Genetic diversity of *Prunus* rootstocks analyzed by RAPD markers

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Abstract

We have used RAPD markers to characterize *Prunus* rootstocks from different species, both commercial, and selected clones from the breeding program at Aula Dei Experimental Station (Zaragoza, Spain). Molecular markers were used to study the genetic variation among different species, and within species. Forty one genotypes were used in this study. They included *P. amygdalo-persica*, and *P. persica* x *P. davidiana* hybrids; *P. cerasifera*, *P. domestica*, and *P. insititia* clones, and other diverse interspecific hybrids, which were divided in three groups according to postulated taxonomic classification. Diversity patterns obtained from 80 RAPD primers were evaluated in a representative subset of genotypes. This screening helped to identify 7 RAPD primers that were selected to produce a combined classification of the whole set of rootstock clones. This analysis successfully clustered rootstocks according to the classification scheme widely used to characterize *Prunus* clones, mainly based on morphological descriptors. Further than that, it supported the alleged origin of some interspecific materials, and confirmed a case of possible misclassification ('Myrobalan 29 C'). A more thorough diversity analysis was conducted within each group of materials, using larger sets of primers (12-14). After this analysis, disjointed clusters were formed for *P. amygdalo-persica* and *P. persica* x *P. davidiana* hybrids in one group, and for Myrobalan (*P. cerasifera*) and Marianna (*P. cerasifera* x *P. munsoniana*) plums in another group. *P. insititia* and *P. domestica* clones, however, formed a jumbled cluster, possibly due to genetic interchange among them during their domestication and breeding history.

Introduction

The genus *Prunus* includes a large array of species, originated mainly in the Northern hemisphere and widely represented in Europe. Botanical classification of species within this genus is sometimes controversial, partly because of the easiness of interspecific hybridization (Dosba et al., 1994), which creates numerous intermediate types, and fades the limits between species.

The *Prunus* breeding program at Aula Dei Experimental Station is mainly directed to the obtention of new stone fruit rootstocks, with specific adaptation to Mediterranean environments. Recently, several clonal rootstocks for stone fruit species have been released (Cambra, 1990; Moreno & Cambra, 1994; Moreno et al., 1995a-c), and some of them are already under extensive commercial exploitation. In general, they are resistant to root asphyxia and lime-induced chlorosis, which occur in compact or highly calcareous soils, respectively.

Traditionally, cultivar identification has relied on morphological and agronomic characteristics of plant materials. Although there is substantial intraspecific variation in vegetative traits, especially leaf and fruit characters, it is difficult to distinguish genotypes on their external morphology alone. In the case of rootstocks, it is very difficult to observe their morphological traits after grafting. Further, these phenotypic characters are generally influenced by environmental factors and the growth stage of the plant. In fruit trees, this requires a lengthy and expensive evaluation during the whole vegetative growth. There is an additional problem to distinguish among cultivars which come from the same cross. In such cases, the relatively

narrow range of variation of morphological traits limits cultivar identification, and different methods must be used.

The development of randomly amplified polymorphic DNA (RAPD) markers, generated by the polymerase chain reaction (PCR) using arbitrary primers, has provided a new tool for the detection of DNA polymorphisms (Welsh & McClelland, 1990; Williams et al., 1990). RAPD analysis has been used to study genetic relationships in a number of fruit trees, including almond (Bartolozzi et al., 1998), apple (Koller et al., 1993), mango (Schnell et al., 1995), olive (Fabbri et al., 1995), papaya (Stiles et al., 1993), peach (Warburton & Bliss, 1996) and plum varieties (Ortiz et al., 1997), as well as apple (Landry et al., 1994), grapevine (This et al., 1997) and peach (Lu et al., 1996) rootstocks. In most cases, data on genetic similarity obtained by RAPD analysis matched classifications based on morphological and agronomic traits.

In this study, we used RAPD markers to characterize *Prunus* rootstocks from different species, for both commercial and selected clones from the breeding program at Aula Dei Experimental Station. Molecular markers were used to study the genetic variation among different species and to determine the genetic similarities among accessions within species.

