Genet Resour Crop Evol (2008) 55:625–642
DOI 10.1007/s10722-007-9269-7

RESEARCH ARTICLE

Genetic diversity analysis of wild *Arracacia* species according to morphological and molecular markers

Raúl Blas · Marc Ghislain · Maria del Rosario Herrera · Jean-Pierre Baudoin

Received: 26 July 2006 / Accepted: 10 July 2007 / Published online: 24 August 2007

Abstract A study of genetic diversity was conducted among four wild *Arracacia* species (*A. elata*, *A. equatorialis*, *A. incisa* and *A. xanthorrhiza*), using three accessions per species, 20 plants per accession referred to as a population, 100 morphological characters and five AFLP primer combinations producing 202 AFLP markers. Genetic diversity was well described using these morphological descriptors and AFLP markers. Analysis of molecular variance showed a total variation of 49.5% which was attributed to differences between species; variation between populations within species amounted for 25.8%, while variation within populations accounted for 24.6%. On the basis of morphological and molecular characteristics, accessions considered previously as *A. equatorialis* could be regrouped with *A. xanthorrhiza*. These comparisons and multivariate analysis resulted in the selection of 28 morphological characters considered as discriminant to identify the *Arracacia* species from Peru. According to our morphological and molecular analysis, three wild species of *Arracacia* genus were clearly identified: *A. elata, A. incisa and A. xanthorrhiza*. However, *A. equatorialis* which was not well identified botanically could be considered as very close to *A. xanthorrhiza*.

Keywords AFLP · *Arracacia* · characterization · descriptors · genetic diversity · taxonomy

R. Blas (✉)
Facultad de Agronomia, Universidad Nacional Agraria, La Molina, Ap. 456, Lima 12, Peru
e-mail: rblas@lamolina.edu.pe

M. Ghislain · M. del Rosario Herrera
Centro Internacional de la Papa, Ap. 1558, Lima 12, Peru

J.-P. Baudoin
Faculté Universitaire des Sciences Agronomiques de Gembloux, Passage des Déportés, 2, Gembloux 5030, Belgique

Introduction

In the genus *Arracacia* Bancroft, the number of species growing from Mexico to Bolivia is not definitive according to the results reported by several authors: 24 species are recorded by Constance (1949), 28 species by Hiroe (1979), 55 species by Pimenov and Leonov (1993), and 30 species by Constance (1997). The "World Umbelliferae Database" in 2005 indicates 72 species (http://www.rbge.org.uk/data/URC/Nomenclature). Most of these species names are provisional (Blas 2000). *Arracacia* genus has been studied mainly in Central and North America (Mexico) (Mathias and Constance 1944, 1968, 1973; Constance and Affolter 1995a, b). In South America, Knudsen (2003) mentioned 10 *Arracacia* species, distributed in the mountainous Andean region of Venezuela, Colombia, Ecuador, Peru and Bolivia. In this region, the last descriptions of new *Arracacia* species were reported in Venezuela (*A. tilletti* Constance and Affolter) and in Colombia (*A. colombiana*

Constance and Affolter) by Constance and Affolter (1995a, b). In Peru, no taxonomical treatment of *Arracacia* specimens was developed for more than half a century, although six species were reported from this country: *A. xanthorrhiza* Bancroft, *A. peruviana* (Wolff) Const., *A. andina* Britt, *A. equatorialis* Const., *A. incisa* Wolff, and *A. elata* Wolff (Mathias and Constance 1962; Brako and Zarucchi 1993). Of these species, *A. xanthorrhiza* is the only cultivated arracacha of northern South America. However, at present, no reliable classification of the *Arracacia* genus from the Andean region is possible due to the poor representation of the above-mentioned species in different herbaria. This is the case for the four taxa: *A. xanthorrhiza*, *A. peruviana*, *A. andina* and *A. equatorialis* for which the botanical descriptions are incomplete and need further investigations.

This work is aimed at the study of *Arracacia* genus in Peru with two specific objectives in mind: to select useful characteristics for the identification of Andean species, and to estimate intraspecific and interspecific diversity. Prior to this study, we separated *A. equatorialis* from *A. xanthorrhiza* by analyzing original descriptions (Constance 1949; Mathias and Constance 1962) and looking at some representations from different herbaria. Distinction between the two taxa was based upon leaflets incision, shape and margin of involucel. On the other side, the species *A. andina* and *A. peruviana* could not be differentiated from *A. xanthorrhiza* due to the fact that they share similar morphological characteristics. The two species *A. elata* and *A. incisa* were clearly differentiated from the other four Peruvian *Arracacia* species (Blas 2005). *A. elata* was distinguished by its sprawling habit, spinulose-serrate leaflet margin, and absence of storage roots. *A. incisa* was identified by its smaller size and ovate to lanceolate, scarious and denticulate-margined involucel, which exceeds the flowers but is shorter than the fruit. On the basis of this preliminary study, four species from Peru were therefore considered in our analysis: *A. elata*, *A. equatorialis*, *A. incisa* and *A. xanthorrhiza*.

Materials and methods

Plant materials

Natural populations of the four wild species, *A. elata*, *A. incisa*, *A. equatorialis* and *A. xanthorrhiza* from eight Peruvian Departments, representative of wild arracacha in Peru, were collected and analyzed (Table 1). Dried herbarium were deposited at the "Herbario MOL" of the Universidad Nacional Agraria La Molina (UNALM) and true seeds, dried with silica gel, were deposited at the "Instituto de Biotecnologia" (IBT-UNALM). Seeds with silica gel were placed into sealed plastic bags and maintained at −20°C. For each species, three accessions and 20 plants per accession were sampled from three localities. The number of 20 plants per accession was determined according to a previous study on sample size estimation in *Arracacia* natural populations (Blas 2005). Taxonomic identification was checked according to the provisional artificial key for *Arracacia* species, proposed by Constance (1949) and to the comparison of our samples with herbarium material.

Morphological data

Morphological data of *Arracacia* species were taken on each area where the accessions were collected (Table 1). The observations on each plant covered 100 (54 qualitative and 46 quantitative) characters previously established by Blas (2005). These characters are indicated in Table 2 and cover the four distinctive morphological parts: the storage roots, the central rootstock, the aerial stems and the leaves (Fig. 1). The morphological descriptors and their states were established according to the provisional key of *Arracacia* species in South America proposed by Constance (1949), the descriptors proposed by Blas (1998), and additional field observations (Blas 2005). Characters of the aboveground parts were recorded at flowering time while those of the underground parts were recorded immediately after harvest. Color of some plant organs was described according to the color chart of the Royal Horticultural Society (1995).

Data analysis

For statistical analysis, data from 20 plants/accession and three accessions/species were used and considered as the Operational Taxonomic Unit (OTU). From data compiled according to the states of descriptors (Table 2), a matrix of 240 plants or OTUs × 100 characters was constructed.

Table 1 *Arracacia* accessions used for morphological and molecular characterization

Species	Accession	Department	Province	District	Locality	Latitude (S)	Longitude (W)	Altitude (m)
A. elata	RBS-130	Cajamarca	Cajamarca	Encañada	Pampa del toro, pin-pin valley	07°02′05.5″	78°12′39.0″	3,500
	RBS-134	Piura	Huancabamba	Canchaque	Quebrada Cascapampa	05°19′01.6″	79°30′48.8″	3,132
	RBS-136	Piura	Huancabamba	Sondorillo	Caserio Ulpamache alto	05°19′18.6″	79°30′16.1″	3,140
A. equatorialis	RBS-93	Ancash	Bolognesi	Chiquian	Umpay-cerro Huayalpampa	10°09′08.7″	77°09′23.8″	3,691
	RBS-96	Junin	Huancayo	Huancayo	Vilcacoto, 10 km from Huancayo city	12°00′31.4″	75°10′10.4″	3,550
	RBS-141	Ayacucho	Huamanga	Chiara	Huambra Huayco river, between Huamanga and Chiara	13°14′19.6″	74°14′25.1″	2,700
A. incisa	RBS-94	Lima	Huarochirí	San mateo	Cerro Comatana	11°48′01.5″	76°19′54.7″	3,044
	RBS-95	Lima	Canta	Canta	Chayoque grande	11°27′21.5″	76°36′28.2″	2,820
	RBS-129	La Libertad	Otuzco	Salpo	Chanchacap, both margins of Río Grande	07°59′28.6″	78°33′21.4″	2,978
A. xanthorrhiza	RBS-124	Huánuco	Huánuco	Cayran	Malconga, Rayan loma valley	06°38′45.0″	78°47′05.0″	2,175
	RBS-138	Piura	Huancabamba	Huancabamba	Caserio Nueva Esperanza	05°18′54.5″	79°29′00.2″	2,863
	RBS-144	Lima	Yauyos	Yauyos	Pallanga, left side of Yungo-Yauyos river, 2.5 km from Yauyos city	12°26′35.8″	75°55′58.5″	3,200

To evaluate the inter- and intra-species variances, descriptors for quantitative traits were analyzed using basic descriptive statistics and variance (ANOVA), with the help of Minitab 13.31 software (2000).

