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RESEARCH ARTICLE

Analysis of the morphological attributes of a sweetpotato collection

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Abstract

The knowledge about the distribution of descriptors of a collection constitutes a useful tool for the management of genetic resources. The object of this work was to evaluate the composition and morphological characterisation of the '*in vitro*' collection kept at the Gene Bank of the Biological Resources Institute (IRB), INTA Castelar, Argentina, to establish conservation criteria and make available useful data for breeding programmes. This collection, comprising 310 sweetpotato clones, includes landraces, worldwide clones, commercial varieties and breeding material. The descriptors, which presented the highest correlation values, were leaf lobe types, the shape of central leaf lobes and general leaf outline. Cluster analyses showed eight major groups with an average similarity of 0.42 (SE $\pm$ 0.005). About 76% of the clones presented unique morphology, whereas 34% of them were distributed in 22 groups that could not be distinguished with this technique. Worldwide germplasm formed a separate group with values of diversity higher than those of the Argentinean clones and no duplicates. A projection of the phenotypic variation among cultivars was obtained through Principal Coordinate Analysis (PCoorA), which confirmed the results obtained by UPGMA analysis, predominant skin colour, secondary skin colour, number of leaf lobes, general leaf outline, petiole pigmentation and predominant colour of vine were the variables that made the highest contribution. Collection composition in reference to flesh and skin colour was also analysed.

Introduction

Sweetpotato [*Ipomoea batatas* (L). Lam] is a hexaploid ($2n = 6X = 90$) species. Although the exact location of the domestication centre is not confirmed, based on the morphological characters of sweetpotato and its related species, the Yucatan Peninsula, Mexico and the mouth of the Orinoco River in Venezuela were postulated as possible origins (Austin, 1988). The dispersal area extends from Central America to the North of Argentina.

There is a demand for a better understanding of the extent of the genetic diversity within germplasm collections and the nature of the genetic relationships between the sweetpotato clones for its rational use. In this way, the morphological characterisation is important if the purpose is to maximise the amount of useful genetic diversity in a germplasm collection, such as *core collection* (Brown, 1989).

Morphological characters, together with physiological, phenological and agronomic descriptors, are the starting point of the characterisation work (Ayres Carvalho & Quesenberry, 2009; Diederichsen, 2009; Gonçalves *et al*., 2009; Hend *et al*., 2009; Karimi *et al*., 2009; Nieto-Ángel *et al*., 2009; Szamosi *et al*., 2009; Vieira *et al*., 2009).

Because these descriptors do not need sophisticated or specific equipment, they constitute a simple approach, and a tool of knowledge of agronomic traits. Descriptors, which are specific for each species, have been largely used in characterisation, differentiation and crop protection. In addition, they have been accepted by the

International Union for the Protection of New Varieties of Plants (UPOV) to record cultivars and evaluate the fulfilling requisites of distinctness, uniformity and stability in different species (Furones-Pérez & Fernández-López, 2009). In sweetpotato, they have been used to evaluate germoplasm collections (Ritschel & Huamán, 2002) and, together with ecogeographical, disease and pest reaction data (Huamán *et al*., 1999), they have been useful to define core collections.

Some of the morphological descriptors are specially important for breeding programmes. Colour is the main attribute for vegetables not only because it influences consumers' preferences (Marsili, 1996), but also because it affects the perception of taste and flavour (Alasalvar *et al*., 2001; Habegger & Schnitzler, 2005). Several descriptors even have functional properties related to nutrition and health. Carotenes and anthocyanins are the most relevant pigments that confer colour and the functional properties reported in sweetpotato. It is well known that carotene can be converted to vitamin A (Olson, 1989) and is responsible for the orange and yellow coloured fruits and vegetables. Anthocyanins, on the other hand, are responsible for the red and purple coloured flesh and skin and have antioxidant properties which play an important role in the prevention of several diseases. Both pigments are ubiquitous natural pigments that serve essential functions (Demming-Adams and Adams, 2002) and may act in plant defence (Bouvier *et al*., 2005).