Materials and Methods

Plant material

The forty-one genotypes used in this study were obtained from the stone fruit rootstock collection maintained at Aula Dei. For practical purposes, the clones were divided into three

groups, shown in Table 1. This classification was based on previous knowledge of taxonomic and morphologic similarity among the clones, and on the authors' expertise. The groups so defined were: 1) peach-based rootstocks, including *P. amygdalo-persica* (West) Redh., and *P. persica* (L.) Batsch. x *P. davidiana* (Carr.) Franch hybrids; 2) Myrobalan and Marianna plums (from here on, Myrobalan-Marianna group), which included six *P. cerasifera* Ehrh. rootstocks, and four one-way interspecific hybrids having *P. cerasifera* as a parent; and 3) slow growing plums (after the denomination proposed by Bernhard & Renaud, 1990), which included ten *P. insititia* L., four *P. domestica* L. rootstocks and one interspecific *P. domestica* L. x *P. spinosa* L. hybrid. With the criteria stated above, it was not possible to ascribe 'Ishtara' and 'Fereley-Jaspi' to any of the three groups. For the RAPD analysis, they were included in the second group since it was the smallest one.

DNA isolation

Young leaves were collected, frozen in liquid nitrogen and subsequently stored at -20ºC until processed. Genomic DNA was isolated from leaf samples using a CTAB (cetyltrimethylethyl amonium bromide) extraction method (Cheng et al., 1997). The extraction buffer contained 2% CTAB, 1.5 M NaCl, 20 mM EDTA, 100 mM Tris pH 8.0, 2% soluble polyvinylpyrrolidone (PVP-40T, Sigma), and 2% 2-mercaptoethanol. It was critical to include 2% PVP in the extraction buffer, to suppress oxidation of phenolic compounds in the initial step, especially for *P. insititia* samples. After ethanol precipitation, the pellet was washed for 1 h with 0.3 M sodium acetate and 0.01 M magnesium acetate, in the presence of 75% ethanol, to remove residual CTAB, salt and other contaminants. The pellet was air dried and dissolved in TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). DNA quality was examined by electrophoresis in 0.8% agarose,

and DNA concentration was quantified on a GeneQuant (Amersham Pharmacia Biotech). For RAPD analysis, aliquots of 10 ng/µl DNA in water were prepared and stored at -20ºC. DNA extraction was carried out in the spring of 1997 for primer screening, and new samples were collected and extracted a year later, 1998, for the study of genetic diversity among rootstock clones.

Primer screening

Eighty 10-mer primers, corresponding to kits F from Operon Technologies (Alameda, Calif.), and kits 7, 9 and 10 from Advanced Biotechnologies Ltd. (Surrey, UK), were initially screened using 6 rootstock clones to determine the suitability of each primer for the study. The rootstocks used were: two *P. amygdalo-persica*, 'Adafuel' and 'Adarcias'; two Myrobalan plums, 'Ademir' and 'Adara'; and two slow growing plums, 'Adesoto 101' and 'PM 105 AD'. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products between and within clones of the rootstock groups defined in the 'Plant Material' paragraph. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded.

DNA amplification

For the RAPD reactions, 20 ng of DNA were used as template in a final volume of 25 µl containing 1x reaction buffer (20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl pH 8.8, 0.01% Tween 20), 1.9 mM $MgCl_2$, 200 µM of each dNTP (Amersham Pharmacia Biotech), 0.4 µM primer and 0.6 U Taq DNA polymerase (Advanced Biotechnologies Ltd., Surrey, UK), overlaid with a drop of

mineral oil (Sigma). An extra reaction containing all the components, plus water instead of template DNA, was included in all the experiments. The DNA amplifications were performed in a PTC-100 programmable thermal cycler (MJ Research, USA) as follows: 1 cycle of 5 min at 93ºC, 45 cycles of 1 min at 93ºC, 1 min at 36ºC, and 2 min at 72ºC (for denaturing, annealing and primer extension, respectively). The last cycle was followed by a final incubation for 10 min at 72ºC, and the PCR products were stored at 4ºC before analysis. The DNA amplification products were analyzed by electrophoresis in 2% agarose gels (NuSieve GTG, FMC, USA or MS-12, Pronadisa, Spain) in 1x TAE buffer for 5 h at 3.5 V/cm. The gels were stained for 20 min with ethidium bromide (1 µg/ml) and destained in water for 15 min. DNA was visualized on a UV transiluminator and photographed using Polaroid type 665 film. Fragment length was estimated by comparison with standard size markers (100 base pair ladder, Amersham Pharmacia Biotech).