For multivariate analysis, each descriptor was standardized by subtracting the mean value from the observed value and dividing by standard deviation, in order to minimize the scale effects (Crisci and López 1983). Then, an exploratory analysis was elaborated with Principal Component Analysis (PCA) (Dagnelie 1986). A correlation symmetric matrix (100 variables × 100 variables) was calculated in order to understand how the characters were associated and grouped. Eigenvalues and Eigenvectors were determined from this matrix, using the NTSYS 2.1 (Rohlf 2000) and Minitab 13.31 softwares. From these values, the accessions were plotted in order to identify population groups. Canonical Discriminant Analysis (CDA) was made using the procedure CANDISC of SAS system v8 (Palm 2000). The objective was to identify characters with high discrimination capacity for genetic diversity evaluation and to allow identification of Peruvian arracacha species.

AFLP marker analysis

DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (1990), adapted and standardized by CIP (1998). For DNA isolation, healthy young leaves were collected from natural populations and dried over silica gel.

The AFLP analysis was carried out according to Vos et al. (1995) and following manufacturer's instructions with minor modification (CIP 1998). Commercial AFLP kits were purchased from Invitrogen™ Life technologies (Carlsbad, California, USA). A sample of 450 ng genomic DNA was digested in 12.5 µM of 5× reaction buffer [50 mM Tris–HCl (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate] with 1 µl *Eco*RI/*Mse*I enzyme combination [1.25 units/µl each in 10 mM Tris–HCl (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 50% glycerol (v/v), 0.1% Triton® X-100]. Following digestion, 12.5 µl of adapter/ligation solution containing 12 µl EcoR I/Mse I adapters, 0.4 mM ATP, 10 mM Tris–HCl (pH 7.5), 50 mM K-acetate and 0.5 µl T4 DNA Ligase (1 unit/µl) was added directly to the DNA digest, incubated 2 h at 20°C,

Table 2 Morphological descriptors used in the diversity analysis of *Arracacia* wild species

Cormels and rootstock

1. Plant conformation: 1 climbing, 2 single, 3 lax, 4 medium, 5 compact (determined according to number of cormels and generative shoot development)
2. Life form: 1 monocarpic, 2 polycarpic [monocarpic plant is a plant that dies after flowering, although it may take several years to flower, and polycarpic plants are fruiting and flowering many times (De Candolle 1835)]
3. Cormels or branches presence: 0 absent, 1 present (1or 2 cormels), 2 (> 2 cormels) (determined in the field at sampling time)
4. Cormels number
5. Cormels surface color: 1 white, 2 yellowish-green, 3 yellow, 4 grayish-orange, 5 beige (brown clear), 6 brown, 7 purple red, 8 grayish purple
6. Cormels shape: 1 hole long tube, 2 conic, 3 ovoid, 4 oblong, 5 oblong long, 6 oblong divided
7. Cormels length (cm)
8. Cormels width (cm)
9. Rootstock shape: 1 hole long tube (root-stock absent), 2 conic 3 oblong-divided, 4 oblong long divided, 5 oblong with few cormels, 6 oblong with many cormels
10. Rootstock length (cm)
11. Rootstock width (cm)

Leaves

12. Leaves length (cm)
13. Leaves width (cm)
14. Pinnas' number of leaves
15. Leaves shape: 1 triangular-ovate, 2 ovate, 3 ovate-lanceolate (determined according to blade projection)
16. Foliage color : 1 clear green, 2 green, 3 dark green, 4 yellowish green, 5 yellowish purple green, 6 purple green, 7 dark purple green
17. Predominant petiole color: 1 clear green, 2 green, 3 yellowish green, 4 grayish orange, 5 grayish red, 6 purple red, 7 grayish purple
18. Secondary petiole color: 0 absent, 1 clear green, 2 green, 3 yellowish green, 4 grayish orange, 5 grayish red, 6 purple red, 7 dark grayish purple
19. Distribution secondary petiole color: 0 absent, 1 superior, 2 medium 3 inferior, 4 equally distributed, 5 irregular
20. Wax in petiole: 0 absent, 1 present (determined at the flowering time)
21. Petiole length (cm)
22. Petiole width (cm)
23. Sheathing shape of petiole: 1 cordate, 2 triangulate, 3 triangulate-long
24. Sheathing color of petiole: 1 white, 2 clear green, 3 yellow green, 4 rose, 5 red purple

Leaflet

25. Main leaflet adaxial color: 1 clear green, 2 green, 3 green-yellowish, 4 purple
26. Main leaflet abaxial color: 1 white, 2 clear green, 3 green, 4 green-yellowish, 5 purple
27. Secondary leaflet abaxial color: 0 absent, 1 green, 2 purple, 3 violet
28. Distribution of secondary leaflet abaxial color: 0 absent, 1 border, 2 veins, 3 rachis, 4 veins and rachis, 5 irregular
29. Leaflet margin color: 0 absent, 1 grayish orange, 2 purple red, 3 grayish purple, 4 dark purple
30. Acumen shape of leaflet: 1subacute, 2 acute, 3 largely acute
31. Leaflet adaxial surface (hairs presence):1 glabra, 2 only in veins, 3 squamose
32. Leaflet shape: 1 triangular-ovate, 2 ovate-oblong, 3 ovate-lanceolate, 4 lanceolate
33. Terminal leaflet length (cm)
34. Terminal leaflet width (cm)
35. Leaflet division (incision of terminal leaflet or lobule): 1 superficial, 2 medium, 3 deeply incised
36. Leaflet margin: 1 mucronate-serrate, 2 serrate, 3 serrate-spinulose
37. Lateral leaflet length (cm)

Table 2 continued

38. Lateral leaflet width (cm)
39. Petiolule length at lower level of leaf, (cm)
40. Petiolule length at upper level of leaf, (cm)

Generative shoot and inflorescence

41. Flower: 0 absent, 1 rarely, 2 medium, 3 frequently
42. Generative shoot color: 1 clear green, 2 green, 3 yellowish green, 4 grayish orange, 5 grayish red, 6 purple red, 7 grayish purple
43. Wax in generative shoot: 0 absent, 1 present
44. Mature umbel color: 1 clear green, 2 green, 3 yellowish green, 4 green purple-red, 5 brown, 6 purple red, 7 violet

Bracteole

45. Involucel number
46. Involucel form: 1 entire conic, 2 oblong-linear, 3 ovate-acuminate, 4 ovate with wing sheathing, 5 oblong-lanceolate, 6 ovate-lanceolate,
47. Involucel margin: 1 entire, 2 narrowly scarious-margined, 3 denticulate-margined
48. Involucel length (mm)
49. Involucel width (mm)
50. Involucre number
51. Involucre form: 1 entire linear or lanceolate, 2 lanceolate with sheath, 3 lanceolate to ovate without sheath, 4 like leaflets
52. Involucre length (mm)
53. Involucre broad (mm)

Umbel

54. Peduncle length (cm)
55. Peduncle width (cm)
56. Number of rays/umbels
57. Number of fertile rays/umbel
58. Ray length (cm)
59. Umbel form: 1 elongated flat, 2 flat, 3 conic flat
60. Umbelulle conformation (form): 1 elongated, 2 medium, 3 compact
61. Generative shoot (GS) length (cm)
62. GS width (cm)
63. Branches number of generative shoot
64. GS number/plant