Argentina germplasm collection of sweetpotato is constituted by landraces, commercial varieties, breeding clones and other materials brought in from other parts of the world (Africa, Asia, USA). Most of the local materials have been collected during three expeditions to the dispersal area of the species in Argentina in collaboration with the International Potato Center (CIP). The decision for making the collections was taken under the threat of genetic erosion, because at that time the cultivar Morada INTA was cultivated practically exclusively. As from 2 years ago, INTA has implemented a programme called 'Family Agriculture'. This programme contemplates small farmers and includes the rescue of neglected crops like sweetpotato, among others. From this initiative, our Gene Bank has to match the demand and morphological traits with the users.

The object of this work was to evaluate the composition and morphological characterisation of the whole sweetpotato collection conserved at the Biological Resources Institute (IRB)–INTA Castelar, Argentina, to establish conservation criteria, generate information to define a core collection, make available useful data for breeding programmes and satisfy the demands of farmers.

Materials and methods

Morphological characters

Data from 310 sweetpotato clones belonging to the *in vitro* collection of sweetpotato at the Gene Bank of IRB-INTA Castelar, Argentina, were considered in this analysis.

A total of 28 descriptors, established by Huamán (1991), including twining (ability of vines to climb stems of plants), growth habit, vine internode diameter and length, predominant and secondary stem colour, vine tip pubescence, leaf lobe, mature leaf size, abaxial leaf vein pigmentation, mature leaf colour, immature leaf colour, petiole pigmentation and length, storage roots arrangement, storage root cortex thickness, storage root shape, storage root defects, predominant skin colour, intensity of predominant colour, secondary skin colour, predominant flesh colour, secondary flesh colour and distribution of secondary flesh colour were analysed in this work. These data were part of the evaluation made by Huamán (1993) and Hompanera *et al*. (1993) and are available at http://servicios.inta.gov.ar/bancos/catalogos.

Data analysis

A simple matching coefficient was selected to construct the similarity matrix. Cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and Principal Coordinate Analysis (PCoorA) was performed with the NTSyS pc v-2.11W computer programme (Rohlf, 1989). Correlations between the similarity matrix and the cophenetic matrix were calculated by Pearson's product-moment and the significance of the correlations tested by Mantel's test (Mantel, 1967) (NTSyS program, *MXCOMP* module). The correlations between the morphological descriptors were computed using the NTSyS pc v-2.11W computer programme.

Diversity evaluation

Simpson (1949) and Shannon & Weaver (1949) diversity indexes were used to measure the phenotypic diversity of each descriptor. For a clear interpretation, descriptors were classified in vein, leaf and storage roots, respectively, as proposed by Ritschel & Huamán (2002). The formula to calculate both indexes is presented below. These indexes have also been used to evaluate *Glycine max* (Perry & McIntosh, 1991), sweetpotato (Ritschel & Huamán, 2002) and *Arachis* collections (Ayres Carvalho & Quesenberry, 2009), among others. The values of these indexes range from 0 to 1, where 1 represents great genetic diversity and 0 represents low, or no genetic diversity.

Shannon–Weaver diversity index: $H = -p_i \log_2(p_i)/\log_2 n$ where n is the number of classes per character and p_i the frequency of the n class of a character.

Simpson diversity index: $D = 1 - sum\,(p_i^2)$

Low values of this diversity index indicate no balanced characters and thus a low level of polymorphism.