Data analysis

Data were recorded as presence (1) or absence (0) of amplified products from the examination of photographic negatives. Each amplification fragment was named by the source of the primer (OP Operon, AB Advanced Biotechnologies), the kit letter or number, the primer number and its approximate size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. Data were analyzed using NTSYS-pc, version 1.80 (Rohlf, 1995). Genetic similarities between pairs of rootstocks were estimated using the Dice coefficient of similarity, also known as the coefficient of similarity of Nei & Li (1979). Similarity matrices were compared using the Mantel matrix-correspondence test (Mantel, 1967). Cluster analyses were carried out on similarity estimates using the unweighted pair-group method, arithmetic

average (UPGMA). The resulting clusters were represented as dendrograms. Cophenetic coefficients (correlation of similarities deduced from the dendrogram with the original similarity matrix) were computed for each dendrogram, after the construction of a cophenetic matrix. The cophenetic matrices were compared using the Mantel matrix-correspondence test. A principal coordinate analysis (Gower, 1966) on the similarity matrix was also performed.

Results and discussion

The primer screening step resulted in 22 primers which detected polymorphisms within the three pairs of rootstocks used. Forty two primers showed polymorphism, but could not distinguish within the three rootstock groups; 12 primers showed monomorphic bands across groups; and 4 gave no amplification products. The reproducibility of the amplification products was tested on template DNA from two independent extractions of the six initial rootstocks, using leaf samples from different years. Also, most of the amplification reactions were duplicated for a large number of clones. Only bands that were consistently reproduced across amplifications were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. This was done to avoid scoring fragments as identical when they were actually different (Thormann et al., 1994).

Two types of comparisons were carried out to evaluate the degree of genetic diversity in the rootstock collection: (1) among groups (which represent mostly groups of species), as well as (2) within groups of common rootstocks.

Among groups

Thirteen of the 22 polymorphic primers identified in the screening step showed complex band patterns and were discarded. From the remaining ones, the seven most informative primers across groups were selected (Table 2, '41 rootstocks' column), and used to evaluate the degree of polymorphism among all rootstock clones. The selected primers generated distinctive products in the range of 170-2400 bp. Bands outside this range were not considered.

A total of 75 amplified fragments (including 5 monomorphic bands) were scored across all rootstocks for the seven selected primers, and were used to estimate relationships among the clones. A matrix of genetic similarities was computed, and used to construct a dendrogram (Figure 1). Genetic similarities between pairs of rootstocks had an average of 0.53 and varied from 0.27 (‘Cadaman’ vs ‘Torinel’) to 1.00 (‘Adesoto 101’ vs ‘Alguazas’). The principal coordinate analysis on the matrix of distances generated two clearly significant axes, which explained 32% and 16% of the total variance, respectively. They appear plotted in Figure 2, which offers a good image of distances and relationships between the main groups, and the intermediate types. The dendrograms represent best the distances among clones occurring in adjacent tips of the classification (Sneath & Sokal, 1973).

The results show a differentiation into three main clusters (Figures 1 and 2), mostly representing the previously defined groups of rootstocks, and in general agreement with the botanical classification of the genus *Prunus* (Dosba et al., 1994). The first cluster contains peach-based rootstocks, of the subgenus *Amygdalus*, and can be divided into two smaller clusters, separating *P. amygdalo-persica* and *P. persica* x *P. davidiana* hybrids. The second and

third main clusters comprise rootstocks belonging to the subgenus *Prunophora*. The first one of these includes Myrobalan and Marianna rootstocks, but clearly separating *P. cerasifera* x *P. munsoniana* hybrids in a distinct group. The last cluster includes slow growing plums of the species *P. insititia* and *P. domestica*.