Flower and fruit

65. Petal color: 1 greenish, 2 reddish brown, 3 dark-purple
66. Pollen grain color: 1 white, 2 rose, 3 red, 4 purple
67. Stylopodium shape: 1 pyramidal, 2 depressed
68. Number of umbels/GS
69. Number of flowers/umbellule
70. Number of hermaphrodite flowers/umbellule
71. Pedicel length (mm).
72. Number of grains/GS
73. Number of grains/umbel
74. Number of grains/umbellule
75. Carpophore insertion: 1 bi-parted at 1/4, 2 bi-parted to base
76. Seed length (mm)
77. Seed width (mm)

Table 2 continued

78. Mericarp transection: 1 semi-rounded, 2 pentagon
79. Fruit shape: 1 rounded, 2 conic, 3 ovoid, 4 ovoid-oblong
80. Ribs: 1 acute, 2 very prominent, 3 very prominent and corky
81. Vittae number in the intervals
82. Vittae number on the commissure
83. Vittae breadth: 1 narrow, 2 medium, 3 broad (determined subjectively by comparison among samples in cross-section of seeds)

Storage Root

84. Root shape: 1 rootlet (not storage), 2 conic, 3 conic long, 4 fusiform, 5 oblong, 6 irregular
85. Number of roots per plant
86. Root length (cm)
87. Root width (cm)
88. Skin thickness: 1 very thin, 2 medium, 3 thick-detaches easily (determined subjectively by comparison among samples at sampling time)
89. Main root surface color: 1white, 2 yellow, 3 clear brown, 4 brown, 5 grayish
90. Secondary root surface color: 0 absent, 1 white, 2 yellow, 3 rose, 4 purple, 5 grayish, 6 black
91. Distribution of secondary root surface color: 0 absent, 1 at base, 2 medium, 3 apex, 4 entire root, 5 irregular
92. Shape of secondary root surface color: 0 absent, 1 stripes, 2 spots
93. Predominant storage root flesh color: 1 white, 2 yellow, 3 purple
94. Secondary storage root flesh color: 0 absent, 1 yellow, 2 rose, 3 purple
95. Distribution of secondary root flesh color: 0 absent, 1 in cambium, 2 pith (central region), 3 cortex, 4 irregular
96. Root oil: 1 very low, 2 medium, 3 abundant (determined subjectively by comparison among samples, 5 min after transection cut of storage root)
97. Root surface: 1 smooth (soft), 2 medium, 3 wrinkled (rough) (determined subjectively by comparison among samples at the sampling time)
98. Branch number of root: 1 rarely (0–1 root division), 2 medium (2–3 root division), 3 dense (>3 root division)
99. Root odor: 1 slight odor, 2 typical odor, 3 like anise (determined subjectively by comparison among samples 5 min after transection cut of storage root)
100. Root flavor: 1 sweet –slightly pungent, 2 medium pungency, 3 very pungent (the taste of storage root was determined subjectively by comparison among samples at the sampling time)

and subsequently diluted 10-fold in 1× TE buffer (10 mM Tris–HCl (pH 8.0), 0.1 mM EDTA).

Preamplification was performed in a total volume of 12.75 µl, containing 1.25 µl of diluted digestion DNA template, 0.125 µM of *Eco*RI + A and *Mse*I + C primers. PCR was performed in a MJ Research PTC-100 thermocycler (Global Medical Instrumentation Inc., Minnesota, USA) using the following temperature profile: 20 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C. The PCR products were diluted 10 times with water and used as templates for the selective amplification.

Five AFLP primer combinations were identified as best for selective amplification: E-ACT/M-CAC, E-AAC/M-CTT, E-ACC/M-CTC, E-ACT/M-CTC and E-ACG/M-CTC. Selective amplification was carried out in a volumen of 10 µl containing 3 µl of diluted preselective PCR product, 0.05 µM of *Eco*RI + ANN and *Mse*I + CNN primers. PCR was performed with the following temperature profile: 12 cycles of 30 s at 94°C, 30 s at 65°C, decreasing −0.7°C/cycle to 56, 60 s at 72°C; followed by 25 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C. Following amplification, 5 µl of formamide loading buffer (98% formamide, 10 mM EDTA, 0.1% each of xylene cyanol and bromophenol blue) was added, and the samples were denatured at 95°C for 5 min. Amplified fragments were separated on 6% polyacrylamide denaturing gels with 29:1 Acrylamide/Bis solution. The gels were pre-run at 110 W for 20 min in 1× TBE (Tris base 1.8%, Boric acid 0.6% and 0.5 M EDTA 0.2%), prior to the loading of 5 µl of sample and, thereafter, run at 110 W for approximately 3.5 h. Following electrophoresis, DNA was detected with silver staining (CIP 1998).

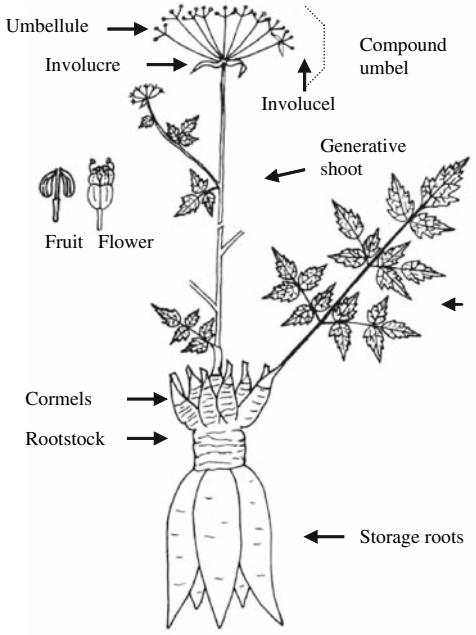

Fig. 1 Plant of cultivated *Arracacia*

Gel scoring and data analysis

Each polymorphic fragment produced by each AFLP primer combination was treated as a unit character and numbered sequentially on the APC film or directly analyzed using Paint Shop Pro 5.01 software (Jasc Corporate, MN, USA). Genotypes were scored for the presence (1) or absence (0) of each fragment, only those fragments with medium or high intensity were analyzed. Fragments with the same mobility on the gel, but with different intensities, were not distinguished from each other when OTUs were compared, so the AFLPs were treated as dominant markers. In order to characterize the capacity of each primer combination, and to reveal polymorphic loci in the germplasm, the polymorphic index content (PIC) = $1 - (faa^2 + fan^2)$ was calculated, where faa^2 is the frequency of the amplified allele and fan^2 is the frequency of the nonamplified allele. The highest value of PIC indicated the most informative AFLP marker. The PIC values for the AFLP markers generated by the same primer combination were cumulated and named AFLP marker index (Ghislain et al. 1997). This index indicates the information content of the AFLP primer by assay. We used Analysis of Molecular Variance (AMOVA) procedure (Excoffier et al. 1992) to estimate variance components at different levels, partitioning the variation among individuals/within population, among populations/within species and among species. This analysis was undertaken with Arlequin ver. 2000, a software for population genetics data analysis, provided by Laurent Excoffier (http://anthropologie.unige.ch/arlequin/; Department of Anthropology and Ecology, University of Geneva, Switzerland).

The genetic analysis was performed using the NTSYSpc 2.1 software (Rohlf 2000). Similarities between OTUs were calculated with the Simple Matching Coefficient (SMC) = $(a + d)/(a + b + c + d)$; where $a = (1,1)$ is the number of common fragments between two accessions, $d = (0,0)$ is the number of fragments absent in both accessions, $c = (0,1)$ and $b = (1,0)$ are the numbers of mismatches (Jaccard 1908, cited by Hancock 2004). According to this distance, the OTUs were plotted using nonmetric multidimensional scaling analysis (Rohlf 2000), in order to identify population groups.