Results

Collection composition in reference to geographic origin

This collection is composed of 80% of Argentine materials (which include landraces, breeding clones and commercial cultivars), 17% of worldwide cultivars and 3% of the clones with missing origin data. The local germplasm comes mainly from the provinces of Misiones (22.97%), Corrientes (22.45%) and Formosa (8.78%). Other local origins with minor contributions are Buenos Aires, Chaco, Jujuy, Entre Rios, Tucumán and Santa Fe. The worldwide group includes materials from the USA, Niger, Puerto Rico, Peru, Japan, China, Brazil, Mexico, Thailand, Ecuador, Cuba and Burundi, most of which were donated by CIP. The origins that make the greatest contributions are the USA (35%) and Niger (24.53%).

Morphological descriptors

Variation among clones was observed for all descriptors (Table 1). Diversity coefficients ranged from 0.061 (for twining) to 0.88 (for colour intensity and vine internode length) with an average value of 0.624 (SE ± 0.038) for *Shannon–Weaver*, and from 0.014 (for twining) to 0.998 (for distribution of secondary flesh colour) with an average value of 0.753 (SE ± 0.055) for Simpson index, for the whole collection. Descriptors were classified into three groups: leaf characters, root characters and vein characters. About 75% of the descriptors presented diversity coefficient values higher than 0.5. Root characters were the most diverse (90% with an index higher than 0.5), followed by leaf characters (80%) and vein characters (75%).

The worldwide germplasm presented values for both diversity indexes higher than those of the local material (Shannon index: worldwide 0.76 (SE ± 0.03), Argentina 0.59 (SE ± 0.04); Simpson index: worldwide 0.64 (SE ± 0.03), Argentina 0.59 (SE ± 0.04).

Correlations between morphological descriptors were calculated. The descriptors which presented the highest correlation values were: type of leaf lobes and shape of central leaf lobes ($r = 0.811$), type of leaf lobes and general leaf outline ($r = 0.798$) and predominant colour of vine and petiole pigmentation ($r = 0.750$). For the root characters group, predominant secondary flesh colour and distribution of secondary flesh colour presented correlation values of 0.587, whereas secondary flesh colour and distribution of secondary flesh colour presented correlation values of 0.631, respectively. Some authors have suggested that only correlation coefficients with absolute values higher than 0.71 should be considered biologically meaningful (Ayres Carvalho & Quesenberry, 2009).

Cluster analysis

Seventy-six per cent (76%) of the clones presented unique morphology whereas 34% were distributed in 22 groups with a similarity value of 1. Because some of these clusters included clones with a correlative denomination, they could be possible cases of duplicates. A possible explanation for duplicates is the nature of propagation and the phenotypic plasticity of the clones. Materials are commonly interchanged between farmers and the same genotype can express itself differentially depending on the environmental and soil conditions. During the collection, some of these materials seemed different, but comparative assays in the same environment revealed they were identical (N.R. Hompanera, personal communication). Worldwide materials were totally discriminated.

The whole collection presented an average similarity value of 0.42 (SE ± 0.005). The local germplasm exhibited a similarity value of 0.48 (SE ± 0.001) between clones, while the worldwide germplasm showed an average similarity value of 0.34 (SE ± 0.004). The similarity between these two groups was 0.27 (SE ± 0.003).

The correlation between the similarity matrix derived from morphological descriptors and the cophenetic matrix was high ($r = 0.8406$) and highly significant (Mantel's test, $P < 0.001$), revealing an accurate and useful graphic representation.

Projections of the phenotypic variation among cultivars were obtained through PCoorA to further support the results of cluster analysis and elucidate the structure of phenotypic variability. The distribution of clones confirmed the results obtained by UPGMA analysis (Fig. 1). Worldwide clones were grouped in a cluster separated from the Argentinean ones (Fig. 2A).

By examining the loadings of variables of the first-three axes of the PCoorA analysis, we observed that predominant skin colour, secondary skin colour, number of leaf lobes, general leaf outline, petiole pigmentation and predominant colour of vine were the variables that made the highest contribution.

