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Analysis of Apple Germplasm in Northwestern Spain

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Abstract. **Spain is the 15th largest apple (*Malus ×domestica* Borkh.) producer in the world with production depending mainly on foreign cultivars. During the 1970s, a germplasm bank of local cultivars was established in Galicia with the aim of preserving the local resources of northwestern Spain. A total of 408 accessions were studied using 89 morphological characters, with 15 corresponding to phenology, 46 to fruit, 7 to flowers, 11 to leaves, 6 to pests and 4 to diseases. Three variable isoenzymes, PGM E.C.5.3.1.9, PGI E.C.2.7.5.1 and EST E.C.2.7.5.1, were analyzed for 405 accessions and 27 commercial cultivars. The main objectives of this work were 1) to evaluate the inter- and intracultivar variability using morphological characters and isoenzymes, 2) to classify the accessions according to the main sources of variability, and 3) to identify repetitions in the germplasm bank. Principal component analysis (PCA) revealed six main sources of variability in the following order: size of fruit, color of skin, acidity, sweetness, harvest time, and attractiveness. The PCA analysis across 350 accessions produced 42 morphological groups. The 3 isoenzymes produced 190 genotype clusters. Combining morphological classification with the isoenzyme genotypes, we found 31 groups of synonyms involving 82 accessions and 8 more possible groups involving 17 accessions. This result allows the elimination of 53 repetitive accessions from the germplasm bank. Six commercial cultivars were identified as the progenitors of eighteen accessions: 'Reineta Blanca' of seven, 'Reina de Reinetas' of two, 'Reineta de Caux' of eight and 'Golden Delicious', 'Golden 4187' or 'Ozark Gold' of one each. Because inter- and intracultivar variability was high and names given by the growers were not reliable, the suggested selection strategy is to select individual clones among and within cultivars to exploit both the inter- and intracultivar genetic variability.**

Spain is the 15th largest apple producer in the world. Apple production reached 719,000 t in 1998; however, Spain imports over 75,000 t per year. Northwestern Spain, Galicia, was an important area of apple production before the 1950s (Lafitte, 1918) based mainly on local cultivars, with production moving subsequently to warmer areas where irrigation was established. At present, commercial production of apples in the four provinces of Galicia is localized in five areas with the total area of production ≈1,000 ha, 2% of the apple acreage in Spain. However, Galicia has 3 million trees in old orchards, which is 45% of the total number of apple trees in Spain, indicating that traditional apple production is predominantly local cultivars. Commercial cultivars planted in Galicia are largely 'Golden Delicious' and 'Reineta' (54% and 22% of the planted acreage, respectively). *Malus sylvestris* is spontaneous (Castroviejo, 1998), and *Malus ×domestica* was introduced many years ago (Boré and Fleckinger, 1997). Since then, both species have hybridized, bringing out new cultivars, more vigorous and less edible than commercial cultivars.

Keulemans (1993) points out the interest in local cultivars for their contribution to variability and better ecological adaptability. Germplasm repositories show the development and improvement of the apple and contain a vast reservoir of desirable genes (Janick et al., 1996). In Spain, apple genetic resources are preserved in three main collections: 1) 56 accessions in Aragón, 2) 365 accessions in Asturias, and 3) 408 accessions in Galicia (Dapena, 1996a, 1996b). Other local collections exist in the Basque Country, Navarra, Cataluña and Mallorca. Only studies about Asturian cultivars have been published (Dapena, 1996b, Coque et al., 1996). Present work has been focused on the Galician Germplasm Bank. Some local cultivars cultivated in Galicia previously are 'Camuesa' (Laffitte, 1918), 'Reineta', 'Repinaldo' and 'Tabardilla' (Lafitte, 1920), and 'Morro de Liebre' (Guinea, 1957).

Descriptors for characterizing and identifying apple cultivars are being developed by the International Union for the Protection of New Varieties of Plants (UPOV, 1974) and the International Board for Plant Genetic Resources (IBPGR, 1982). Multivariate analysis has been used to study germplasm of perennial fruit species: *Prunus armeniaca* L. (Pérez-Gonzales, 1992), *P. persica* (L.) Batsch. (Pérez et al., 1993), *P. cerasus* L. (Hilling and Iezzoni, 1988), *Juglans regia* L. (Fjellstrom et al., 1994), *Castanea sativa* Mill. (Pereira et al., 1996a, 1996b), *C. dentata* Borkh (Huang et al., 1998) and *C. pumila* Mill. (Dane and Hawkins, 1999). This technique has also been used to study variability in crop species such as *Lolium perenne* (Oliveira and Charmet, 1992), *Zea mays* L. (Goodman and Bird, 1977; Llauradó and Moreno-González, 1993), *Phaseolus vulgaris* L. (Martin and Adams, 1987) and *Eleusine coracana* (L.) Gaertn. (Hussaini et al., 1977). Isoenzymes were first used for identification of apple cultivars by Chyi and Weeden (1984), Menendez et al. (1986a, 1986b), Weeden and Lamb (1985), and Manganaris (1989).

The objectives of our work were 1) to determine the inter- and intracultivar variability using morphological characters and isoenzymes, 2) to classify the cultivars according to the main origins of variability, allowing the pre-selection of the best accessions for further trials, and 3) to identify duplications in the Galician Germplasm Bank.

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Materials and Methods

Plant material. Between 1978 and 1981, the four Galician provinces (A Coruña, Lugo, Ourense and Pontevedra) were visited to identify the local cultivars growing between sea level and 1000 m. Scions were collected and grafted onto MM.106 rootstock, and after 1 year were planted at 4 × 1.5 m and trained according to the Lepage system (Ascasíbar, 2000). Two trees of each accession were planted at the Galician Germplasm Bank of apple cultivars, located in the Centro de Investigaciones Agrarias de Mabegondo, A Coruña, 20 m above sea level. A total of 408 accessions were studied using morphological characters and isoenzymes (Table 1). To identify possible synonyms with important cultivars that could have been introduced in Galicia, 27 commercial cultivars were included in the isoenzyme study (Table 1).

Isoenzymes analysis. Three isoenzyme systems used by Manganaris (1989) and Batlle (1993) were studied in 405 accessions and 27 commercial cultivars. Two systems were evaluated in starch [phosphoglucoisomerase (PGI; E.C.5.3.1.9) and phosphoglucomutase (PGM; E.C.2.7.5.1)], and one in acrylamide [esterase (EST; E.C.3.1.1.)]. PGM, PGI, and EST were selected for clonal identification in this study because of their high variability in our collection. Our profiles for Pgm1, Pgm2, Pgm4, and Pgm5 corresponded with those assigned by Weeden and Lamb (1987), and Est1 and Est4 with Manganaris (1989) and Manganaris and Alston (1992). PGI was recorded as in Fig. 1. Leaves from each one of the two trees by accession were collected during the growing season to prepare extractions. Each accession was studied at least two times.

Morphological traits. For observations on fruit, 10 typical fruit were selected out of a minimum of 20 from two trees, excluding terminal fruit as defined by IBPGR (1982). Twenty-four morphological characters (evaluated in 1995–99 for phenology and diseases and in 1996–99 for fruit) were preselected from a previous principal component analysis (PCA) of >89 characteristics (Ascasíbar, 2000) indicated by UPOV (1974) and IBPGR (1982) as discriminant in apple. These 24 characters had the highest Eigen vectors in the first 40 principal components, which accounted for 80% of the variance. Nine were quantitative (1 to 9 below), and fifteen were qualitative and scored as class variables (10 to 24 below). The quantitative and qualitative variables can be used for classification analysis as suggested by Kaufman and Rosseeuw (1990) and Rubin (1967), and were used for PCA in peach (Pérez et al., 1993) and chestnut (Pereira et al., 1996a). A new binomial variable can be created for each of the classes of qualitative traits, where the values 1 or 0 indicate presence or absence of the observed quality in the individual phenotype being recorded (Kaufman and Rosseeuw, 1990). Thus, the fifteen qualitative traits were split up into 74 binomial variables.

From 1995 to 1999, the 24 traits recorded were as follows.