Results and discussion

Morphological analysis

The univariate analysis of variance (ANOVA) for each of the 46 quantitative variables is detailed in Blas (2005). Data show highly significant differences ($P < 0.001$) between species for 43 characters, significant differences ($P < 0.05$) for one character [pinnas' number of leaves (14)] and no significant differences for two characters [involucre number (50) and vittae number on the commissure (82)]. The ANOVA between populations in each species show similar tendencies. Populations of *A. xanthorrhiza* show highly significant differences for 40 characters, no significant differences for five characters [rootstock length (10), rootstock width (11), involucel number (45), peduncle width (55) and number of hermaphrodite flowers/umbellule (70)]; one character [petiolule length at upper level of leaf (40)] does not show variation. Populations of *A. equatorialis* show highly significant differences for 26 characters, and no significant differences for 20 characters; all characters show variation in this taxon. Populations of *A. incisa* show highly significant differences for 18 characters, no significant differences for 27 characters; only one character does not show variation

[petiolule length at upper level of leaf (40)]. Populations of *A. elata* show highly significant differences for eight characters, no significant differences for 28 characters, four characters [petiolule length at lower level of leaf (39), petiolule length at upper level of leaf (40), branches number of generative shoot (63), branch number of root (98)] do not show variation. The following six characters [cormels number (4), cormels length (7), cormels width (8), rootstock length (10), rootstock width (11), number of storage roots per plant (85)] are absent in the populations of *A. elata*.

With this analysis, we observe a high morphological diversity among the material analyzed at both between-species (*A. elata*, *A. equatorialis*, *A. incisa* and *A. xanthorrhiza*) and within-species (between populations or accessions) levels. This morphological diversity is higher in populations of *A. xanthorrhiza*, *A. equatorialis* and *A. incisa* than in populations of *A. elata*.

In general, in these species, a high variability is found for most of the morphological descriptors. However, this variability could have been inflated by environmental conditions as the data were recorded in different regions and ecosystems, at the time of our collecting missions.

Principal Component Analysis (PCA)

In the Principal Component Analysis, the first three principal components (PC) show respectively 35.3%, 10.9% and 8.1% of the total variability. These low values mean that the populations and characters analyzed have high variability.

According to the first two PC, three groups of distribution are observed in plotting the 240 genotypes (Fig. 2). Group I represents the three populations of the species *A. elata* (accessions: RBS-130, RBS-134 and RBS-136); this group is positively related to the second component. *A. elata* is morphologically easy to identify by the absence of storage roots, rootstocks and cormels, by its plant size (more than 3 m), its ovoid seed and its involucel shape and size.

Group II includes only a single population of the species *A. xanthorrhiza* (accession RBS-138); this population is isolated from the other *A. xanthorrhiza* populations; this group is positively related to the first component and negatively to the second component. This population is a wild form accession, morphologically very closely related to the cultivated ones and characterized by a high quantity of seeds and well-developed storage roots.

In Group III, eight populations are positively related to the first component, covering the three species: *A. equatorialis* (RBS-93, RBS-96 and RBS-141), *A. incisa* (RBS-94, RBS-95 and RBS-129), and *A. xanthorrhiza* (RBS-124 and RBS-144). All genotypes of this group have storage roots, rootstocks and cormels. The RBS-95 and RBS-129 accessions of *A. incisa* are shown to be related to other populations corresponding to *A. equatorialis*, e.g. individuals corresponding to RBS-129 accession appear to be more related to accession RBS-141 of *A. equatorialis*. Concerning the three populations of *A. equatorialis*,

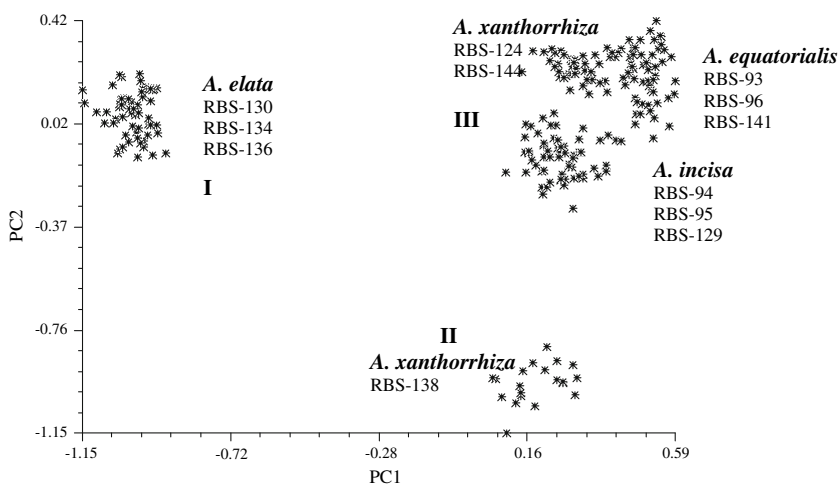

Fig. 2 Distribution of the 240 plants belonging to the four *Arracacia* species according to the first two PC

two accessions are grouped together, e.g. RBS-93 and RBS-141; the third population (accession RBS-96) is more related to *A. xanthorrhiza* populations.

The two populations corresponding to *A. xanthorrhiza* are grouped with the two different related species, i.e. *A. incisa* and *A. equatorialis*. For example, individuals corresponding to RBS-124 accession are clustered in different groups; some individuals being more related to *A. equatorialis* and others to *A. incisa.* However, inside Group III, differentiation between individuals is not easy, rendering necessary the definition of more discriminant characters. Such characters would be useful to better define groups and to obtain a reliable identification of the species. As a result, this analysis does not enable to differentiate clearly between populations corresponding to the three species *A. equatorialis*, *A. incisa* and *A. xanthorrhiza*.

Canonical Discriminant Analysis (CDA)

Three variables (of the 100 used) are eliminated due to a very low variability in the basic descriptive statistics and PC analyses previously made. These characters are: petiole length at upper level of the leaf (40), secondary storage root flesh color (94) and distribution of secondary storage root flesh color (95). In the CDA, the first three components explain all the variability (Table 3); the first component explains more than 92% of the total variation while the second component explains more than 5% of the total variation. Consequently, characters with great discriminant capacity for the genetic diversity analysis in arracacha are those that display high vector with the first and the second component of the canonical variables.

The criterion applied to identify discriminant characters is the absolute value of the Eigenvector coefficient (r) between the character and the canonical variable (Table 4). This coefficient should be $r \geq 0.5$. Considering this criterion, descriptors are reduced to fifty discriminant characters. Among the latter, the most important are in decreasing order the leaflet adaxial surface (31), stylopodium shape (67), number of rays per umbel (56), leaflet margin (36), storage root shape (84), rootstock width (11), vittae breadth (83), number of flowers per umbellule (69), cormel width (8), generative shoot length (61), plant conformation (1), petiolule length at lower level of leaf (39), cormels shape (6), and mericarp transection shape (78).

In the CDA, some characters used in the original descriptions to differentiate the *Arracacia* species do not have high discriminant values. This is the case for fruit length, fruit width, fruit shape, leaf length, and leaf width. On the other side, other characters in our analysis appear to have more discriminant power. These characters correspond to vegetative parts (cormels, rootstock and storage root) and generative parts (generative shoots, flowers and fruits). The characters corresponding to the underground vegetative parts were not taken into consideration in the original descriptions of the Andean *Arracacia* species, partly because most samples in the herbarium lack representations of these organs.

Considering the 50 selected descriptors, the 12 populations were analyzed in order to test the discriminant power of characters and to determine cluster of individuals.

These selected descriptors allow us to obtain a good clustering among the 240 plants of the 12 analyzed populations (Fig. 3). These populations are clearly separated into three groups: Group I includes three populations of *A. elata*, Group II includes three populations of *A. incisa* and Group III includes six populations corresponding to *A. equatorialis* and *A. xanthorrhiza*.