The results of the analysis showed that the first-three principal components explained 28.66% of the total variation, accounting for 14.23%, 7.94% and 6.49%, respectively. The most important traits found to explain the variation were predominant skin colour, associated with C1 (axis 1) and the general leaf outline, associated with C2 (axis 2). Although a low percentage of variation

Table 1 Observed frequencies of descriptors and Shannon–Weaver and Simpson index

Vine Characters

Index	Twining	Plant Type	Vine Internode Length	Vine Internode Diameter	Predominant Colour of Vine	Secondary Colour of Vine	Vine Tip Pubescence
Shannon–Weaver	**0.061**	**0.777**	**0.886**	**0.431**	**0.806**	**0.541**	**0.776**
Argentina	0.037	0.443	0.891	0.467	0.869	0.511	0.761
Worldwide	0.267	0.757	0.627	0.780	0.890	0.884	0.831
Simpson index	**0.014**	**0.464**	**0.591**	**0.321**	**0.753**	**0.307**	**0.594**
Argentina	0.0078	0.268	0.595	0.278	0.746	0.570	0.6774
Worldwide	0.087	0.592	0.936	0.543	0.766	0.681	0.702

Leaf Characters

Index	Type of Leaf Lobes	Shape of Central Leaf Lobe	Mature Leaf Size	General Leaf Outline	Number of Leaf Lobes	Abaxial Leaf Vein Pigmentation	Immature Leaf Colour	Petiole Pigmentation	Mature Leaf Colour	Petiole Length	Flowering Habit
Shannon–Weaver	**0.651**	**0.692**	**0.283**	**0.673**	**0.845**	**0.800**	**0.794**	**0.784**	**0.294**	**0.517**	**0.731**
Argentina	0.643	0.560	0.125	0.513	0.846	0.739	0.860	0.792	0.110	0.479	0.772
Worldwide	0.668	0.631	0.481	0.670	0.650	0.864	0.753	1.036	0.717	0.671	0.939
Simpson	**0.568**	**0.671**	**0.199**	**0.598**	**0.678**	**0.718**	**0.732**	**0.780**	**0.218**	**0.557**	**0.509**
Argentina	0.569	0.557	0.076	0.458	0.679	0.679	0.741	0.749	0.053	0.436	0.622
Worldwide	0.558	0.555	0.442	0.589	0.674	0.757	0.654	0.821	0.631	0.633	0.707

Storage Root Characters

Index	Storage Roots Arrangement	Storage Root Cortex Thickness	Storage Root Shape	Storage Root Defects	Predominant Skin Colour	Secondary Skin Colour	Intensity of Predominant Colour	Secondary Flesh Colour	Predominant Flesh Colour	Distribution of Secondary Flesh Colour
Shannon–Weaver	**0.670**	**0.489**	**0.569**	**0.421**	**0.840**	**0.543**	**0.88**	**0.584**	**0.754**	**0.631**
Argentina	0.597	0.569	0.541	0.394	0.779	0.473	0.830	0.504	0.673	0.635
Worldwide	0.734	0.845	0.673	0.782	0.923	0.619	0.988	0.551	0.986	0.918
Simpson	**0.597**	**0.572**	**0.592**	**0.462**	**0.810**	**0.643**	**0.575**	**0.709**	**0.737**	**0.521**
Argentina	0.570	0.540	0.526	0.346	0.764	0.456	0.536	0.506	0.696	0.591
Worldwide	0.590	0.649	0.666	0.705	0.837	0.518	0.658	0.498	0.849	0.691