1) Time of beginning of bud burst (C3) (Fleckinger, 1964). Bud burst date, such that full blooming and harvest time were calculated using the effective thermal index (ETI) defined as the accumulated day-degrees above 7.5 ºC from 15 Feb. to bud burst, full blooming, and harvest time (Hidalgo, 1980).
2) Full blooming (F2) (Fleckinger, 1964). See above.
3) Harvest time (K) (Fleckinger, 1964). See above.
4) Weight (We). Average fruit weight in grams.
5) Length (Le). Average fruit length in mm (UPOV, 1974).
6) Width (Wi). Average fruit width in mm (UPOV, 1974).
7) Depth of calyx cavity (Dc) in millimeters (UPOV, 1974).
8) Width of calyx cavity (Wc) in millimeters (UPOV, 1974).
9) Width of stalk cavity (Wp) in millimeters (UPOV, 1974).
10) Fruit shape (Sh). The following scores were assigned according to the IBPGR (1982) classification: 1.0, globose; 1.1, globose-conical; 1.2, short globose conical; 2.0, flat; 2.1, flat-globose (oblate); 3.0, conical; 3.1, long conical; 3.2, intermediate-conical; 4.0, ellipsoid; 4.1, ellipsoid-conical (ovate); 5.0, oblong; 5.1, oblong-conical; 5.2, oblong-waisted.
11) Intensity of *Podosphaera leucotricha* infection on trees (Ph) at three levels (IBPGR, 1982; Lateur and Populer, 1994).
12) Intensity of *Venturia inaequalis* infection on trees (Vt) at three levels (IBPGR, 1982; Lateur and Populer, 1994).
13) Intensity of *Venturia inaequalis* infection on fruit (Vfr) at three levels (IBPGR, 1982; Lateur and Populer, 1994).
14) Shininess of skin: absent (Ab); moderately shiny (Ms); very shiny (Vs) (UPOV, 1974).
15) Fruit attractiveness (At). The following scores were assigned according to the IBPGR (1982) classification: 1, very low; 2, low; 3, intermediate; 4, high; 5, very high.
16) Russet amount (Ru). The following scores were assigned according to the IBPGR (1982) classification: 1, 0%; 2, 2% to 12%; 3, 13% to 25%; 4, 26% to 37%; 5, 38% to 50%; 6, 51% to 62%; 7, 63% to 75%; 8, 76% to 87%; 9, 88% to 100%.
17) Position of russeting: around calyx (P1); around stalk cavity (P2); on cheeks (P3); overall (P4) (UPOV, 1974).
18) Russet type: extremely fine (K1); very fine (K2); intermediate (K3); scaly (K4); cracked (K5) (IBPGR, 1982).
19) Sweetness of flesh: absent (S1); weak (S2); strong (S3); medium (S4) (UPOV, 1974).
20) Acidity of flesh: weak (A1); strong (A2); medium (A3) (UPOV, 1974).
21) Flavor of flesh: aromatic (F1); insipid (F2); aniseed (F3) (UPOV, 1974).
22) Intensity of over color of skin: no over color (I1); deep (I2); intermediate (I3); pale (I4) (UPOV, 1974).
23) Kind of over color: streaked (C1); splashed (C2); washed out (C3); complete (C4) (IBPGR, 1982).
24) Color: yellow (Ye); bicolor (Bi); ochre (Oc); red (Re); green (Gr) (IBPGR, 1982).

Statistical analysis. With the isoenzymes, a cluster analysis (UPGMA) was performed based on euclidean distance computed between each pair of trees using the PROC CLUSTER of SAS over the allelic frequencies. An analysis of variance (ANOVA) was conducted to determine the effects of the cultivar and trees within the cultivar for 11 traits: bud burst (C3); full blooming (F2); harvest time (K); average fruit weight (We); average fruit length (Le); average fruit width (Wi); depth of calyx cavity (Dc); width of calyx cavity (Wc); width of stalk cavity (Wp); intensity of *Phodosphaera leucotricha* infection (Ph); and intensity of *Venturia inaequalis* infection (Vt). The procedure PROC GLM of SAS for unbalanced data was used, and the following model was applied: $X_{i(m)jk} = \mu + C_m + T_{i(m)} + Y_j + (CY)_{mj} + (TY/C)_{i(m)j} + \varepsilon_{i(m)jk}$, where $X_{i(m)jk}$ is the observation of the tree i (i = 1 to 24) within the cultivar m (m = 1 to 287) in the year j (j = 1 to 5) and the sample k (k = 1 to 10); m is the mean of all the observations; C_m, $T_{i(m)}$, Y_j, $(CY)_{mj}$, $(TY/C)_{i(m)j}$, and $\varepsilon_{i(m)jk}$ are the effects of the cultivar m, the tree i within the cultivar m, the year j, the interaction cultivar × year, the interaction tree within cultivar × year, and the error associated to the sample k in the observation $i(m)jk$, respectively. All effects are assumed to be random. The error terms for testing the significance of the mean squares for cultivars and trees within cultivars are the cultivar × year and the tree within cultivar × year interactions, respectively.

For the 49 traits selected to classify the Galician cultivars,

Table 1. Apple accessions and commercial cultivars established in the Germplasm Bank of the Centro de Investigaciones Agrarias de Mabegondo, A Coruña, Spain.

Codes	Accession	Codes	Accession
1	Roja De Julio	50	Manzana De Julio
2	Blanca De S. Juan	51	Tabardilla
3	Unknown	52	Blanca Plana
4	Ollo Landoy	53	De Fraga
5	Pero	54	Tres En Cunca
6	Marafouza	55	Tabardilla
7	Allo Mouro	56	Muy Grande
8	Ardio	57	Blancas
9	Parafreir (Suiza)	58	Tres En Cunca
10	Francesa Muy Grande	59	Manzana De Sada
11	Repinaldo	60	Amarillas
12	Grande Plana	61	Santiaguesa
13	Dulce	62	Muy Grande Aplanada
14	Comadronas	63	Tabardilla
15	Reineta	64	Temperá
16	Temperá	65	Manzana De Invierno
17	José Antonio	66	Tabardilla
18	Grande Dulce	67	Repinaldo
19	Tres En Cunca	68	Reineta
20	Blanca De Julio	69	De Cera
21	Tabardilla	70	Pero Mingán
22	Tres En Cunca	71	Grande Plana Estriada
23	Fariñentas	72	Sangre De Toro
24	Grande Plana Verde	73	Blanca Rojo
25	Grandísima Amarilla	74	Tabardilla
26	Roja De Julio	75	Repinaldo
27	Tardía	76	Tercer Premio "76"
28	De Cera	77	Arriscadas
29	Camoesa	78	Cascuda
30	Tabardilla	79	José Antonio
31	Jose Antonio	80	Blanca
32	De Mayo Blanca	81	Pero
33	Mazá Fresca	82	Camba
34	De Pera	83	Pequeña Verde
35	Roja De Julio	84	Sangre De Toro
36	Rogelia	85	Tres En Cunca
37	Romana	86	Plátano
38	José Antonio	87	Tardía
39	Pero	88	Tabardilla
40	Blanca Estriada	89	Camoesa
41	Camoesa	90	Roja De Julio
42	De Príncipe	91	Unknown
43	Morenas	92	Blanca De Julio
44	Asturias	93	Roja Estriada
45	Tres En Cunca	94	Reineta
46	Cobadia	95	Repinaldo
47	Santa María	96	De Príncipe
48	Grande Plana	97	Temperá
49	Santiaguesa	98	Temperá

PCA on the correlation matrix of the accession means was carried out using the procedure PRINCOMP of SAS. For the principal components with an Eigen Value over 2 ($\lambda_k > 2$), a cluster analysis was performed using the Mahalanobis' generalized distance.

Results and Discussion

ISOENZYMES. Our results for Pgm4 and Pgm5 agreed with those shown by Manganaris (1989), denominated in that work as Pgm3 and Pgm4, for 'Gala', 'Golden', 'Granny Smith' and MM.106 (Table 2). For Est1 and Est4 there were coincidences with the cultivars previously studied by Manganaris (1989) and Manganaris and Alston (1992) 'Gala', 'Golden', 'Granny Smith', MM.106 and M.9, but not for 'Belle de Boskoop', perhaps due to a mistake in selecting the accession we used for this study.

A total of 12 alleles and two nulls were found (Table 3). Allelic frequencies for Pgm1a, Pgm1b+c and Pgm1d were very similar between Galician accessions and *Malus ×domestica* studied by

Table 1 (continued). Apple accessions and commercial cultivars established in the Germplasm Bank of the Centro de Investigaciones Agrarias de Mabegondo, A Coruña, Spain.

