## Short Communication

# Genetic Diversity between Yacon Landraces from Different Countries Based on Random Amplified Polymorphic DNAs

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**Abstract**: Random amplified polymorphic DNA (RAPD) markers are widely used for evaluating the genetic relationship of crop germplasm. Five different landraces of yacon (*Smallantus sonchifolius* (Poepp. and Hendl.) H. Robinson; *Asteraceae*) collected in various countries and showing different morphological traits were investigated using a total of 61 decamer primers. A total of 282 RAPD markers were scored and 28.7% of them were polymorphic at least within landraces. RAPD markers generated by one primer (OBP14) discriminated between all landraces. Markers were used to calculate genetic similarity coefficient and to build a dendrogram representing the genetic relationship between analysed landraces. The results suggest that RAPD markers could be used as a reliable tool to perform fingerprinting studies in *Smallantus sonchifolius* genome. This is the first report on the use of RAPDs to evaluate genetic distance and to distinguish between different landraces in yacon.

**Keywords**: *Smallantus sonchifolius*; yacon; RAPDs; fingerprinting

For the assessment of genetic diversity molecular markers have been generally superior to morphological traits and biochemical markers (Melchinger *et al.* 1991). At the same time molecular markers could be useful, in particular in crops, to investigate genome structure (Hemmat *et al.* 1994; Davis & Yu 1997), germplasm characterisation (Cardeña *et al.* 2003), to define crossing combinations and cultivar identification (Luro *et al.* 1995; Millan *et al.* 1996; Cao & Oard 1997; Martelli *et al.* 1999). Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir 1990). Molecular Marker Based Genetic Diversity Analysis (MMGDA) also has a potential for assessing changes in genetic diversity over time and space. Yacon *Smallantus sonchifolius* (Poepp. and Hendl.) H. Robinson; *Asteraceae* is a perennial herb 1.5–3 m tall with the root system composed of 4–20 freshly edible tuberous storage roots weighing up to 2 kg, originally cultivated in South America. The parenchyma accumulates sugars and, in some cases, pigments typical of certain landrace groups. According to pigments, the flesh

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colour varies considerably: white, cream, white with purple striations, purple, pink and yellow. Flower production is more reduced in yacon than in other wild *Smallantus* species. Reduced flowering and fruit set are features commonly present in other clonally propagated tuber crops. During yacon evolution, continued vegetative propagation and selection for root yield may have impaired flowering and fruit set. Although yacon is a clonal crop, there is some morphological and physiological variation. However, this variation may reflect to some extent the phenotypic plasticity expressed in the contrasting environments where it is grown rather than genetic variation. It is very difficult to differentiate some yacon landraces from a wide geographical range, from Ecuador to Argentina, when they were grown in the same environment (Grau & Rea 1997). Yacon was considered by the early Andean inhabitants as a fruit and it has a relatively low energy value despite its juiciness and sweet taste. In South America, Bolivia, Brazil and Argentina, yacon roots and leaves are commonly consumed by people suffering from diabetes or various digestive or renal disorders and this ethnobotanical use was confirmed by recent scientific research (Aybar *et al.* 2001; Simonovska *et al.* 2003). Recently, the interest in this crop has increased due to its good post-harvest life if managed properly (Ohyama *et al*. 1990), exceptional qualities for low-calorie diets thanks to its abundant content of fructooligosaccharides that humans cannot digest in the colon, the absence of starch and medicinal properties, (Inoue *et al.* 1995; Hondo *et al.* 2000; Aybar *et al.* 2001). Yacon was originally cultivated in South America, and it has been introduced into New Zealand, Japan, Germany and the Czech Republic. The productivity and other valuable agronomic traits of yacon strongly suggest that it is a species with a great potential, moreover the cultivation of this plant hardly needs any pesticides (Lin *et al.* 2003). The evaluation of the genetic relationship between ecotypes or cultivars by molecular strategies could be useful for planning breeding programs, mainly to select segregating populations and for patent protection or patent demand. Compared with a majority of other roots and tubers, the knowledge of yacon is rather limited and many fundamental aspects of its biology and agronomy are virtually unknown. At the moment, nothing is reported on yacon genome fingerprinting, making this report to be the first to describe the significance of RAPD markers in *Smallantus sonchifolius* genome analysis. The aim of this study is to investigate genetic variability in five different landraces selected in various countries showing different morphological traits.