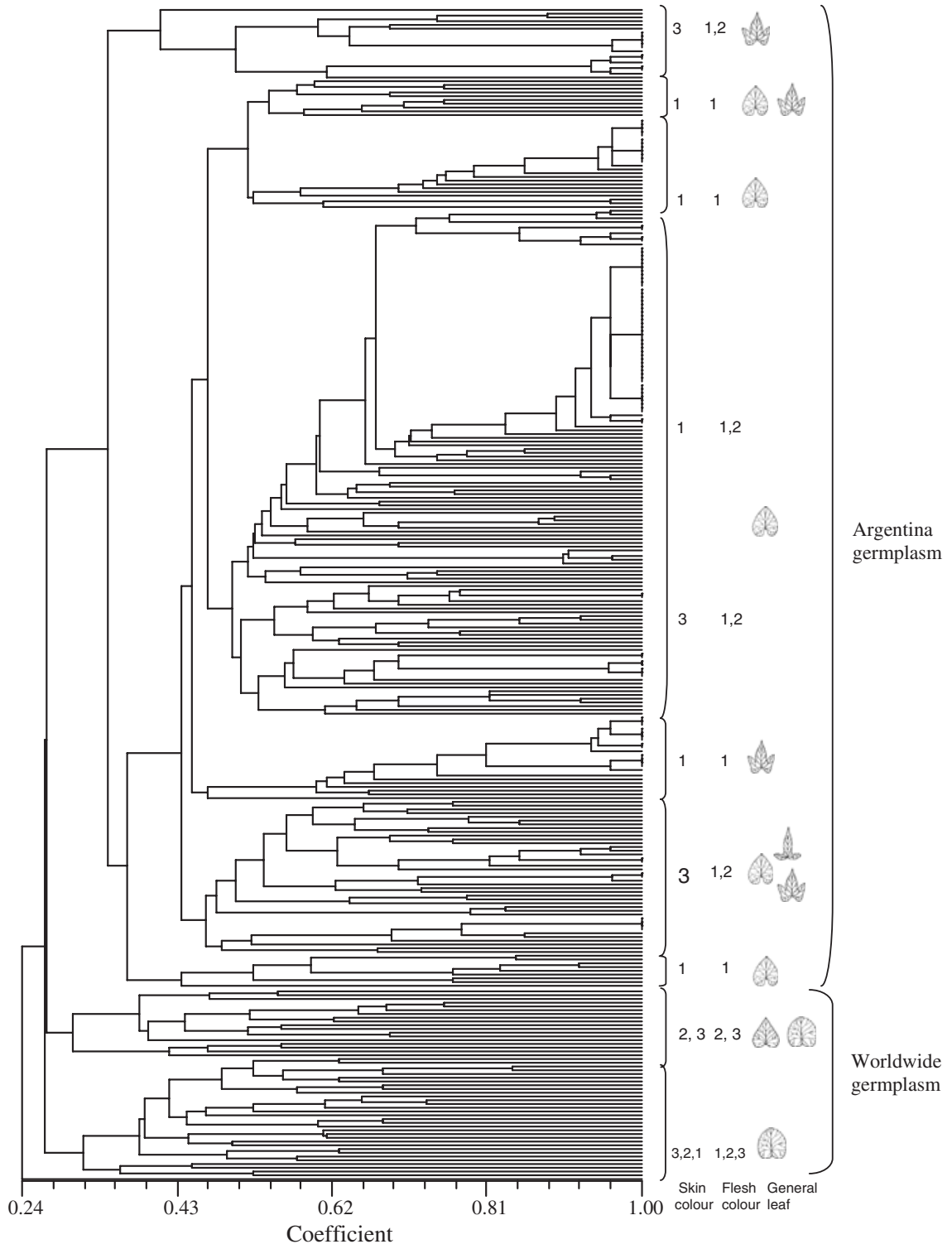

Figure 1 Dendrogram showing cluster analysis of 310 sweetpotato clones of the *in vitro* collection of IRB based on morphological descriptors (categories were simplified in groups for a better visualisation – for skin colour: 1 includes white, cream and yellow, 2 orange and brownish orange and 3, purple, pink, red, purple red and dark purple; for flesh colour: 1 includes white, cream and dark cream, 2 pale yellow and dark yellow and 3 includes pale, medium and dark orange).