Codes	Accession	Codes	Accession
99	Verdosa Septiembre	148	Temperá
100	Verdosa Septiembre	149	Unknown
101	Pontellas	150	Unknown
102	Blanca De Julio	151	De Invierno
103	Unknown	152	Unknown
104	De Julio	153	Unknown
105	Unknown	154	De Libra
106	Repinaldo	155	De Noya
107	Rayas	156	Blanca Plana
108	Negras	157	Roja Muy Fuerte
109	Roja	158	Unknown
110	Unknown	159	Tabardilla
111	Troncocónicas	160	Unknown
112	Tres En Cunca	161	Unknown
113	Blandas Rayas	162	Mediana Agosto
114	Repinaldo	163	Agosto
115	Tardía	164	Tabardilla
116	Agosto	165	De Septiembre
117	Blanca	166	Tabardilla
118	Pero	167	Agosto
119	Peraza	168	Unknown
120	Unknown	169	Unknown
121	Temperá	170	Roja Plana
122	Pedra Dura	171	Unknown
123	Unknown	172	Tres En Cunca
124	Ollo Blanco	173	Muleiriña
125	Tempera	174	Tabardilla
126	José Antonio	175	Tabardilla Antigua
127	Roja	176	Pero
128	De S.Juan	177	Esperiega Ademuz
129	Pero	178	Tabardilla
130	Maza Dura	179	Unknown
131	Unknown	180	Unknown
132	Agosto	181	Tabardilla
133	Unknown	182	Rabala
134	Castellana	183	Unknown
135	Santiaguesa	184	Parecida De Cera
136	Unknown	185	Do Cú Negro
137	Unknown	186	Unknown
138	Unknown	187	Tabardilla
139	Repinaldo	188	Unknown
140	Unknown	189	Unknown
141	De Rayas	190	De Canle
142	Unknown	191	Parecida De Cera
143	De Julio	192	Tabardilla
144	Unknown	193	Tabardilla
145	Pero	194	Verdellas
146	Unknown	195	Duras
147	Tabardilla	196	Parecida De Cera

Wagner and Weeden (2000) (Table 3), and Pgm1c and Pgm1d in commercial cultivars agreed with those studied by Manganaris (1989). No references were found for allelic frequencies in Pgm2 and Pgm4, but we found variability as Weeden and Lamb (1987) established for those *loci* (Table 3). Pgm5b showed a higher frequency in Galician cultivars in respect to Pgm5a as was shown in other studies (Wagner and Weeden, 2000; Manganaris, 1989) (Table 3). Finally, Galician accessions presented very similar allelic frequencies for EST in comparison with Manganaris (1989) results. Our accessions are not closely related to other *Malus* species, since the alleles we found corresponded only with those found by other authors for *Malus* ×*domestica* (Table 3).

Eight different phenotypes were found for PGI (Fig. 1). The most frequent, numbered 2, was found in 78% of the individuals studied, and it was also the most frequent one encountered by Weeden and Lamb (1985) and Manganaris (1989); the one numbered 6, present in 7.4% of our accessions, also was reported previously by Chevreau (1984); and number 5, present only in

Table 1 (continued). Apple accessions and commercial cultivars established in the Germplasm Bank of the Centro de Investigaciones Agrarias de Mabegondo, A Coruña, Spain.

Codes	Accession	Codes	Accession
197	Unknown	246	Unknown
198	Parecida Repinaldo	247	Unknown
199	Folla	248	Pata De Mula
200	Rayada De Asturias	249	Café
201	Negras	250	Louriñán
202	Tardía	251	Xaneira
203	San Lorenzo	252	Reineta
204	De S.Juan	253	Repinaldo
205	Tres En Rama	254	Vizcaíña
206	Unknown	255	Unknown
207	Mariñas	256	Toca Pandeiro
208	Unknown	257	Roja De Verano
209	Unknown	258	Roja De Verano
210	Morro De Liebre	259	De Puebla
211	Santa María	260	Unknown
212	Unknown	261	Unknown
213	Tabardilla	262	Unknown
214	De Cedo	263	Golden Gallega
215	Unknown	264	Camoesa
216	Do Prado Da Cima	265	De S.Juan
217	Fariñentas	266	Parda
218	Camoesa	267	Unknown
219	Pero	268	Unknown
220	Repinaldo	269	Unknown
221	Redondela	270	Unknown
222	Unknown	271	Unknown
223	Freixeirana	272	Unknown
224	Reineta Blanca	273	Unknown
225	Pimienta	274	Unknown
226	De Rioboi	275	Unknown
227	Tabardilla	276	Unknown
228	Tabardilla	277	Espleje
229	Tardía	278	Paxeta
230	Temperá	279	Cregueira
231	Tres En Cunca	280	Caguleira o Presente
232	Unknown	281	Parece 'Cox'
233	Bico Negro	282	Albariña
234	Faba	283	Unknown
235	Unknown	284	San Antonio
236	Pero	285	Piolla
237	Unknown	286	Riscadas
238	De Invierno	287	Riscadas
239	Santa María	288	Unknown
240	Unknown	289	Luneda
241	Unknown	290	Costoya
242	Amarilla Julio	291	Altonobles
243	Reineta	292	Dulce
244	De S.Juan	293	Dos Enfermos
245	Do Apostol	294	Mazá de todo o ano

0.9% of our accessions, was also shown by Manganaris (1989). Our PGI profiles for commercial cultivars also agreed with those reported by Manganaris (1989) and Weeden and Lamb (1985).

Morphological characters. Using PCA over the Correlation Matrix of the 49 morphological characters studied, the first six principal components (PCs) accounted for 46% of the variance with Eigen values over 2 (Table 4). Main sources of variability, i.e., with the highest Eigen vectors in each PC, were as follows.

PC1: size of fruit: length (Le); width (Wi); depth of calyx cavity (Dc); width of calyx cavity (Wc); and width of stalk cavity (Wp)

PC2: color: discriminating yellow (Ye) and bicolor (Bi)

PC3: russet (Ru), kind of russet (P2, P4) and intensity of the over color (I3)

PC4: sweetness (S4), acidity (A1, A2) and flavor (F2)

PC5: intensity of the over color (I1, I2) and type of over color complete (C4)

Table 1 (continued). Apple accessions and commercial cultivars established in the Germplasm Bank of the Centro de Investigaciones Agrarias de Mabegondo, A Coruña, Spain.

Codes	Accession	Codes	Accession
295	Unknown	344	Tabardilla
296	Unknown	345	Rubia
297	Unknown	346	Unknown
298	Parecida A Golden	347	Bilbaína
299	Unknown	348	Temperá
300	Blanca	349	De Invierno
301	De Pataca	350	Perdices
302	De Septiembre	351	Unknown
303	Unknown	352	Unknown
304	Repinaldo	353	Unknown
305	Parecida R.Encarnada	354	Unknown
306	Unknown	355	Parecida De Cera
307	Unknown	356	Unknown
308	Unknown	357	Reina De Reinetas
309	Tardía	358	Belarda
310	Verde Helada o Glasé	359	Belarda
311	Tardía	360	Pero
312	Ramona	361	Tabardilla
313	Unknown	362	Unknown
314	Unknown	363	Unknown
315	Unknown	364	De S.Roque
316	Unknown	365	Coruñesa
317	Dulce Septiembre	366	Sangre De Toro
318	Pero	367	Unknown
319	Unknown	368	Unknown
320	Unknown	369	Temperá
321	Bella Colorada	370	Unknown
322	Ferroi	371	Unknown
323	Leuros	372	Mandarina
324	Unknown	373	Sangre De Toro
325	Pero	374	Unknown
326	De Septiembre	375	Rabal
327	Temperá	376	Unknown
328	Unknown	377	Unknown
329	Coro	378	Unknown
330	Unknown	379	Unknown
331	Unknown	380	Unknown
332	Parecida Starking	381	Unknown
333	Parecida Carapanón	382	Unknown
334	Reineta	383	Unknown
335	Regalada	384	Unknown
336	Unknown	385	Unknown
337	Unknown	386	Unknown
338	Espiño Mol	387	Unknown
339	De Pera	388	Unknown
340	Piel De Sapo	389	Santa María
341	Dulce	390	Unknown
342	Unknown	391	Unknown
343	Unknown	392	Unknown

PC6: phenology (C3, F2 and K)

Infection by *Podosphaera* and *Venturia* had little influence in the first six PCs. That could be because our collection had a relatively low susceptibility to *Podosphaera* (only 32% of the accessions had a medium level of infection, and 9% had a high level of infection). The level of susceptibilty to *Venturia* was low (only 6% and <1% of the collection showed medium and high susceptibility in leaves, respectively; and 4% had medium level of infection on fruit).

Mean values for fruit weight varied between 10 and 500 g, with an average value of 112 g (Ascasíbar, 2000). Bicolor apples are the most frequent in the germplasm bank (48%), followed by yellow (43%), and less frequently red (5%), green (3%) and brown (1%), with brown corresponding to the skin completely covered by russeting. In Galicia brown skin is denominated by 'Tabardilla'. Acidity was strong in 11% of the samples, medium in 36%, and low in 54%. Of the samples classified, 45% were sweet, 16% very sweet, while 39% were considered as not sweet or mildly sweet.

Table 1 (continued). Apple accessions and commercial cultivars established in the Germplasm Bank of the Centro de Investigaciones Agrarias de Mabegondo, A Coruña, Spain.