## MATERIALS AND METHODS

***Plant material***. Five landraces of yacon (*Smallantus sonchifolius* (Poepp. and Hendl.) H. Robinson) named with numbers increasing from 1 to 5, were collected in four countries. The landraces were selected for their different morphological traits (Table 1) and then grown in the same environmental conditions in the Czech Republic after vegetative propagation. They have been grown in the Czech Republic since 1994 at the experimental farm of the Czech University of Agriculture in Prague and in the Potato Research Institute in Havlíčkův Brod.

***DNA extraction***. Genomic DNA was extracted from approximately 500 mg of young leaf tissue. The leaf tissue was ground to a fine powder in

Table 1. Origin and morphological traits of yacon landraces used in this study

| Origin | Code | Height[a] (mm) | Leaf colour | External root colour | Root flesh colour | Rhizome colour | Flower production |
|---|---|---|---|---|---|---|---|
| New Zealand | 1 | 1330 | green | cream | orange | purple | No |
| New Zealand | 2 | 1350 | dark green | purple | crystal white | purple | Yes |
| Germany | 3 | 1050 | light green | cream | crystal white | white with purple striations | No |
| Ecuador | 4 | 1410 | dark green | purple | white | purple | Yes |
| Bolivia | 5 | 1500 | dark green | purple | white | purple | Yes |

[a]Trait observed at a harvest time approximately 5.5 months after planting out (2000–2004)

liquid nitrogen. Total genomic DNA was extracted using acetyltrimethylammonium bromide (CTAB) protocol described by Eldredge *et al.* (1992) and then purified using Wizard DNA Clean up System (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. DNA concentrations were estimated by a spectrophotometric assay.

***PCR procedure***. The 10-mers used as random primers (Table 2) in the PCR were purchased from Operon Technologies (Alameda, California, USA) and Applied Biosystems (Foster City, California, USA). Conditions for DNA amplifications were standardised for all primers. *Taq* DNA polymerase, together with 10× concentrated PCR buffer and $MgCl_2$ solution, was supplied by Promega. Approximately 25 ng of genomic DNAs were amplified, moreover each 25 µl PCR reaction contained, 0.1mM each of dATP, dCTP, dGTP, dTTP, 2.0mM random primer and 0.5U *Taq* DNA polymerase in 1× PCR buffer (10mM Tris-HCl pH 9.0, 50mM KCl, 0.1% Triton X-100). The RAPD reactions were carried out in an MJ Research PTC-200 thermal cycler under the following conditions: 94°C for 4 min and 40 s, followed by 40 cycles at 94°C for 20 s, 36°C for 60 s, ramp + 0.2°C/s, 72°C for 60 s, and final extension 72°C for 9 min. The amplification products were mixed with 3 µl of loading dye (62.5 mM EDTA, 20% w/v Ficoll 400, 0.1% w/v bromophenol blue, 0.1% w/v xylene cyanol and 0.2% w/v orange G), separated in 1.5% TAE agarose gel (SERVA, Heidelberg, Germany), run for 3.5 h at 80 V, stained with ethidium bromide and photographed under UV light with Polaroid films. RAPD experiments were repeated twice, and only reproducible DNA fragments ranging from 500 to 2000 were considered. Negative controls, without DNA, were included in each experiment in order to verify that neither self-amplification nor DNA contamination occurred.

***Statistical analysis***. PCR fragments were scored and each was used to build binary a matrix by considering the presence (1) or absence (0) of bands in each of the five landraces. Several calculations were made to compare them with respect to their RAPD marker profile. The proportion of polymorphic loci ($P$) was calculated according to the formula: $P = n/N \times 100\%$, where $n$ is the number of polymorphic bands within the group of landraces and $N$ is the total number of bands that was analysed. This coefficient was recommended for the evaluation of genetic similarities when using RAPD data (Lamboy 1994). The evaluation of similarity coefficient and cluster analysis was carried out with the Popgene 32 software package (available at http://www.ualberta.ca/~fyeh/download.htm). Genetic distance was estimated by Nei's (1972) genetic identity and genetic distance and Nei's (1978) unbiased genetic identity and genetic distance. The estimation is made for multiple populations' dendrogram drawing a dendrogram based on Nei's genetic distances using the unweighted pair-group method arithmetic averages (UPGMA). This program is an adoption of program NEIGHBOR of PHYLIP version 3.5c by Joe Felsenstein. Similarity estimates were analysed by UPGMA, the resulting clusters were expressed as dendrograms.