The three populations identified in *A. equatorialis* are not clearly separated from *A. xanthorrhiza*, indicating that the former taxon could not deserve the status of a species. Accordingly, populations of *A. equatorialis* could be classified together with populations of *A. xanthorrhiza*, because the

Table 3 Eigenvalue of the first three canonical variables and their proportion of variations

CAN = Canonical variables, *P* = probability

CAN	Eigenvalue	Difference	Proportion	Cumulative	*P*
1	984.047	926.183	0.928	0.928	0.0001
2	57.864	38.764	0.055	0.982	0.0001
3	19.100		0.018	1.000	0.0001

Table 4 The 50 screened characters and the Eigenvector coefficient (r) between the character and the canonical variable

CAN	Ch*	r	Ch*	r	Selected descriptors	
1	31	0.933	71	−0.719	*Cormels and rootstock*	49. Involucel width
	67	0.933	46	−0.712	1. Plant conformation	51. Involucre form
	56	−0.904	65	0.707	3. Cormels presence	54. Peduncle length
	36	−0.894	60	0.696	4. Cormels number	56. Number of rays/umbels
	84	0.890	37	−0.693	6. Cormels shape	57. Number of fertile rays
	11	0.879	48	−0.686	7. Cormels length	58. Ray length
	83	−0.868	9	0.670	8. Cormels width	59. Umbel form
	69	−0.867	98	−0.668	9. Rootstock shape	60. Umbelulle conformation (form)
	8	0.864	66	0.628	10. Rootstock length	61. Generative shoot (GS) length
	61	−0.864	22	−0.588	11. Rootstock width	64. GS numbers/plant
	1	0.858	63	0.585		65. Petal color
	39	0.844	49	−0.578	*Leaves*	66. Pollen grain color
	6	0.826	3	0.578	15. Leaves shape	67. Stylopodium shape
	78	0.814	32	−0.563	22. Petiole width	69. Number of flower/umbellule
	10	0.798	47	−0.559	25. Main leaflet adaxial color	71. Pedicel length
	99	0.796	38	−0.553	26. Main leaflet abaxial color	78. Mericarp transection
	58	−0.787	91	0.530	30. Acumen shape of leaflet	80. Ribs of fruit
	96	0.770	26	0.516	31. Leaflet adaxial surface	81. Vittae number in the intervals
	59	0.767	30	−0.513	32. Leaflet shape	83. Vittae broad
	57	−0.758	4	0.513	36. Leaflet margin	
	64	−0.756	88	0.512	37. Lateral leaflet length	*Storage Root*
	7	0.750	15	0.507	38. Lateral leaflet width	84. Root shape
	81	0.736	25	−0.501	39. Petiolule length at lower	86. Root length
	54	−0.727	86	−0.500		88. Skin thickness
2	80	0.645			*Inflorescence and fruit*	91. Distribution of secondary root surface color
3	51	0.570			46. Involucel form	96. Root oil
					47. Involucel margin	98. Branch number of root
					48. Involucel length	99. Root odor

Ch* = character number, following the arrangement of Table 2 (see Materials and methods), CAN = canonical variable

discriminant descriptors overlap between both taxa. This overlap in characters expression could be due to the natural gene flow between the two taxa and the production of viable hybrid seeds. To support this hypothesis, it is interesting to note that Knudsen (2003) obtained viable hybrid seeds in artificial crosses between cultivated and wild forms of *A. xanthorrhiza* in Cajamarca (Peru). Crosses could have occurred spontaneously between *A. xanthorrhiza* and *A. equatorialis* as the two taxa have the same geographical distribution. As *A. xanthorrhiza* was botanically described first, its status as species has to be preserved, should one decide to merge the two taxa in a single species. On the other part, populations of *A. incisa* and *A. elata* are clearly separated allowing easily their identification with the selected descriptors. The CDA shows that 48 characters out of the 50 present an eigenvector coefficient $r \geq 0.5$ with the first three canonical variables. The exception is for two characters: cormels number (4) and distribution of secondary root surface color (91). In spite of their high vector coefficients, we decided to discard the following characters: plant conformation (1), cormels length (7), cormels width (8), rootstock length (10), rootstock width (11), leaves shape (15), petiole width (22), main leaflet adaxial color (25), main leaflet abaxial color (26), lateral leaflet length (37), lateral leaflet width (38), petiolule length at lower level of leaf (39), involucel margin (47), involucre form (51), peduncle length (54), number of

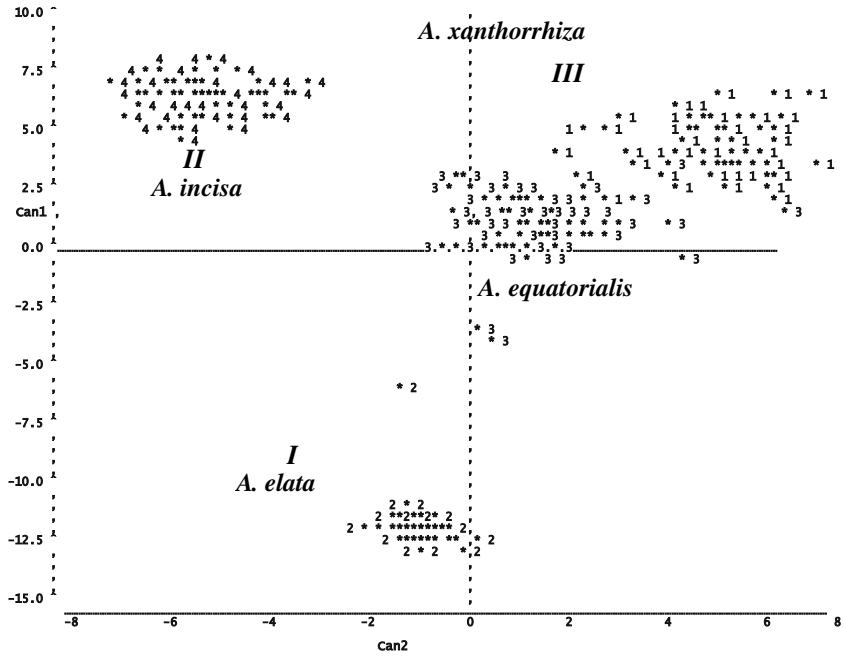

Fig. 3 Plotting of 240 plants with 50 selected descriptors according to the first two canonical variables (1 = *A. xanthorrhiza*, 2 = *A. elata*, 3 = *A. equatorialis*, and 4 = *A. incisa*)

fertile rays (57), umbelulle conformation (60), pollen grain color (66), number of flower/umbellule (69), ribs of fruit (80), vittae number in the intervals (81), vittae breadth (83), root shape (84), root length (86), skin thickness (88), root oil (96), branch number of root (98), and root odor (99).

This is justified by the fact that we do not know the age of the evaluated material. As natural populations of arracacha are perennial plants, samples coming directly from natural areas do not represent a homogenized material. For instance, the length and the width of cormels, rootstocks and storage roots are "strongly" influenced by the environmental conditions, the soils where such material is found and the plant age. In addition, the color of the petioles and leaflets are not stable characters. However, these characters could be useful in some diversity analysis, such as at within population level.

On the basis of this consideration (CDA, Eigenvector coefficient), we selected 28 characters for identification of the *Arracacia* species from Andean region (Table 5). We also decided to include nine characters although they had in the CDA Eigenvector coefficient $r < 0.5$: life form (2), leaflet division (35), number of umbels/GS (68), number of grains/GS (72), carpophore insertion (75), seed length (76), seed width (77), fruit shape (79), and predominant storage root flesh color (93). We consider these characters as very discriminant within each species due to their stability. Then, the discriminant capacity of these selected descriptors will be useful in further

Table 5 The 28 selected morphological descriptors for the identification of *Arracacia* wild species

Descriptors	
Cormels and rootstock	56. Number of rays/umbels
2. Life form	58. Ray length
3. Cormels presence	59. Umbel form
6. Cormels shape	61. Generative shoot (GS) length
9. Rootstock shape	64. GS numbers/plant
	65. Petal color
Leaves	67. Stylopodium shape
30. Acumen shape of leaflet	68. Number of umbels/GS
31. Leaflet adaxial surface	71. Pedicel length
32. Leaflet shape	72. Number of grains/GS
35. Leaflet division	75. Carpophore insertion
36. Leaflet margin	76. Seed length
	77. Seed width
Inflorescence and fruit	78. Mericarp transection
46. Involucel form	79. Fruit shape
48. Involucel length	
49. Involucel width	*Storage Root*
	93. Predominant storage root flesh color

Arracacia species identification from the Andean region and the analysis of natural populations.