Codes	Accession	Codes	Accession
393	Unknown	401	Unknown
394	Unknown	402	Unknown
395	Ros Alargada	403	Parecida Golden
396	Gravillán	404	Parecida G. Smith
397	Ros Redonda	405	Unknown
398	Unknown	406	Unknown
399	Unknown	407	E1
400	Unknown	408	E2
Commercial cultivar		Commercial cultivar	
1001	Belle de Boskoop	1018	Erovan
1002	Buluaga	1019	Golden 4187
1004	Gala	1020	MM.106
1005	Gala Imperial	1021	M.9
1007	Golden Delicious	1023	Oregon
1008	Granny Smith	1024	Ozark Gold
1009	Red Chief	1025	Peas Good
1010	Reina de Reinetas	1026	Red Gala
1011	Reineta Blanca	1027	Red Spur
1012	Reineta de Caux	1028	Reineta Encarnada
1013	Reineta Gris	1029	Reineta Regil
1015	Teórica	1030	Summer Red
1016	Cardinal	1031	Top Red
1017	Cooper		

The UPGMA cluster analysis carried out on 350 accessions produced 42 groups (Fig. 2) when the dendrogram is cut at a Mahalanobis distance of 0.6 (Ascasíbar, 2000). This distance is less than the 1.0 Mahalanobis distance used by Goodman and Bird (1977) to define maize races or the 0.8 used by Pereira et al. (1996a) to define chestnut cultivars. It is the same distance used by Llauradó and Moreno-González (1993) to group local land races of maize using similar methodology and analysis. In our results, this distance was useful to classify local apple cultivars and to find synonyms.

Morphology and Isoenzymes. The cultivar effect had less significance than the tree within-cultivar effect, suggesting that the names assigned by growers to the trees included in the collection (Table 5) were inconsistent. These results were confirmed with isoenzymes and multivariate analysis, since only four of 251 names were repeated two or more times: 1) accessions 41 and 264 of five named 'Camoesa' were identical; 2) accessions 191 and 196 of four named 'Parecida de Cera'; 3) accessions 117, 175, 178, 192 and 193 in one group and 213 and 228 in another of 24 named 'Tabardilla'; and 4) accessions 85 and 112 in one group and 205 and 231 in another of 10 named 'Tres en Cunca.'

Comparing clusters obtained by isoenzymes (Table 2) and morphology (Fig. 2), indicates repeats in the Germplasm Bank (Table 6). These accessions show the same isoenzyme profile and the same morphological characteristics. For example, accessions number 10, 15, 59, 96, 107, 146 and 202 have the same variation by isoenzymes (cluster 1, Table 2), but only accessions 59, 96, 107 and 146 were grouped in the same cluster using morphological characteristics (cluster 6, Fig. 2), which means that these four accessions are repeats in the Germplasm Bank. In this way, we have found 32 groups of synonyms with a total of 82 trees involved (Table 6). We will maintain only one tree per group of synonyms. There are eight other groups of possible synonyms, but the present database was not complete enough to classify them by morphological characters.

Commercial cultivar 'Reineta Blanca' (code 1011) could be inferred as the origin of accessions codified as 58, 117, 175, 178, 192, 193 and 301, while 30 and 208 may have originated from 'Reina de Reinetas' (code 1010), since they clustered in the same isoenzyme and morphological groups (Table 6). This situation may be related to the importance of the production of 'Reineta' apple type in northern Spain.

'Summer Red' (code 1030) is in isoenzyme group 2 (Table 2) but this cultivar cannot be the progenitor of accessions 85, 112, 121, 134, 191, 196, 247, 260, 271, 296, 319, and 368 because all

Fig. 1. Phosphoglucoisomerase phenotypes found in the germplasm bank and number of accessions with the same phenotype.

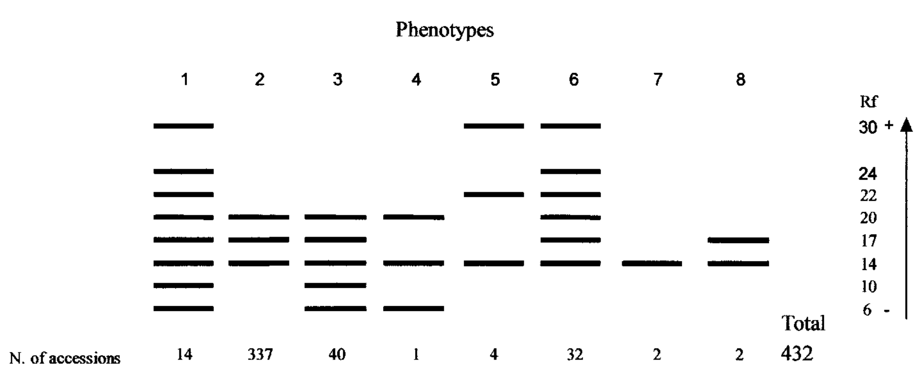

Table 2. Allelic profiles for PGM and EST isoenzymes and PGI phenotype for 406 apple accessions and 27 commercial cultivars.

Cluster	Accessions code
1	10, 15, 59, 96, 107, 146, 202
2	14, 18, 34, 45, 52, 60 63, 85, 112, 115, 121, 134, 184, 191, 196, 197, 206, 247, 260, 271, 296, 319, 364, 368, 1030
3	16, 19, 73
4	22, 23, 24, 210, 237, 266, 354, 405
5	17, 31, 38, 179
6	1, 35, 49, 50, 61, 64, 128, 133, 135, 159, 188, 204, 258, 265
7	9, 36, 136, 144, 302, 303, 376.2, 386, 1012
8	28, 57, 105, 292, 300, 1018, 1027
9	8, 65, 101, 363
10	11, 70, 242, 244, 304, 331
11	53, 71, 76, 77
12	13, 84, 125
13	30, 88, 208, 213, 228, 252, 281, 333, 384, 395, 1010
14	21, 91, 229, 272, 344, 348, 1009, 1020
15	74, 98, 103, 104, 130, 154, 156, 160, 181, 238, 282, 310, 338
16	27, 106
17	26, 116, 153, 200, 215
18	58, 117, 120, 175, 178, 183, 192, 193, 263, 301, 320, 397, 1011, 1021
19	7, 119, 124
20	108, 126
21	39, 131, 287, 367, 378
22	86, 137, 369
23	110, 138, 158, 167, 170, 171, 245, 307, 321
24	113, 141, 230, 290, 326, 337, 345
25	68, 145
26	69, 149, 305
27	82, 152, 371
28	56, 155, 163, 165, 195
29	123, 161, 289
30	162, 172
31	122, 173, 293, 399, 406
32	177, 185, 186, 335
33	169, 199, 268, 274, 291, 339
34	79, 201
35	54, 205, 231, 255, 283
36	139, 211, 312, 318
37	87, 214, 259, 262
38	127, 221, 250, 343
39	219, 225
40	224, 227, 297, 1007, 1015, 1019, 1024
41	209, 235
42	40, 239
43	233, 248, 254, 295
44	41, 264
45	100, 279, 361
46	46, 280
47	241, 284, 351
48	218, 285
49	203, 286
50	142, 288
51	251, 294
52	4, 298
53	90, 308
54	261, 313
55	309, 329
56	212, 334
57	314, 342, 377
58	350, 353
59	349, 356

PGI	Pgm1	Pgm2	Pgm4	Pgm5	Est1	Est4
2	aa	aa	aa	aa	ab	ab
2	ac	aa	nn	ab	ab	ab
2	aa	aa	nn	ab	aa	aa
2	ac	aa	nn	ab	bb	aa
3	ab	aa	aa	aa	bb	ab
2	ac	aa	nn	ab	bb	ab
2	ab	aa	nn	ab	bb	aa
2	ac	aa	nn	ab	ab	bb
2	cc	aa	aa	aa	ab	aa
2	ab	aa	nn	aa	bb	aa
2	cc	aa	nn	ab	ab	ab
2	ac	aa	aa	bb	ab	aa
2	aa	aa	nn	ab	ab	ab
2	aa	aa	nn	ab	bb	aa
2	ac	aa	nn	ab	ab	ab
2	ab	aa	nn	aa	ab	aa
2	ab	aa	aa	aa	ab	ab
2	ab	aa	nn	ab	ab	ab
3	ab	aa	aa	ab	ab	ab
3	ab	aa	aa	aa	ab	ab
2	ac	aa	nn	aa	ab	aa
3	ac	aa	nn	ab	aa	aa
2	ac	aa	aa	ab	ab	ab
2	aa	aa	nn	aa	ab	ab
2	cc	aa	nn	bb	bb	aa
2	aa	aa	nn	bb	bb	ab
6	ab	aa	nn	aa	ab	ab
2	aa	aa	aa	ab	ab	ab
3	ac	aa	aa	aa	ab	ab
1	ac	aa	aa	bb	ab	ab
2	ac	aa	aa	aa	ab	ab
2	ac	aa	nn	bb	ab	ab
2	ac	aa	nn	ab	ab	aa
3	aa	aa	aa	aa	bb	ab
1	ac	aa	aa	bb	ab	aa
2	ac	aa	aa	aa	ab	bb
2	abc	aa	aa	ab	ab	ab
2	ac	aa	nn	aa	ab	bb
2	ac	aa	nn	aa	bb	aa
2	aa	aa	nn	ab	bb	ab
2	cc	aa	nn	aa	aa	aa
2	abc	aa	nn	ab	bb	ab
1	aa	aa	aa	aa	ab	aa
3	ac	aa	nn	ab	ab	ab
2	ac	aa	nn	bb	ab	aa
2	ab	aa	aa	ab	bb	aa
6	ac	aa	nn	ab	ab	aa
2	ac	aa	aa	ab	bb	aa
2	abc	aa	nn	ab	ab	aa
2	aa	aa	nn	aa	ab	aa
2	cc	aa	aa	aa	ab	ab
2	ab	aa	aa	ab	bb	ab
2	aa	aa	nn	bb	ab	ab
2	cd	aa	nn	ab	ab	ab
2	aa	aa	aa	ab	ab	bb
6	aa	aa	nn	aa	bb	aa
2	aa	aa	aa	bb	bb	aa
2	bb	aa	aa	ab	aa	aa
2	cc	aa	aa	bb	ab	aa