## RESULTS

A total of 61 decamer primers (Table 2) were screened with the DNA samples from the five landraces. Of them, 28 provided at least one polymorphic band, the remaining primers were monomorphic (Table 2). The fragments ranged from about 300 to 3000 base pairs (bp), but our results suggest that fragments out of the range between 500 and 2000 bp were rarely reproducible. The amplification products ranging from 500 to 2000 bp that yielded only strong, sharp and repeatable bands

Table 2. Used primers and schematic description of results

| Primers | Number of primers | Monomorphic bands | Polymorphic bands | Total scorable bands |
|---|---|---|---|---|
| AB2 1, 3–7 | 6 | 17 | 5 | 22 |
| CS 12, 13, 15, 16, 31 | 5 | 18 | 12 | 30 |
| OPA 1–20 | 20 | 78 | 17 | 95 |
| OPB 1–12, 14–20 | 19 | 47 | 39 | 86 |
| OPD 5, 7 | 2 | 6 | 0 | 6 |
| OPI 1–9 | 9 | 36 | 7 | 43 |

Table 3. RAPD similarity matrix of landrace groups, Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

| Landrace | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | **** | 0.77 | 0.89 | 0.81 | 0.83 |
| 2 | 0.26 | **** | 0.82 | 0.92 | 0.91 |
| 3 | 0.12 | 0.20 | **** | 0.85 | 0.83 |
| 4 | 0.21 | 0.08 | 0.16 | **** | 0.94 |
| 5 | 0.18 | 0.10 | 0.19 | 0.06 | **** |

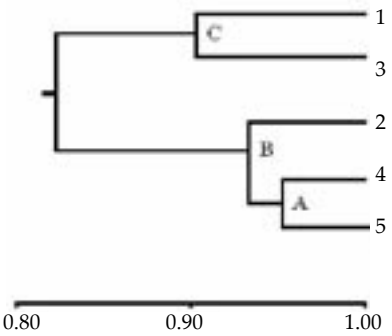


Figure 2. Dendrogram of the five yacon landraces generated by UPGMA cluster analysis of similarity matrix based on 282 RAPD markers

were scored to build a binary matrix. A total of 282 bands (Table 2) were detected with the mean number of 4.9 accountable fragments per primer and 28.7% of them were polymorphic (Table 2). The similarity values based on RAPDs ranged from 0.77 to 0.94 (Table 3) with an average similarity value of 0.86. The highest value 0.94 is between the varieties coming from South America and the lowest between the two New Zealand varieties (Table 3). Primer OPB14 (Figure 1) was able to give a specific pattern for each landrace. The number of rare RAPD markers was low; only eighteen markers were landrace specific, five for landrace 1, six for 2, two for each 3 and 4, and 3 for 5, respectively. One band obtained with OPA01 primer was specific for the South American varieties. The binary matrix was used to build a dendrogram (Figure 2). Several calculations were made to compare the obtained results, dendrograms were also built using only markers produced by polymorphic primers, or produced by a single series of Operon Technologies primers.