Differences among *Arracacia* species of Peru

Figure 4 illustrates the differences in vegetative and generative structures of these Peruvian species, considering stylopodium, carpophore, fruit, umbel, leaflet acumen, involucel, cormel and rootstock.

Arracacia elata is not likely to be confused with any of the Peruvian *Arracacia* species. In general, *A. elata* displays spinulose-serrate leaflet margin, ovoid fruit with acute apex, and ovate-lanceolate involucel.

A. incisa can be distinguished by the length of the involucel which exceeds the flower, the conspicuously scarious margins, and the semi-rounded seeds. In addition, *A. incisa* is characterized by a short plant size, combining short petioles and small and incised leaflets. Additionally, the taxon is geographically isolated, being present in hilly and dry areas of the Peruvian western and central highlands of Quechua region. *A. incisa* is closely related to *A. xanthorrhiza* through the shared compressed basal stem structures (cormels), the ramification of the flowering stem and the perennial growth habit.

A. xanthorrhiza includes cultivated form, as well as monocarpic and polycarpic wild forms. The *A. xanthorrhiza* cultivated form has many storage roots and well developed cormels, two properties distinguishing this form from other *Arracacia* specimens belonging to the group with the storage root. The *A. xanthorrhiza* monocarpic form differs from the *A. xanthorrhiza* polycarpic form and *A. xanthorrhiza* cultivated form by the monocarpic behavior (the root dying away after the end of flowering), the presence of one (or rarely 2) cormel and one (rarely 2) reproductive shoot per plant. In addition, this reproductive shoot is very vigorous and shows many ramifications that carry up to 1,000 seeds (Blas 2005).

A. xanthorrhiza polycarpic form differs from *A. xanthorrhiza* cultivated form, in having few and small cormels, and often a splitting fibrous root. *A. xanthorrhiza* polycarpic form is related to *A. xanthorrhiza* monocarpic form, but differ in some characters: the presence of cormels with several shoots, the few umbels or low amount of seeds/generative shoot, the short plant size and the perennial behavior. Apparently, the *A. xanthorrhiza* polycarpic form appears to be the most closely related to *A. xanthorrhiza* cultivated form, sharing a perennial habit and similar ramifications of the flowering shoot, leaves, involucel and fruit shape.

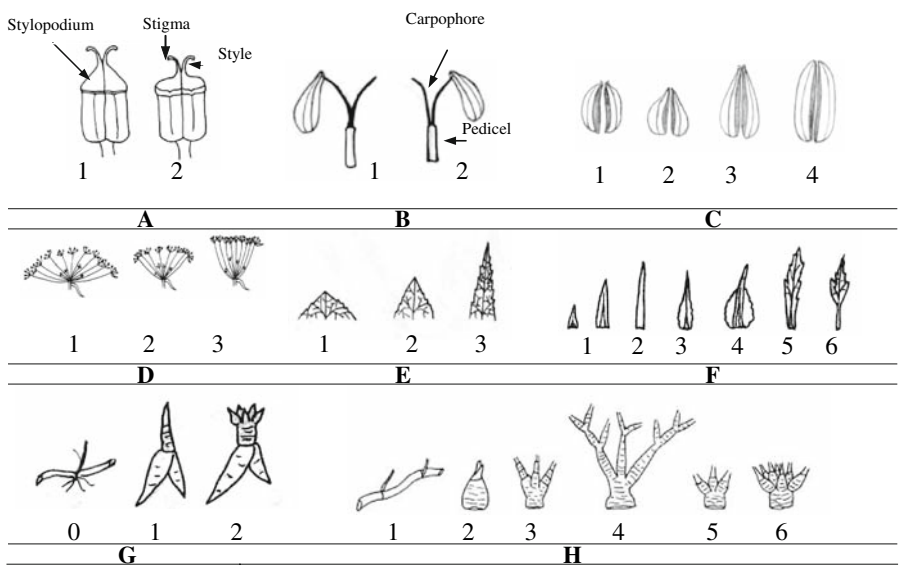

Fig. 4 Some morphological descriptors for the identification of *Arracacia* species: (**A**) Stylopodium shape; (**B**) Carpophore insertion; (**C**) Fruit shape; (**D**) Umbel form; (**E**) Acumen shape of leaflet; (**F**)involucel form; (**G**) Cormels presence and (**H**) Rootstock shape

Molecular analysis

The five most informative screened primer combinations (E-ACT/M-CAC, E-AAC/M-CTT, E-ACC/M-CTC, E-ACT/M-CTC and E-ACG/M-CTC) generate 202 AFLP reliable and reproducible markers. The highest values of AFLP marker index, showing the information content of the combinations of AFLP primers by assay, concern E-ACC/M-CTC, followed by E-ACT/M-CTC (Table 6). These two primers will be used in subsequent fingerprinting research in arracacha. Within species, very few unique polymorphic fragments are observed. *A. elata* shows three unique polymorphic fragments, representing only 1.5% of the total polymorphic fragments. No distinctive fragments are found for the species with storage roots, previously identified as *A. incisa*, *A. equatorialis* and *A. xanthorrhiza* (Table 6).

In general, the number of polymorphic bands is higher for *A. elata* than for the other species (*A. incisa*, *A. equatorialis* and *A. xanthorrhiza*). *A. elata* presents 78.5% polymorphic bands of the total polymorphic fragments. *A. incisa, A. equatorialis* and *A. xanthorrhiza* show 37.6%, 44.1% and 47.6% polymorphic bands respectively. Polymorphism among populations within species presents the same tendency in the four analyzed species, varying from 36.5 to 60.2%, 24.8 to 30.5%, 13.3 to 28.7% and 23.2 to 26.7%, respectively for *A. elata*, *A. incisa, A. equatorialis* and *A. xanthorrhiza* (Table 7).

The AMOVA analysis shows highly significant differences ($p = 0.000$, determined from a 10,000 replication bootstrap) among the four species, among populations within species and within populations. The analysis shows that 49.5% of the total variation is attributed to differences between species, while variation among populations within species is 25.8% of the total variation and the variation within populations accounts for 24.6% of the total variation (Table 8). This relatively high degree of genetic differentiation recorded between species, could be attributed to the geographic boundaries among species that do not facilitate gene flow among them. The high degree of variation within the populations could be explained by the widespread occurrence of wind pollination and breeding systems that promote outcrossing. Indeed, in arracacha, the presence of protogyny hinders self-fertilization and promotes outcrossing (Hermann 1997). Arracacha appears most likely to be a facultative outbreeder, giving progenies of genotypes with new genetic combinations. Another possible source for such pronounced genetic variation within populations might also result from hybridisations between wild and cultivated forms of *A. xanthorrhiza* or between *A. xanthorrhiza* and closely related species.

Three main groups are found after plotting the 12 populations with 202 AFLP markers according to nonmetric multidimensional scaling analysis (Fig. 5). The first group corresponds to the three populations of *A. elata* (RBS-130, RBS-134 and RBS-136). Individuals from each population are grouped closely, confirming previous results in the morphological analysis.

The second group corresponds to the three populations of *A. incisa* (RBS-94, RBS-95 and RBS-129); individuals from each population are grouped closely.

The third group includes six populations corresponding to the two species previously identified as *A. xanthorrhiza* (RBS-124, RBS-138, RBS-144) and *A. equatorialis* (RBS-93, RBS-96 and RBS-141). A population of *A. equatorialis* (RBS-96) is closely related to *A. xanthorrhiza*. The same tendency is reported from the morphological analysis.