Table 2 (continued). Allelic profiles for PGM and EST isoenzymes and PGI phenotype for 406 apple accessions and 27 commercial cultivars.

Cluster	Accessions code	PGI	Pgm1	Pgm2	Pgm4	Pgm5	Est1	Est4
60	355, 357, 390, 1001	2	ab	aa	nn	ab	ab	aa
61	147, 362, 1013	2	ab	aa	nn	bb	ab	ab
62	62, 366	2	cc	aa	nn	bb	ab	aa
63	341, 373	2	ac	aa	nn	aa	aa	aa
64	80, 375	2	ac	aa	nn	ab	aa	aa
65	78, 376.1, 400	2	bc	aa	nn	bb	ab	aa
66	129, 380	2	cc	aa	nn	bb	ab	bb
67	97, 383	6	cc	aa	nn	ab	ab	ab
68	166, 387, 1002, 1016	2	ac	aa	nn	aa	ab	ab
69	189, 388	6	aa	aa	nn	aa	ab	ab
70	132, 396	8	ac	aa	nn	ab	ab	aa
71	83, 408	6	ac	aa	nn	aa	ab	ab
72	1004, 1005	2	aa	aa	nn	aa	ab	bb
73	352, 1008	2	aa	aa	nn	aa	aa	ab
74	1017, 1031	2	ac	aa	nn	ab	bb	bb
75	29	3	ac	aa	nn	ab	bb	aa
76	42	3	ab	aa	aa	ab	bb	ab
77	25	2	aa	aa	aa	aa	bb	ab
78	109	3	ac	aa	aa	bb	ab	aa
79	95	3	ac	aa	aa	aa	ab	bb
80	5	3	aa	aa	nn	aa	ab	aa
81	234	3	ac	aa	nn	aa	ab	ab
82	249	3	aa	aa	aa	ab	ab	ab
83	256	3	ac	aa	nn	aa	bb	aa
84	277	2	ab	aa	aa	ab	ab	ab
85	226	3	ac	aa	aa	ab	ab	bb
86	299	2	ac	aa	aa	ab	ab	bb
87	328	3	ab	aa	aa	ab	bb	aa
88	374	2	ab	aa	aa	aa	bb	ab
89	114	2	ac	aa	aa	aa	ab	aa
90	379	3	ac	aa	aa	aa	ab	aa
91	398	3	aa	aa	nn	ab	ab	ab
92	157	3	ac	aa	nn	aa	bb	ab
93	1025	2	ac	aa	nn	aa	bb	ab
94	20	6	cd	aa	nn	ab	ab	aa
95	32	6	cd	aa	nn	ab	ab	ab
96	33	2	acd	aa	nn	ab	ab	aa
97	47	2	bb	aa	nn	aa	ab	aa
98	43	6	ab	aa	nn	ab	bb	ab
99	51	6	ab	aa	nn	ab	bb	aa
100	92	6	cc	aa	nn	ab	ab	aa
101	99	2	ab	aa	nn	ab	ab	bb
102	102	6	ac	aa	nn	ab	ab	ab
103	44	2	cc	aa	nn	bb	bb	bb
104	93	2	cc	aa	aa	ab	ab	ab
105	150	2	cc	aa	aa	bb	ab	ab
106	168	2	bc	aa	nn	bb	ab	ab
107	174	2	abc	aa	nn	bb	ab	ab
108	12	2	ab	aa	aa	ab	aa	ab
109	182	2	ab	aa	aa	aa	aa	ab
110	140	6	aa	aa	nn	ab	bb	bb
111	187	6	aa	aa	nn	ab	bb	ab
112	190	2	ac	nn	aa	ab	ab	ab
113	194	2	ac	nn	aa	ab	aa	ab
114	176	2	cc	aa	aa	ab	aa	ab
115	216	2	ac	aa	aa	ab	aa	ab
116	240	6	aa	aa	nn	ab	ab	aa
117	143	2	ab	aa	nn	ab	bb	ab
118	243	2	ab	aa	nn	bb	bb	ab

Table 2 (continued). Allelic profiles for PGM and EST isoenzymes and PGI phenotype for 406 apple accessions and 27 commercial cultivars.

Cluster	Accessions code	PGI	Pgm1	Pgm2	Pgm4	Pgm5	Est1	Est4
119	246	2	aa	aa	nn	ab	ab	aa
120	276	6	aa	aa	nn	aa	bb	ab
121	278	2	ab	aa	nn	aa	ab	ab
122	322	2	aa	aa	aa	ab	bb	aa
123	332	2	aa	aa	aa	aa	ab	aa
124	340	7	ac	aa	nn	ab	ab	aa
125	306	2	ac	aa	nn	ab	aa	ab
126	346	2	aa	aa	nn	ab	aa	ab
127	336	2	cc	aa	aa	ab	ab	aa
128	273	2	ab	aa	nn	aa	aa	ab
129	360	2	ab	aa	aa	ab	ab	bb
130	81	2	ac	aa	aa	bb	aa	aa
131	365	2	ac	aa	aa	ab	aa	aa
132	323	2	ab	aa	nn	bb	aa	ab
133	392	2	bb	aa	nn	bb	aa	ab
134	55	5	acd	aa	nn	aa	ab	aa
135	393	5	ac	aa	nn	aa	ab	aa
136	382	2	ac	aa	nn	bb	ab	bb
137	401	2	ac	aa	nn	bb	bb	bb
138	347	2	abcd	aa	nn	ab	bb	aa
139	403	2	abcd	aa	nn	bb	bb	aa
140	257	2	acd	aa	nn	ab	bb	ab
141	1023	2	acd	aa	nn	ab	ab	ab
142	1026	2	ab	aa	nn	aa	ab	bb
143	404	2	ab	aa	aa	aa	aa	bb
144	1028	2	ab	aa	aa	aa	ab	bb
145	267	3	abc	aa	aa	aa	bb	ab
146	381	2	cc	aa	nn	aa	ab	ab
147	148	2	ac	aa	aa	bb	ab	ab
148	118	2	abc	aa	aa	aa	ab	ab
149	48	3	aa	aa	aa	aa	bb	aa
150	72	2	cd	aa	nn	bb	ab	bb
151	385	2	ac	aa	nn	aa	aa	ab
152	75	2	ac	aa	aa	aa	bb	ab
153	324	2	ab	aa	nn	bb	bb	aa
154	311	2	ab	aa	nn	aa	bb	ab
155	370	2	cd	aa	nn	bb	ab	aa
156	407	2	aa	aa	nn	ab	aa	bb
157	1029	6	ab	aa	nn	ab	ab	aa
158	389	2	aa	aa	nn	bb	ab	aa
159	111	2	dd	aa	nn	ab	ab	ab
160	6	6	cc	aa	aa	aa	aa	aa
161	217	6	cc	aa	aa	ab	aa	ab
162	151	1	aa	aa	nn	aa	ab	aa
163	220	2	aa	nn	aa	ab	ab	bb
164	327	2	ac	nn	aa	ab	bb	bb
165	330	2	ab	aa	aa	bb	aa	aa
166	89	3	abc	aa	nn	ab	ab	ab
167	358	3	bc	aa	nn	aa	ab	ab
168	164	2	ab	nn	nn	ab	ab	ab
169	359	2	ab	nn	aa	ab	ab	ab
170	37	6	ab	aa	nn	aa	aa	aa
171	275	6	aa	aa	aa	ab	ab	aa
172	66	5	ac	aa	aa	aa	ab	aa
173	207	3	ac	nn	aa	ab	aa	aa
174	232	3	ab	aa	nn	aa	aa	aa
175	253	2	cd	aa	nn	ab	bb	aa
176	317	2	ac	nn	aa	bb	ab	aa
177	391	6	ab	aa	aa	aa	ab	ab

Table 2 (continued). Allelic profiles for PGM and EST isoenzymes and PGI phenotype for 406 apple accessions and 27 commercial cultivars.

