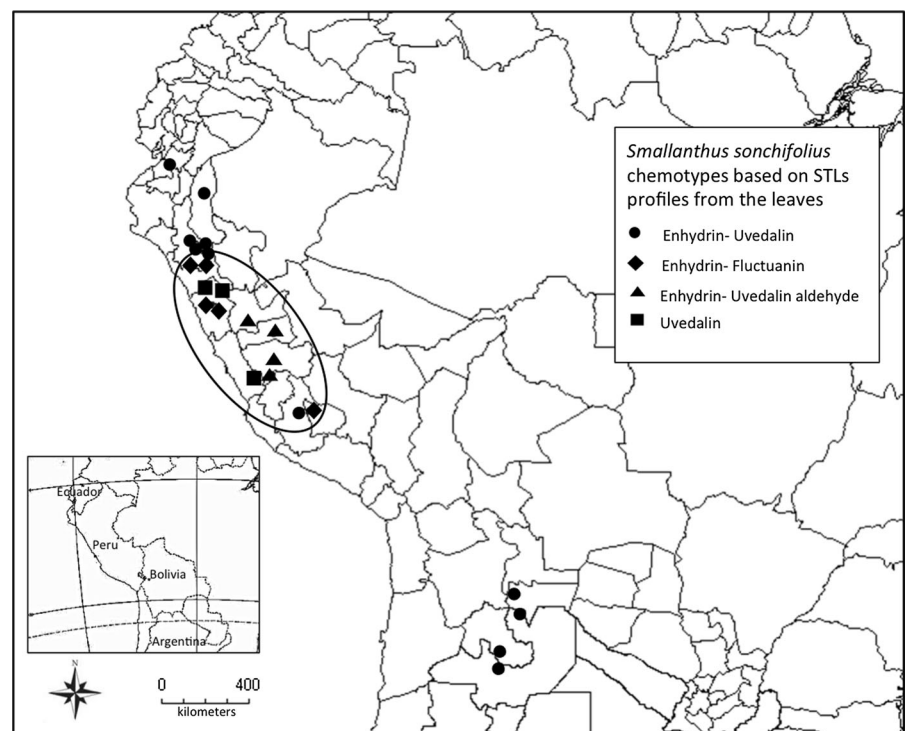
Mansilla et al. (2006) studied genetic diversity among 22 yacon accessions from different Peruvian

locations cultivated at CIP. They found a low level of polymorphism, as expected because of the high level of vegetative propagation and the low level of seed set of this crop, nevertheless they were able to group the accessions in seven clusters based on similarities of RAPD markers. They found that within the studied material, the central Peruvian region (Departments Huánuco y Pasco, Lima, Junín, Ancash) showed the highest genetic diversity, in accordance with the results presented here.

**Fig. 3** UPGMA cluster analysis of 25 *S. sonchifolius* accessions, based on the STLs content obtained from the leaves



**Fig. 4** Sites of origin of the accessions of *S. sonchifolius*. Circle indicates the region with the highest chemodiversity



Milella et al. (2005) studied RAPD markers from material cultivated in New Zealand, Germany, Ecuador and Bolivia. They found material from Bolivia to Ecuador, as well as one of the materials from New Zealand, had a common genetic ancestry and a high level of similarity.

Svobodová et al. (2013) compared a set of 29 accessions of *S. sonchifolius*, and three wild relatives using ISSR markers. They clearly separated the wild relatives from all *S. sonchifolius* samples, which remained close to each other, confirming the clonal origin and thus a very low genetic variability within the species.

The region extending from northern Bolivia to central Peru has been reported as the area with the largest clonal diversity, and where native Quechua (*Llaqon*, *llacum*, *llacuma* or *yacumpi*) and Aymara (*Aricoma* or *aricama*) names are used (Grau and Rea 1997). The central region of Peru, in the eastern humid slopes of the Andes, which showed the highest STL chemodiversity, is thought to be the center of domestication of yacon (Grau and Rea 1997). Interestingly, accessions from Ecuador (UNT LIEY 97-2) and Argentina (UNT LIEY 97-3, UNT LIEY 06-4 and UNT LIEY 06-5) had the same STLs profiles with very high similarity values (Fig. 3), with enhydrin as majoritarian STL and uvedalin as the second compound in importance in the foliar rinse extracts. It is possible that the wider distribution of this chemotype (Fig. 4) is due to human selection, apparently due to a highest root yield, leading to expand the distribution of this chemotype. According to Grau and Rea (1997), the species could have been introduced in Ecuador, and probably Argentina, with the Inca conquest, only decades earlier than the Spanish invasion.

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