Table 6 Primer combinations, marker index and polymorphic bands in each studied species

Primers	Polymorphic bands	Marker index	Unique fragments inside species			
			A. elata	*A. incisa*	*A. equatorialis*	*A. xanthorrhiza*
E-ACC/M-CTC	67	24.0	0	0	0	0
E-ACT/M-CTC	40	16.1	1	0	0	0
E-AAC/M-CTG	33	13.40	0	0	0	0
E-ACT/M-CAC	31	12.55	1	0	0	0
E-AAC/M-CTT	31	11.86	1	0	0	0
Total	202		3	0	0	0

Table 7 Polymorphic bands for each population and species

Species	Populations	Per population		Per species	
		Polymorphic loci	% poly[a]	Polymorphic loci	% poly
A. elata	RBS-130	54	36.5	80	78.4
	RBS-134	73	56.6		
	RBS-136	77	60.2		
A. incisa	RBS-93	34	24.8	32	37.6
	RBS-96	36	28.6		
	RBS-141	36	30.5		
A. equatorialis	RBS-94	39	28.7	49	44.1
	RBS-95	19	13.5		
	RBS-129	20	13.3		
A. xanthorrhiza	RBS-124	36	26.7	50	47.6
	RBS-138	34	23.4		
	RBS-144	32	23.2		

[a] poly = polymorphism

Table 8 AMOVA results for variation among species, among populations within species and within populations

Source of variation	d.f.*	Sum of squares	Variance components	Percentage of variation	*P* (value)
Among species	3	1251.008	5.87880	49.53	0.000
Among populations within species	8	514.200	3.06757	25.84	0.000
Within populations	228	666.600	2.92368	24.63	0.000
Total	239	2431.808	11.87005		

* d.f. = degree of freedom, *P* = probability

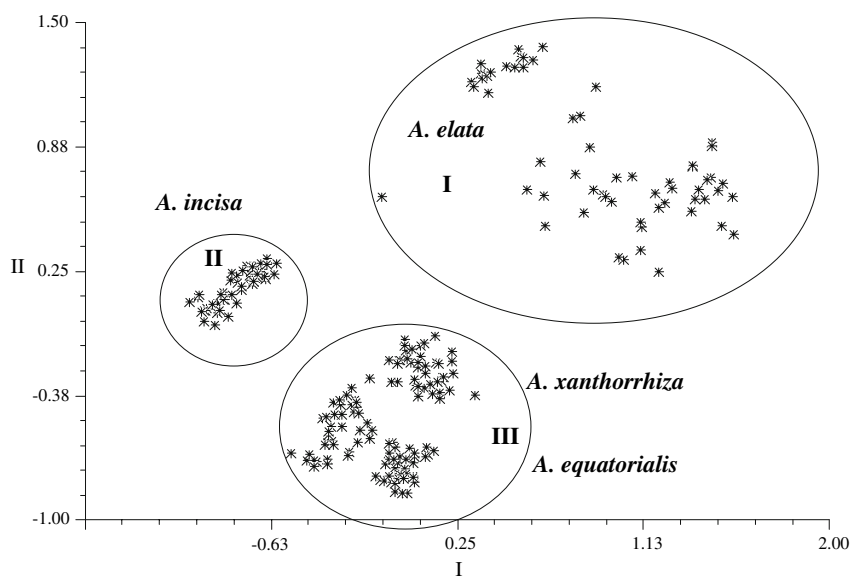

Fig. 5 Plotting 240 plants of 4 *Arracacia* species according to nonmetric multidimensional scaling analysis with 202 AFLP markers

On the basis of morphological and molecular markers analysis, it is difficult to separate *A. xanthorrhiza* and *A. equatorialis*, indicating the closely related connection between them. Consequently, the identified taxon *A. equatorialis* could be considered as a different form within the *A. xanthorrhiza* species.

On the other side, *A. incisa* is a taxon deserving the status of species, in concordance with Constance's (1949) study; this conclusion is drawn from our morphological and molecular analysis. Therefore two species are recognized in the Andean region: *A. incisa* and *A. xanthorrhiza*; both of them have storage roots. One of them, *A. xanthorrhiza*, includes cultivated and wild forms; while the second, *A. incisa*, includes only wild forms. The *A. xanthorrhiza* cultivated form has many storage roots and well developed cormels, two properties distinguishing this form from other *Arracacia* specimens belonging to the group with the storage root (Blas 2005).

Essential factors to clarify *Arracacia* taxonomy

The clarification of the wild Andean *Arracacia* taxonomy is not an easy task due to the influence of various parameters, such as introgressive hybridisation between distinct species and hybrid speciation, phenotypic plasticity within species, morphological convergence among species and presence of the same ploidy level in the Andean *Arracacia* species (Blas et al. 1997; Blas 2005).

In particular, the extent and effect of hybridisation and introgression in wild *Arracacia* do not facilitate the botanical separation between some taxa and the phyletic understanding of the genus. The taxonomical differentiation among the wild Andean species is even more complicated when cross-compatible cultivated arracacha varieties occur in the vicinity of these wild populations: as those cultivated varieties are more variable phenotypically due to both anthropical and environmental influences, gene flow between the cultivated and wild forms hinders seriously the taxonomical identification of the Andean wild *Arracacia*. Hybridisation between Andean wild and cultivated arracacha genotypes have produced extensive hybrid swarms; some of them have been recognized as taxa while others are still considered as intermediate forms. This explains why some species associated to the Andean region like *A. incisa* and *A. xanthorrhiza*, show a complex pattern of variation with not well defined boundaries between genotypes of this group. This complex pattern might also result from the two following factors:

- Phenotypic plasticity which induces polymorphism in some morphological traits influenced by environmental components; this is illustrated by the high variation in leaf shape observed in several of the concerned taxa, depending on their ecological habitats;
- Weediness which is common in many representatives of this group displaying a preference for disturbed habitats (such as roadsides, boundaries of cultivated fields, or ancient ruins of the Peruvian civilization).

Domestication process of *Arracacia*

Andean South America seems to be the place of domestication of arracacha, although the genetic diversity of the genus *Arracacia* in other regions like Mexico is particularly high. According to Bukasov (1930), arracacha was domesticated in the southern region of Colombia where he found different varieties of arracacha. At present, in South America, the highest diversity of *Arracacia* wild species was found in Colombia and Venezuela. Nevertheless, these wild species are not closely related to *A. xanthorrhiza*, due to the lack of storage root. The wild species *A. incisa* and the wild forms of *A. xanthorrhiza*, bearing storage roots and cormels and resembling more closely the cultivated arracacha (*A. xanthorrhiza*), present high genetic diversity in the Andean region, from where they probably originated. *Arracacia xanthorrhiza* is widely distributed through Bolivia, Peru and Ecuador from 2,000 to 4,000 m; whereas *A. incisa* is confined to the highland areas between the central and southern regions of Peru (Blas et al. 2007). *A. incisa* is the species more closely related to *A. xanthorrhiza* (Blas 2005).

So far it is not possible to elucidate clearly the origin of cultivated arracacha. Chromosome or gene mutations, selection in the wild forms, as well as hybridization between wild forms or between

A. incisa and *A. xanthorrhiza* are all hypothetical ways to explain the origin of the cultivated arracacha. One of the farmers we met during our field survey in the region of Cajamarca considered that a selection of wild forms during three consecutive cycle at small farm level could lead to the development of domesticated form.

Evidences of Andean domestication are indicated by the uses of wild arracacha in medicinal or religious activities and as plants providing animal and human food since pre-Inca's time (Blas 2005). There are some historical references about pre-Columbian cultivation of arracacha in Ecuador, Colombia and Peru, which were documented by Spaniard chroniclers during the conquest of South America (Cobo 1956). In addition, there is some archeological evidence, such as Nazca pottery designs more or less 2,000 years old (Yacovleff and Herrera 1934). This pre-Inca culture developed in the Central and South Peru, covering coastal and highland parts. Only, in this region, the species *A. incisa* considered as the most closely related to *A. xanthorrhiza* is found. The precise site of arracacha origin is difficult to establish in this context, but undoubtedly arracacha was domesticated in the Andean region, perhaps in the Southern region of Peru. This area corresponds to Ayacucho, Apurimac, Huancavelica, Cuzco Departments, the cradle of ancient cultures of the Inca Empire like Nazca and Chancas.