Cluster	Accessions code	PGI	Pgm1	Pgm2	Pgm4	Pgm5	Est1	Est4
178	236	2	cd	aa	nn	ab	aa	bb
179	269	1	ac	aa	aa	ab	bb	ab
180	3	3	bc	aa	nn	bb	ab	bb
181	67	2	bc	aa	nn	aa	bb	aa
182	372	4	bc	aa	nn	ab	ab	ab
183	94	6	cc	aa	nn	bb	aa	aa
184	222	2	aa	nn	aa	aa	aa	ab
185	394	3	bc	aa	nn	aa	aa	bb
186	198	7	cc	aa	aa	aa	ab	bb
187	325	2	cc	aa	aa	aa	bb	bb
188	270	6	abc	aa	nn	bb	bb	ab
189	402	1	cc	aa	nn	aa	bb	ab
190	223	5	ab	aa	aa	ab	ab	ab

Table 3. Allelic frequencies of 405 Galician apple accessions and 25 commercial cultivars in comparison with previous studies.

Alleles	Accessions	Commercial cultivars	*Malus ×domestica* (Wagner and Weeden, 2000)	Cultivars (Manganaris, 1989)	*Malus* spp. (Manganaris, 1989)
Pgm1a	0.016	0.013	0.015	---	---
Pgm1b	0.329	0.173	Pgm1b + Pgm1c	---	---
Pgm1c	0.130	0.200	0.455	0.254	0.500
Pgm1d	0.524	0.733	0.530	0.746	0.308
Pgm2a	0.978	1.000	---	---	---
Pgm2n	0.022	0.000	---	---	---
Pgm4a	0.351	0.040	---	0.038	0.194
Pgm4n	0.649	0.960	---	0.962	0.639
Pgm5a	0.579	0.640	0.604	0.583	0.417
Pgm5b	0.421	0.360	0.369	0.417	0.306
Pgm5c	---	---	---	---	0.083
Pgm5d	---	---	---	---	0.194
Est1a	0.422	0.340	---	0.446	0.346
Est1b	0.578	0.660	---	0.554	0.411
Est1c	0.000	0.000	---	0.000	0.177
Est1d	0.000	0.000	---	0.000	0.056
Est1f	0.000	0.000	---	0.000	0.001
Est4a	0.665	0.440	---	0.604	0.583
ESt4b	0.335	0.519	---	0.384	0.383
Est4n	0.000	0.000	---	0.012	0.034

of those accessions produce yellow apples (Table 6, cluster 1 and 10); and accessions 18, 52, and 63 produce bicolor apples (Table 6, cluster 4 and 16).

German cultivar Reineta de Caux (code 1012) is defined as yellow with red stripes (Lafitte, 1920) and could be a progenitor of the bicolor accessions codified as 9, 36, 136, 144, 302, 303, 376.2, and 386.

'Erovan' or 'Early Red One' (code 1018) and 'Red Spur' (1027) were clustered in isoenzyme group 8 (Table 2) with accessions 28, 57 and 300, which produce yellow apples, and with 105 and 292, which produce bicolor apples.

'Red Chief' (code 1009) and rootstock MM.106 (code 1020) were clustered in isoenzyme group 14. They appear not to be related to accessions 21, 91, 229, 272, and 348, which produce bicolor apples, and 344, which produces yellow fruit.

'Golden Delicious' (code 1007), 'Golden 4187' (code 1019) and 'Ozark Gold' (code 1024) showed the same profile of isoenzymes (Table 2), and they could be the origin of accession 297, which also produces yellow apples. They are not related with 'Teórica' (code 1015), Asturian cultivars producing red apples used for cider production, or with accessions 224 and 227, producing bicolor apples.

'Belle de Boskoop' (code 1001) from Netherlands produces bicolor apples like accession 390 (Table 2), which presents the same isoenzymes, but with the over-color in streaks rather than complete.

Apples from 'Reineta Gris' (code 1013) are completely covered with russeting, a characteristic also presented by accession 147, with which it could be related because it presents the same profiles in isoenzymes (Table 2, cluster 61). Accession 362 produces bicolor apples quite different to 'Reineta Gris'.

Accession 352 showed the same isoenzymes as 'Granny Smith' (code 1008) but it produces longer apples quite different from 'Granny Smith' (Table 2, cluster 73).

Results shown in Table 7 allowed us to pre-select some accessions for further trials to promote the use of local cultivars for local market. Accessions in morphological clusters 5, 7, 9, 17, 22, 23, 24, 25, 26, 28, 30, 31, 32, 34, 35, 36, 37, 39, and 41, and 19 accessions of cluster 12, (for a total of 133 accessions) have the lowest interest for apple production due to the small size of their

Table 4. Eigen values, eigen vectors and accumulated variance associated with the first seven principal components (PCs) estimated from the correlation matrix of 49 variables.

Variable[z]	PC1	PC2	PC3	PC4	PC5	PC6	PC7
C3[z]	–0.11	–0.08	–0.10	0.08	–0.06	0.35	–0.07
F2	–0.13	–0.06	–0.12	0.10	–0.05	0.33	–0.08
K	–0.04	–0.14	–0.03	0.20	0.04	0.42	–0.03
We	0.37	0.08	–0.04	0.10	0.08	0.11	0.06
Le	0.31	0.09	–0.11	0.08	0.06	0.15	0.05
Wi	0.38	0.10	–0.04	0.10	0.08	0.09	0.02
Dc	0.32	0.05	–0.06	0.06	–0.04	–0.04	0.12
Wc	0.35	0.05	–0.01	0.05	0.02	–0.01	–0.03
Wp	0.34	0.06	–0.05	0.05	0.10	0.11	–0.01
Sh	–0.10	–0.01	0.00	–0.06	–0.12	0.09	0.18
Ph	0.04	–0.07	0.04	–0.08	–0.06	–0.20	–0.06
Vt	–0.04	0.03	–0.03	–0.02	0.03	–0.01	0.06
Vfr	0.02	0.00	0.09	0.08	–0.04	–0.04	0.01
Ab	0.05	–0.26	0.27	0.06	0.01	0.01	0.15
Br	–0.04	0.20	–0.29	–0.03	–0.07	–0.04	–0.24
Vb	–0.01	0.15	0.06	–0.06	0.15	0.07	0.23
At	0.22	0.20	0.04	0.02	0.13	0.16	–0.09
Ru	0.08	–0.25	0.33	0.06	0.03	0.03	0.04
P1	0.03	0.07	–0.10	0.02	–0.05	–0.14	0.29
P2	–0.03	0.25	–0.28	–0.08	–0.05	–0.06	0.10
P3	0.00	0.06	–0.06	–0.16	0.16	–0.01	0.17
P4	0.03	–0.26	0.29	0.09	0.04	0.09	–0.12
K1	–0.08	0.07	–0.01	0.00	0.07	0.03	0.04
K2	–0.12	0.07	–0.19	0.10	–0.07	0.19	0.13
K3	0.04	0.00	0.06	–0.09	–0.05	–0.09	–0.28
K4	0.10	–0.19	0.17	0.02	0.09	0.02	0.17
K5	0.10	0.00	0.07	–0.04	0.05	–0.19	0.02
S1	–0.02	0.05	0.00	0.21	0.12	–0.31	0.14
S2	–0.14	0.01	–0.07	0.19	0.12	–0.02	–0.11
S3	0.07	0.00	0.03	–0.13	–0.08	0.23	–0.10
S4	0.10	–0.06	0.06	–0.26	–0.15	0.00	0.15
A1	0.03	–0.10	–0.04	–0.40	–0.17	0.21	0.26
A2	–0.03	0.08	0.04	0.29	0.14	–0.09	–0.40
A3	–0.01	0.05	0.01	0.23	0.07	–0.23	0.19
F1	–0.15	–0.03	–0.10	0.30	0.18	0.07	0.30
F2	0.15	0.03	0.08	–0.25	–0.15	–0.01	–0.29
F3	0.01	0.00	0.04	–0.14	–0.08	–0.14	–0.05
I1	–0.07	0.20	0.12	–0.20	0.36	0.07	–0.02
I2	–0.01	0.18	0.17	0.17	–0.38	–0.01	0.07
I3	0.08	–0.29	–0.29	–0.03	0.00	–0.05	–0.04
C1	–0.03	0.22	0.18	0.14	–0.28	0.10	0.04
C2	0.03	–0.02	–0.01	–0.05	–0.05	–0.07	0.03
C3	0.09	–0.23	–0.27	0.01	–0.02	–0.11	–0.02
C4	–0.09	0.10	0.10	–0.23	0.40	0.09	–0.04
Ye	0.07	–0.31	–0.26	0.00	0.01	–0.09	–0.04
Gr	–0.03	–0.04	–0.08	0.04	0.03	0.10	0.00
Bi	–0.04	0.31	0.23	0.05	–0.17	0.05	0.04
Oc	0.02	–0.07	0.15	0.06	0.07	0.02	0.00
Re	–0.07	0.05	0.05	–0.18	0.35	0.03	0.00
Eigen value	5.62	5.39	3.83	2.72	2.54	2.32	1.83
Accumulated variance (%)	12	23	30	36	41	46	50