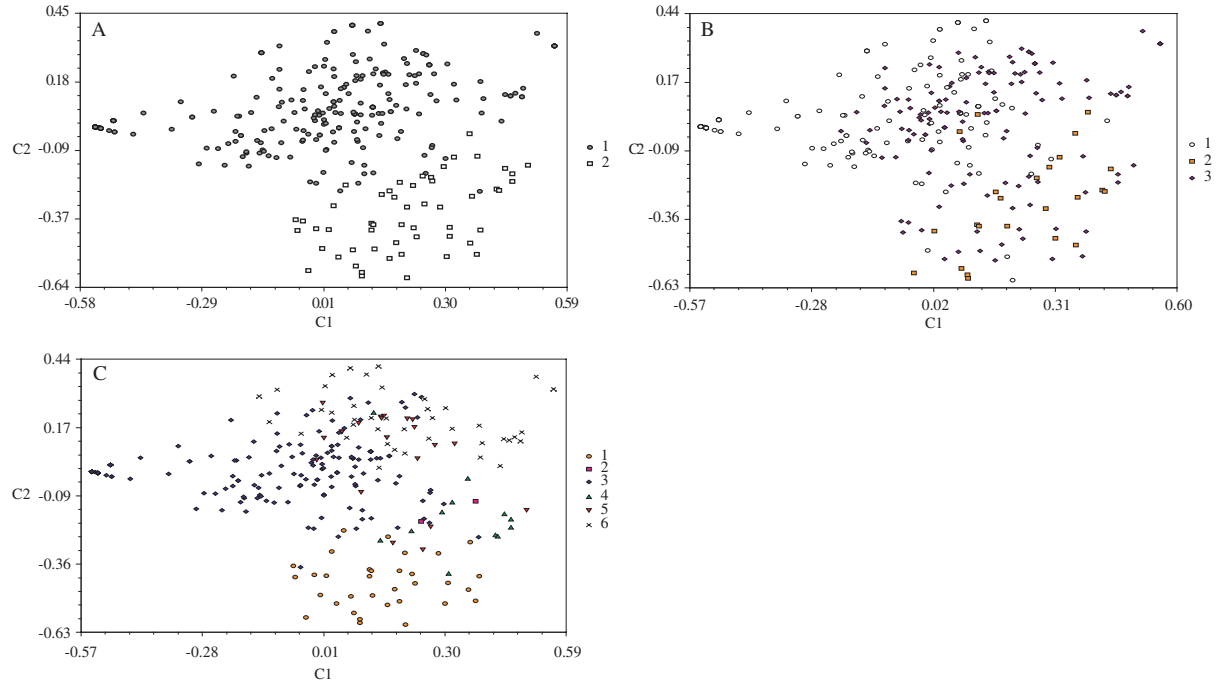

Figure 2 Dispersion of 310 sweetpotato clones in the plots 1−2 of the PCoorA based on morphological descriptors. (A) Visualisation by geographic precedence: 1 Argentina germoplasm, 2 worldwide germplasm. (B) Visualisation by predominant skin colour (categories were simplified in three groups for a better visualisation: 1 white, cream and yellow, 2 orange and brownish orange and 3 purple, pink, red, purple red and dark purple).(C) Visualisation by general leaf outline: 1 rounded, 2 reniform (kidney-shaped), 3 cordate (heart-shaped), 4 triangular, 5 hastate (trilobular) and 6 lobed.

is explained, aggregations can be visualised in reference to these two descriptors (Fig. 2B and Fig. 2C).

Colour composition of the collection

As colour is one of the main attributes of this vegetables on which breeding programmes focus importantly, we analysed the collection composition in reference to this morphological descriptor in flesh and skin.

In reference to flesh colour (Table 2), cream and pale yellow are the predominant colours in the collection, and belong in their vast majority to local germplasm. Material introduction through breeding programmes in the past was oriented to obtain different clones from known ones that were consumed locally. Orange flesh materials have the lowest percentages and they are a contribution from worldwide germplasm.