Conclusions

The 28 most discriminant morphological characters were selected to identify *Arracacia* species. These descriptors contributed to the distinction between the three species known from Peru: *A. elata*, *A. incisa* and *A. xanthorrhiza*. Each species was also well differentiated along its evolutionary process. For example, *A. elata* grows in eastern humid highlands, while *A. incisa* is geographically confined to the hilly and dry area of Peruvian central and western highlands (no rain for 7 months). The species *A. equatorialis* and *A. xanthorrhiza* share the same ecological and geographical distribution throughout all Peruvian Inter-Andean valleys (no rain for 5 months), which favors constant gene flow between them (Blas 2005) and even the likely production of viable hybrid seeds. Differentiation between these two taxa is not easy due to overlapping of morphological characters. The high morphological diversity among the species (*A. elata*, *A. equatorialis, A. incisa* and *A. xanthorrhiza*) and between the different populations analyzed in each of them could also be the result of environmental conditions, as samples were collected and evaluated in different regions. Molecular characterization (with 202 AFLP markers) in this material also showed a high diversity among species and inside populations. The magnitude of genetic differentiation between and within species suggests that the population size must be large enough to maintain such levels of genetic variation over time. The genetic analyses presented here could be used to develop conservation strategies for the species, for example through the definition of appropriate units for plant genetic resources management. In order to help explain the role of such units of conservation, the nature of (seed, pollen) dispersal mechanism, the pattern of gene flow within and between populations or species and their effects on reproductive and demographic processes should be further investigated. Patterns of variation in quantitative genetic traits should also be clarified in the building of conservation plans.

Morphological and molecular marker analysis do not separate obviously *A. xanthorrhiza* and *A. equatorialis*, indicating the close relation between them. For this reason, the populations corresponding to *A. equatorialis* could be considered as populations of *A. xanthorrhiza* and therefore the taxon might not deserve the status of a species. Consequently, our morphological and molecular analysis identified clearly at this stage three wild species of *Arracacia* genus: *A. elata, A. incisa and A. xanthorrhiza*. However, to clarify taxonomical differentiation, particularly between *A. xanthorrhiza* and *A. equatorialis*, it will be useful to validate the most discriminant characters among the studied populations, by comparing their performance and stability in multilocation trials.

Analysis of the wild arracacha genetic diversity for agronomical traits such as resistance to drought and decay of storage roots is also important to improve the cultivated arracacha. Incorporation of these desirable characteristics into existing cultivars will

be useful to overcome the limiting agricultural factors of this Andean neglected crop.

Acknowledgements This research was supported by grants from Coopération Universitaire au Développement (CUD) and Direction Générale de la Cooperation Internationale, Belgium.

References

Blas R, Arbizu C, Rodriguez G (1997) Número de cromosomas de la arracacha (*Arracacia xanthorrhiza* Bancroft). Anales Cientificos 32:44–54

Blas R (1998) Caracterización y evaluación de arracachas cultivadas (*Arracacia xanthorrhiza* Bancroft) del Perú. Tesis M. Sc., Universidad Nacional Agraria La Molina. Lima, Perú, 100 pp

Blas R (2000) Diversité génétique et éléments de physiologie chez l'arracacha (*Arracacia xanthorrhiza* Bancroft). Mémoire de fin d'études du Diplôme d'Etudes Approfondies, Faculté Universitaire des Sciences Agronomiques de Gembloux, Belgique, 67 pp

Blas R (2005) Diversity of *Arracacia* species in Peru. Ph.D. thesis. Gembloux Agricultural University, Belgium. 154 pp

Blas R, Hermann M and Baudoin J-P (2007) Analysis of the geographic distribution and relationships among Peruvian *Arracacia* wild specie. Genet Resour Crop Evol (in press)

Brako L, Zarucchi J (1993) Catálogo de las angiospermas y gimnospermas del Perú. Vol. 45, Missouri Botanical Garden, St. Louis, Missouri, USA, 1286 pp

Bukasov SM (1930) Las plantas cultivadas de Mexico, Guatemala y Colombia. Leningrado, Rusia. Traducido de la versión inglesa de M.H. Byleved por Jorge León (1985). CATIE Unidad de Recursos Genéticos, Turrialba, Costa Rica, 168 pp

Centro Internacional de la Papa (CIP) (1998) Molecular biology laboratory protocols: plant genotyping. Herrera MR, Zhang D, Ghislain M (eds) Genetic Resources Department, training manual, CIP, 2nd edn, Lima, Peru, 40 pp

Cobo PB (1956) Biblioteca de autores españoles, desde la formación del lenguaje hasta nuestros dias. Tomo I, Libro IV Estades, Artes Graficas. Madrid, España, pp 154–199

Constance L (1949) The South American species of *Arracacia* (*Umbelliferae*) and some related genera. Bull Tor Bot Club 76(1):39–52

Constance L (1997) An instance of East–West confusion in Chinese *Umbelliferae*, or *Arracacia* out of Asia. Edinb J Bot 54(1):99–104

Constance L, Affolter JM 1995a. *Arracacia ravenii* (*Apiaceae*), a new species from southern Mexico. Novon 5:22–23

Constance L, Affolter JM 1995b. Three new species and a new combination in *Arracacia* Bancroft (*Umbelliferae*/*Apiaceae*). Brittonia 47(3):320–327

Crisci J, López M (1983) Introducción a la teoría y práctica de la taxonomía numérica. Serie de biología No.26, Secretaria General de la Organización de los Estados Americanos (OEA). Programa Regional de Desarrollo Científico y Tecnológico, Washington D.C., 132 pp

Dagnelie P (1986) Analyse statistique à plusieurs variables. 3eme réimpression, Les Presses Agronomiques de Gembloux, Gembloux, Belgique, 362 pp

De Candolle A (1835) Introduction à l'étude de la Botanique, traité élémentaire de cette science. Tome I, Librairie Encyclopédique de Roret, Paris, France, 534 pp

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred for metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Ghislain M, Zhang D, Fajardo D, Huamán Z, Hijmans R (1997) Genetic diversity analysis in a cultivated Andean potato, *S. phureja* Juz. et Buk. The International Potato Center (CIP), Program Report 1995–1996. Lima, Peru, pp 77–83

Hancock JF (2004) Plant evolution and the origin of crop species, 2nd edn. CABI Publishing, Wallingford, UK, 313 pp

Hermann M (1997) Arracacha (*Arracacia xanthorrhiza* Bancroft). In: Andean roots, tubers: Ahipa, arracacha, maca and yacon. Promoting the conservation and use of underutilized and neglected crops. In: Hermann M and Heller J (eds) Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy, pp 75–172

Hiroe M (1979) *Umbelliferae* of the World. Ariake Book Company, Tokyo, Japan, 2129 pp

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaudoise Sci Nat 44:223–270

Knudsen SR (2003) Reproduction biology of the Andean root crop arracacha (*Arracacia xanthorrhiza* Bancroft var. *xanthorrhiza*) and the taxonomic status of the South American *Arracacia* Bancroft species with special emphasis on the position of the cultivated arracacha and related wild species. Ph.D. thesis. Botanical Section, Department of Ecology, The Royal Veterinary and Agricultural University. Copenhagen, Denmark, 150 pp

Mathias ME and Constance L (1944) *Arracacia.* North American Flora. The New York Botanical Garden. 28B (Part 1): 90–102

Mathias ME and Constance L (1962) *Arracacia* Bancroft. In: Mathias ME and Constance L (eds) Flora of Perú. Field Mus Nat Hist Bot 13(1) Part V-A: 13–19

Mathias ME, Constance L (1968) Two new apioid *Umbelliferae* from southern Mexico. Collectanea Botanica 7(Fasc. II, no. 43):759–766

Mathias ME, Constance L (1973) New and reconsidered Mexican *Umbelliferae*. Contr Univ Michigan Herbar 11(1):1–24

Palm R (2000) L'analyse de la variance multivariée et l'analyse canonique discriminante: principes et applications. Notes de statistique et d'informatique 2000/1, Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique, 40 pp

Pimenov MG, Leonov MV (1993) The genera of the *Umbelliferae* . Royal Botanic Gardens, Kew, UK, 156 pp

Rohlf F (2000) NTSYS PC: Numerical taxonomy and multivariate analysis for the IBM PC microcomputers (and

compatibles), version 2.1 user guide. Applied Biostatistics Inc. Stony Brook, New York, USA, 39 pp

Royal Horticultural Society (RHS). (1995) Colour Chart. London, UK

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23(21):4407–4414

Yacovleff E, Herrera F (1934) Racacha. En: El mundo vegetal de los antiguos peruanos. Botánica Etnológica. Revista del museo nacional, Lima, Perú 3(3):309–310