[z]See list of abbreviations in Materials and Methods.

fruit. Fifteen accessions from cluster 12 (numbers 54, 73, 83, 87, 129, 172, 177, 181, 205, 238, 268, 297, 298, 331, and 384) and 204 accessions from clusters 1, 2, 3, 4, 6, 8, 10, 11, 13, 14, 15, 16, 18, 19, 20, 21, 27, 29, 33, 38, 40, and 42 produced apples with more than 100 g per unit. Among those 217 accessions, 128 were bicolor, 73 produce yellow apples, 10 red, 3 green, and 3 ochre due to russeting. Ninety accessions (avoiding repetitions showed in Table 6) of 128 producing bicolor apples, produce sweet fruit. Eighteen of these also produce fruit with medium acidity. If we eliminate the repetitions shown in Table 6 and those accessions

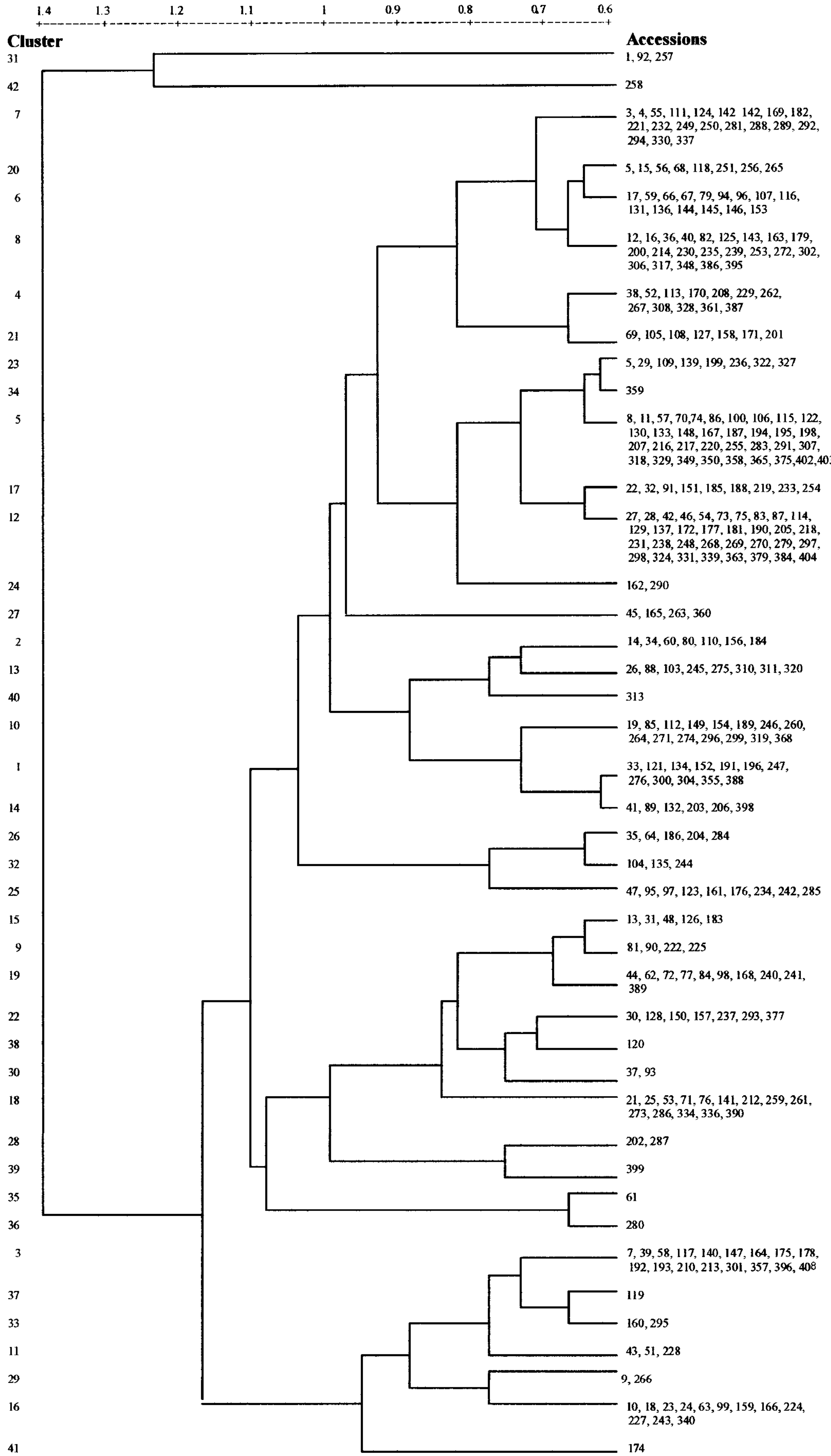

Fig. 2. Average linkage dendogram of 350 accessions of apple from northwestern Spain based on the Mahalanobis generalized distance of morphological data.

Table 5. F values from the analysis of variance of several morphological traits for the sources of variation cultivar, tree within cultivar, year and interactions cultivar × year and tree (cultivar) × year.

Variable[z]	Cultivar	Tree (cultivar)	Year	Cultivar × year	Tree (cultivar) × year
C3	1.31^{NS}	2.25****	124.30****	1.15^{NS}	0.68^{NS}
F2	1.20^{NS}	2.82****	79.88****	1.55***	0.68^{NS}
K	1.74***	7.33****	0.60^{NS}	4.61****	0.33^{NS}
We	1.37*	5.68****	2.57^{NS}	1.21^{NS}	5.19****
Le	1.59**	3.03****	4.85**	1.03^{NS}	6.29****
Wi	1.56**	4.05****	6.64***	1.22^{NS}	6.98****
Dc	1.68**	2.07****	102.28****	0.97^{NS}	5.71****
Wc	1.51**	4.86****	21.97****	1.36**	3.63****
Wp	1.44*	2.81****	11.82****	1.32*	4.30****
Ph	1.21^{NS}	2.70****	18.29****	0.91^{NS}	1.35****
Vt	0.74^{NS}	1.81****	13.41****	1.03^{NS}	1.04^{NS}

[z]See list of abbreviations in Materials and Methods.

NS,*,**,***,****Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001 or 0.0001, respectively.

Table 6. Synonyms found in the germplasm bank of Mabegondo using cluster analysis over allelic variation and morphological classification with 49 traits.

Synonyms	Isoenzyme clusters	Morphological cluster	Total synonyms
59, 96,107, 146	1	6	4
121, 134, 191, 196, 247,	2	1	5
14, 34, 60, 184	2	2	4
85, 112, 260, 271, 296, 319, 368	2	10	7
18, 63	2	16	2
23, 24	4	16	2
35, 64, 204	6	26	3
144, 136	7	6	2
36, 302, 386	7	8	3
11, 70	10	5	2
53, 71, 76	11	18	3
272, 348	14	8	2
74, 130	15	5	2
181, 238	15	12	2
103, 310	15	13	2
116, 153, 215	17	6	3
58, 117, 175, 178, 192, 193, 301, 1011 (‘Reineta Blanca’)	18	3	7
158, 171	23	21	2
167, 307	23	5	2
326, 345	24	15	2
123, 161	29	25	2
268, 339	33	12	2
54, 205, 231	35	12	3
255, 283	35	5	2
221, 250	38	7	2
224, 227	40	16	2
40, 239	42	8	2
233, 254	43	17	2
142, 288	50	7	2
212, 334	56	18	2
314, 342	57	15	2
Total			82
Other possible synonyms			
30, 208, 1010 (‘Reina Reinetas’)	13	11, ?, ?[z]	3
213, 228	13	11 (wrongly 3), 11	2
252, 395	13	?, 8	2
82, 152	27	8, 8 (wrongly 1)	2
41, 264	44	10, 26	2
129, 380	66	12, ?	2
97, 383	67	23, ?	2
189, 388	69	1, 10	2
Total possible synonyms			17

[z]? = undetermined morphological cluster.