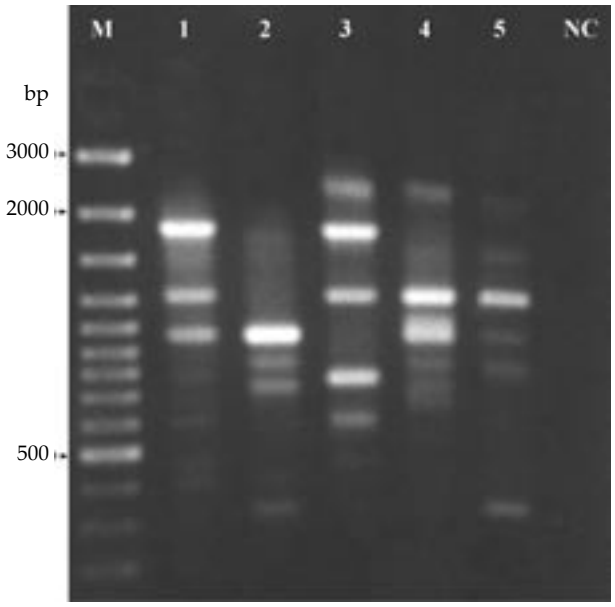


Figure 1. RAPD profiles generated by the primer OPB14. The number corresponding to the landrace is at the top of each lane. Negative control (NC) was added and lane M is the molecular weight marker (MBI Fermentas, Lithuania)

## DISCUSSION

Five different yacon landraces were analysed, and they show 28.7% of polymorphic bands. As expected, the similarity values are very high because of the high level of vegetative propagation, the low level of flower production and the long-term selection for desired agronomic traits during yacon evolution. Dendrograms were built using different binary matrixes: all scored fragments, only OPA (20 primers) primer series band pattern, only OPB (19 primers) primer series band pattern. Notably, all three dendrograms showed the same grouping, meaning that in this case not so many primers were necessary to determine the genetic relationship between selected landraces. The dendrogram (Figure 2) shows that the two South American varieties are closely related (group A), which is congruent with all morphological traits (leaf colour, root flesh colour and rhizome colour, root external colour, presence of flowering) and the plant origin, and both were closely related to landrace 2 collected in New Zealand, the other grouping suggests that varieties 1 and 3 are correlated (group C), despite their origin and some morphological traits. In fact varieties 1 and 3 differ in leaf colour, root flesh colour and rhizome

colour, conversely they are similar in root external colour and in the absence of flowering. As reported by Melchinger *et al*. (1991), RAPD markers were more superior to morphological traits for the assessment of the genetic relationship investigation. The low number of rare bands between landraces, only eighteen, suggests that the plant material analysed in this study shares a common genetic ancestry and possesses a high similarity level. For these reasons the distribution of the landraces within the clusters does not fully respect their geographical origin. We demonstrate that RAPD markers are a useful tool for the identification of yacon landraces and our results suggest that the landraces investigated in this study were closely related. This work represents the first report on the use of RAPDs to evaluate genetic distance and to distinguish between different varieties in yacon. A larger study, analysing cultivated and wild yacon landraces, would be necessary to confirm this hypothesis and to evaluate more accurately the diversity available for yacon breeding.

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## Souhrn

Milella L., Salava J., Martelli G., Greco I., Cusimamani E.F., Viehmannová I. (2005): **Genetická diverzita mezi krajovými odrůdami jakonu pocházejícími z různých zemí detekovaná metodou náhodně amplifikovaných polymorfních DNA**. Czech J. Genet. Plant Breed., **41**: 73–78.

Náhodně amplifikované polymorfní DNA (RAPD) markery jsou široce používány k hodnocení genetické příbuznosti genových zdrojů kulturních rostlin. Pět krajových odrůd jakonu (*Smallantus sonchifolius* (Poepp. a Hendl.) H. Robinson; *Asteraceae*), pocházejících z různých zemí a vykazujících odlišné morfologické znaky, bylo analyzováno pomocí 61 náhodných primerů. Celkem bylo hodnoceno 282 RAPD markerů, z nichž 28,7% vykazovalo polymorfismus mezi studovanými krajovými odrůdami. RAPD markery vytvořené primerem OPB14 rozlišily všechny krajové odrůdy jakonu. Byl proveden výpočet koeficientu genetické podobnosti a sestaven dendrogram ukazující příbuznost mezi vybranými krajovými odrůdami. Získané výsledky naznačují, že je možné použít RAPD markery pro fingrprintink genomu *Smallantus sonchifolius*. Příšpěvek je první sdělení o použití RAPD markerů pro odlišení krajových odrůd jakonu a hodnocení genetických vzdáleností mezi nimi.

**Klíčová slova**: *Smallantus sonchifolius*; jakon; RAPDs; fingrprintink

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