A lack of clones with homogeneous purple flesh roots was observed. However, 3.9% of the clones are strongly pigmented with anthocyanins, which are distributed in scattered spots and rings near the cortex. Purple flesh root materials were extensively developed in Japan for industrial use as colour dyes. The incorporation of materials with this characteristic to the collection will be important not only for practical applications but also for genetic studies.

In reference to skin colour, white, cream and purple red are the predominant colours in the collection, with a similar frequency distribution, and belong in their vast majority to local germplasm. Orange and brownish-orange skin materials have the lowest percentages and are a contribution from worldwide germplasm (Table 3).

The diversity indexes for both predominant skin colour and predominant flesh colour presented values

Table 2 Collection composition and distribution in reference to flesh colour

Flesh Colour	% Whole Collection[a] (310)	% of Clones Argentina[a] (257)	% of Clones Worldwide[a] (53)
White	9.74 (30)	76.67 (23)	23.33 (7)
Cream	43.18 (134)	90.98 (122)	9.02 (12)
Dark cream	8.77 (27)	74.07 (20)	25.93 (7)
Pale yellow	23.05 (71)	90.14 (64)	9.86 (7)
Dark yellow	5.52 (17)	58.82 (10)	41.18 (7)
Pale orange	1.62 (5)	100.00 (5)	0.00 (0)
Intermediate orange	1.95 (6)	0.00 (0)	100.00 (6)
Dark orange	2.27 (7)	0.00 (0)	100.00 (7)
With anthocyanins	3.90 (12)	100.00 (12)	0.00 (0)

[a] Absolute values are shown in parenthesis.

Table 3 Collection composition and distribution in reference to skin colour

Skin Colour	% Whole Collection[a] (310)	% of Clones Argentina[a] (257)	% of Clones Worldwide[a] (53)
White	27.51 (85)	100 (85)	0 (0)
Cream	25.29 (67)	95.5 (64)	5.97 (3)
Yellow	2.59 (8)	0 (0)	100 (8)
Orange	1.94 (6)	33.3 (2)	66.67 (4)
Brownish orange	3.24 (10)	20 (2)	80 (8)
Pink	7.12 (22)	90.9 (20)	9.09 (2)
Red	10.03 (31)	58.1 (17)	48.39 (14)
Purple red	22.01 (68)	86.8 (59)	13.24 (9)
Dark purple	3.88 (12)	58.3 (7)	41.67 (5)

[a]Absolute values are shown in parenthesis.

Table 4 Diversity values of predominant skin and flesh colour of local and worldwide germplasm

	Germplasm Origin	Shannon–Weaver	Simpson
Predominant skin colour	Argentina	0.779	0.764
	Worldwide	0.923	0.837
Predominant flesh colour	Argentina	0.673	0.696
	Worldwide	0.986	0.849

in worldwide germplasm higher than those in the local germoplasm, a fact that reflects higher variability in this first group (Table 4).

Discussion

In this analysis, we obtained a wide landscape of our collection and its morphological descriptors. Most of the morphological descriptors used for characterisation were useful as diversity index indicators. The diversity indices presented high values for most of the descriptors, thus indicating balanced characters and a high level of polymorphism. Root characters were the most diverse descriptors (90% with an index higher than 0.5), followed by leaf characters (80%) and vein characters (75%). In accordance with Ritschel & Huamán (2002), aerial parts presented the lowest values of diversity. In reference to vein characters, the material presented either slight or no twining, whereas the internode diameter presented scarce variation. Leaf characters presented more variability, except for mature leaf size and colour, which are either medium or large and either green or slightly purple, respectively. For root descriptors, storage root defects was the only descriptor that presented a diversity value below 0.5.