Table 7. Classification of 350 Galician accessions of apple in 42 clusters by the six main origins of variability corresponding to the six first principal components of a principal component analysis over the correlation matrix with 49 morphological variables.

Cluster	Accession no.
1	33, 121, 134, 152, 191, 196, 247, 276, 300, 304, 355, 388
2	14, 34, 60, 80, 110, 156, 184
3	7, 39, 58, 117, 140, 147, 164, 175, 178, 192, 193, 210, 213, 301, 357, 396, 408
4	38, 52, 113, 170, 208, 229, 262, 267, 308, 328, 361, 387
5	8, 11, 57, 70, 74, 86, 100, 106, 115, 122, 130, 133, 148, 167, 187, 194, 195, 198, 207, 216, 217, 220, 255, 283, 291, 307, 318, 329, 349, 350, 358, 365, 375, 402, 403
6	17, 59, 66, 67, 79, 94, 96, 107, 116, 131, 136, 144, 145, 146, 153, 155, 173, 209, 215, 223, 277, 278, 346, 354, 374
7	3, 4, 55, 111, 124, 142, 169, 182, 221, 232, 249, 250, 281, 288, 289, 292, 294, 330, 337
8	12, 16, 36, 40, 82, 125, 143, 163, 179, 200, 214, 230, 235, 239, 253, 272, 302, 306, 317, 348, 386, 395
9	81, 90, 222, 225
10	19, 85, 112, 149, 154, 189, 246, 260, 264, 271, 274, 296, 299, 319, 368
11	43, 51, 228
12	27, 28, 42, 46, 54, 73, 75, 83, 87, 114, 129, 137, 172, 177, 181, 190, 205, 218, 231, 238, 248, 268, 269, 270, 279, 297, 298, 324, 331, 339, 363, 379, 384, 404
13	26, 88, 103, 245, 275, 310, 311, 320
14	41, 89, 132, 203, 206, 398
15	13, 31, 48, 126, 183, 211, 314, 321, 326, 342, 345, 366, 391
16	10, 18, 23, 24, 63, 99, 159, 166, 224, 227, 243, 340
17	22, 32, 9, 151, 185, 188, 219, 233, 254
18	21, 25, 53, 71, 76, 141, 212, 259, 261, 273, 286, 334, 336, 390
19	44, 62, 72, 77, 84, 98, 168, 240, 241, 389
20	6, 15, 56, 68, 118, 251, 256, 265
21	69, 105, 108, 127, 158, 171, 201
22	30, 128, 150, 157, 237, 293, 377
23	5, 29, 109, 139, 199, 236, 322, 327
24	162, 290
25	47, 95, 97, 123, 161, 176, 234, 242, 285
26	35, 64, 186, 204, 284
27	45, 165, 263, 360
28	202,287
29	9, 266
30	37, 93
31	1, 92, 257
32	104, 135, 244
33	160, 295
34	359
35	61
36	280
37	119
38	120
39	399
40	313
41	174
42	258

[z]In parentheses less frequent colors classified in the same group.
[y]Harvest time: 1 = from end of July to September; 2 = September; and 3 = October.

possibly originating from the commercial cultivars mentioned above, 74 accessions from the bicolor group could be selected that produce fruit of desirable sweetness and acidity for the fresh market. Among the 73 accessions with yellow apples, 45 of them produce fruit that is desirable in terms of sweetness. Nine red apple accessions also produce fruit that is desirable in terms of sweetness. Considering apples for cider production, which require more than medium acidity, we may consider 10 accessions from the bicolor group, seven from the yellow group, one from the red group and one from the green group, which amounts to a total of 19 accessions.

Conclusions

Information presented here will be useful for breeders looking for specific characteristics and for restructuring the Galician

Level of discrimination and type of discriminating trait					
1	2	3	4	5	6
Wt	Color	Acidity	Sweetness	Harvest time	Attractiveness
>100 g	Yellow	Weak	Medium-strong	1,2,3[y]	3.3
>100 g	Bicolor	Medium	Medium-weak	1,2	3.3
>100 g	Yellow (and Green)[z]	Weak	Medium-strong	2,3	2.6
>100 g	Bicolor	Weak	Medium-strong	1,2,3	3.4
<100 g	Yellow (Green and Bicolor)	Medium	Weak	1,2,3	2.5
>100 g	Bicolor	Medium-weak	Medium-weak	1,2,3	3.1
<100 g	Bicolor	Weak	Medium-weak	1,2,3	2.6
>100 g	Bicolor	Medium-weak	Medium-strong	1,2,3	2.9
<100 g	Bicolor	No acid	Medium-strong	1,2	2.7
>100 g	Yellow	Weak	Medium-strong	1,2,3	3.3
>100 g	Ochre	Medium	Medium	2,3	2.8
<100 and > 100 g	Yellow (and Green)	Weak	Medium	1,2,3	2.4
>100 g	Yellow (and Bicolor)	Medium-strong	Weak	1,2,3	3
>100 g	Yellow	No acid	Medium-strong	2,3	3
>100 g	Bicolor	Weak	Medium-strong	1,2,3	3.3
>100 g	Bicolor	Weak	Strong	2,3	2.7
<100 g	Yellow	No acid	Medium-strong	1,2,3	2.3
>100 g	Bicolor	Medium	Medium-weak	1,2,3	3.6
>100 g	Red (and Bicolor)	Weak	Medium-weak	1,2,3	3.5
>100 g	Bicolor	Medium-strong	Weak	1,2,3	2.8
>100 g	Bicolor	No acid	Medium	1,2,3	3.7
<100 g	Bicolor	Medium-strong	Medium-weak	1,2,3	2.8
<100 g	Yellow (and Bicolor)	Weak	Weak	2,3	1.8
<100 g	Green	No acid	Medium	2,3	2.5
<100 g	Yellow	No acid	Medium-strong	1,2,3	2.1
<100 g	Bicolor (and Yellow)	Medium	Medium	1,2	2.3
>100 g	Yellow (Green and Bicolor)	Medium	Weak	2,3	3.1
<100 g	Bicolor	Medium-weak	Weak	3	3
>100 g	Bicolor	Strong	Weak	2,3	2.7
<100 g	Red	Medium	Weak	2,3	2.9
<100 g	Bicolor	Strong	No sweet	1	2.5
<100 g	Bicolor	No acid	Strong	1	2.6
>100 g	Yellow	No acid to acid	Medium-weak	3	2.3
<100 g	Yellow	Medium	Weak	3	1
<100 g	Red	Weak	Medium	1	1.8
<100 g	Red	No acid	Medium-strong	2	2.7
<100 g	Yellow	Medium	Weak	3	3
>100 g	Bicolor	Medium-strong	Weak	2,3	2.8
<100 g	Bicolor	No acid	Medium	3	2
>100 g	Bicolor	Medium-strong	Weak	1,2,3	3.5
<100 g	Yellow	No acid	Strong	1,2	2
>100 g	Red	Medium	Not sweet	1	2

Germplasm Bank. Using the main sources of variability found using PCA, a classification based on easily recognizable characteristics can be drawn from the results. Six hierarchical levels are suggested: 1) size of fruit; 2) color of skin; 3) acidity; 4) sweetness; 5) harvest time (PCA clustered samples in three groups of ripening: 1, from the end of July to September; 2, September; and 3, October); and 6) attractiveness. These levels give rise to 42 morphological groups (Table 7) that correspond to the groups that the UPGMA cluster analysis recognized. A total of 350 accessions could be classified. With the three isoenzymes studied (PGM, PGI, and EST), 406 accessions (the two replications numbered as 376 in the germplasm bank were different) and 21 commercial cultivars were grouped into 190 clusters. Joining information about morphological characters and isoenzymes, we have detected 31 groups of synonyms, involving 82 accessions, and eight more possible groups, involving 17 accessions. These results allow the elimination of 53 accessions from the germplasm bank.

Six commercial cultivars could be determined as the progenitors of 18 accessions: 'Reineta Blanca' of seven, 'Reina de Reinetas' of two, 'Reineta de Caux' of eight and 'Golden Delicious', 'Golden 4187' or 'Ozark Gold' of one. We can assume that the rest of the accessions are local selections from populations resulting from the hybridization of cultivars introduced from other areas as other authors have suggested (Boré and Fleckinger, 1977). This hypothesis can be supported by the similar allelic frequencies found in Galician accessions, and indicates that they could come from the same background as the commercial cultivars studied by other authors.

This characterization of the germplasm bank will allow for the immediate propagation of clones with desirable commercial characteristics, and will aid in the selection of useful breeding materials. Because inter- and intracultivar variability was high and names given by the growers were unreliable, the suggested selection strategy is to select individual clones among and within cultivars to exploit both the inter- and intracultivar genetic variability.

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