Although these useful descriptors constitute the simplest approach, they are limited not only in number but also because of the environmental influence and developmental stage. However, it is plausible to focus on the evaluation of some descriptors. Colour evaluation could be implemented using a colorimeter and colour charts could be designed as a reference, similar to those developed by CIP for potatoes. The level of correlation between descriptors shows that all of them are informative to a different degree and gives non-redundant information. In spite of the good level of diversity revealed by the morphological descriptors evaluated in this work, they were able to discriminate only 76% of the clones. There is a need to perform more sensitive techniques, such as molecular analysis, either to confirm or discard duplicates. Molecular markers provide an objective method for diversity evaluation that overcomes these limitations.

The local germplasm presented levels of variability lower than those of the introduced germplasm. This is supported not only by the diversity index values found but also by the grouping analysis. All worldwide clones were discriminated from each other, whereas 40% of the local materials were found to be identical. We considered a value higher than that found in other collections. By analysing 324 native clones from Brazil with 25 descriptors, Ritschel & Huamán (2002) found only 20% of duplicates.

In agreement with Tairo *et al*. (2008), as there is no naming system for cultivars, we found that the same name was given to different cultivars and vice versa. Materials collected in different locations presented high similarity. Tairo *et al*. (2008) attribute this situation to material exchanged between farmers. In this process, different farmers sometimes give different names making reference to a certain morphological attribute, locality or name of the person who brought it. Sometimes, the loss of identity occurs when material is transferred from one zone to another.

The results of cluster analysis matched those obtained with PCoorA and one of the descriptors responsible for spatial arrangement was skin root colour. This is coincident with its non-homogeneous distribution. Some of the colour variants were observed to be absent both in the local and worldwide clones. This distribution also reflects the consumers' preferences from the zones where the germplasm was collected (in the case of Argentina clones) and the interest of the breeding programmes to incorporate new materials.

Our results reveal a relatively low genetic diversity among local clones and lower distances in comparison to worldwide germplasm. However, the diversity analyses between these two groups show a greater variation within groups than between groups. Differences between the diversity found in Misiones and Corrientes in contrast to

Santiago del Estero, Córdoba and Buenos Aires depended on the end use of the production. In the region of Misiones and Corrientes the production is oriented to self-consumption and local markets. Landraces are the most common materials cultivated. White flesh clones present low water content (Boy, 1987) and can be stored in the field until use. The second group includes zones which send their production to large scale markets far from farms. In these zones the farming plots have large extensions, and the production is limited to a few commercial varieties. There is a bias towards materials with purple skin, which is more resistant to post-harvest manipulation and transportation.

The high diversity among the worldwide clones reflects the different origin of the clones. Material from several countries in America, Africa and Asia have made their contribution to the collection.

Because local market preferences are biased towards purple skin colour, breeding programmes have been focused on introducing materials with this characteristic for many years. The vast majority of the orange coloured skin in our bank comes from the USA, but there is not a wide diffusion yet, due partially to the absence of determination in market classes and to the fact that producers are not associated in an organisation that can invest in advertising (Marti, 2008). The biased composition must be reversed by introducing germplasm with characteristics useful for different applications so that the germplasm bank has the highest variability possible. The incorporation of materials with orange pigments, precursors of vitamin A and purple pigments with high antioxidant content is a target for further production of 'functional food'.

The evaluation of morphological descriptors allows establishing criteria for the implementation of a *core collection*. The knowledge of the frequency distribution of descriptors constitutes a useful tool for the management of genetic resources. The correlation of variables helps to administer evaluation resources in collections with a large number of materials. An exhaustive analysis of diversity with molecular markers is necessary to evaluate the conserved genetic base and to confirm putative duplicates. Molecular characterisation of the whole collection, together with morphological characterisation, will give a complete view of the diversity of the conserved materials and will allow planning of future introductions, avoiding genetic base narrowing. Based on these data, we will be able to make decisions on conservation strategies and design future analysis. Breeding programmes will also be benefited with the knowledge of the characteristics available in the conserved materials, and will be able to widen and diversify the applications of this crop.

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