Average genetic similarity within clusters was 0.728. Between clusters, average values were smaller. The most distinct group was the peach-based rootstocks, which presented average similarities of 0.396 and 0.360 with Myrobalan-Marianna plums, and slow growing plums, respectively. On the contrary, the average similarity between the two *Prunophora* groups was relatively larger (0.540). It was possible to identify unique products common to peach-based rootstocks (AB9-06-1300 or AB10-03-1650), or both Myrobalan-Marianna and slow growing plums (OPF-08-1150), but there were no unique products for each of the last two groups. This result agrees with the hypothesis of Crane & Lawrence (1952) who suggested that *P. insititia* and *P. domestica* (both hexaploids) are hybrids between *P. spinosa* (tetraploid) and *P. cerasifera* (diploid) species. Two RAPD bands were present in all slow growing plum rootstocks, and absent in the Myrobalan-Marianna (though they were present in some peach-based rootstocks).

'Fereley-Jaspi', 'Ishtara' and 'Miral 3278 AD', all of them interspecific hybrids, were the most distinct clones, based on their botanical description (Table 1), and agronomic characteristics. In the combined analysis, 'Fereley-Jaspi' (*P. salicina* x *P. spinosa*), was clustered together with the slow growing plums (but with the highest aggregation level, Figure 1), though it was included in the Myrobalan-Marianna group of clones during the RAPD evaluation. Actually, it had an intermediate position between the two groups of plums, (its average genetic similarity with the slow growing plums group was 0.687, and 0.633 with the

Myrobalan-Marianna group), which is evident from Figure 2. ‘Ishtara’ is a complex interspecific hybrid, whose male parent was a natural *P. cerasifera* x *P. persica* hybrid (Renaud et al., 1988). This rootstock shared a band (AB9-18-1500) with all (but one) hybrids of *P. persica* parentage, which was absent from all other plum rootstocks, thus confirming the possible presence of *P. persica* in its pedigree. Its position in Figure 2, near the Myrobalan-Marianna group, but in the direction of peach-based rootstocks, agrees with the double dose of *P. cerasifera* and the single dose of *P. persica* in the pedigree of ‘Ishtara’. ‘Miral 3278 AD’ is a natural hybrid of unknown parentage, whose morphology resembles Myrobalans, but has some almond-like characteristics. Thus, a *P. cerasifera* x *P. amygdalus* origin has been proposed for this clone (M.A. Moreno, unpublished). It had two bands which were absent from the Myrobalan-Marianna group, and occurred in some peach-based rootstocks, and in some slow growing plums. Therefore, the marker analysis partly supports the proposed hypothesis, i.e., that its pedigree includes other species besides *P. cerasifera*, but does not provide further insight on their identity.

Many species of *Prunophora* hybridize fairly easily among themselves, and with the subgenus *Amygdalus* (Ramming & Cociu, 1990), producing many types of interspecific hybrids. This fact, favored by the presence of auto-incompatibility systems, hinders taxonomic studies, as the borders between species are unclear. Actually, the latest trends in breeding stone fruit rootstocks are based on the production of interspecific hybrids (like ‘Ishtara’ or ‘Fereley-Jaspi’, among others), aiming to put together favorable traits which occur in different species. Also, the pedigree of most of the clones is unknown, due to lack of parental control. These facts justify the combined analysis of rootstocks belonging to different *Prunus* species by means of molecular markers, as the most appropriate method to systematically address the organization of *Prunus* germplasm.

The analysis among groups successfully clustered rootstocks according to the classification scheme widely used to characterize *Prunus* clones, based on morphological descriptors, and according to the classification presented in the Materials and Methods section. Polymorphism among the rootstocks is large, since we have been able to separate them using seven 10-mer primers. These results agree with those of Graham & McNicol (1995) who correctly separated 13 species of *Rubus* into 3 subgenera, based on RAPD data of ten primers. The cophenetic correlation coefficient was 0.950 suggesting a very good fit of the dendrogram with the similarity matrix. Similar results have been reported in studies with olive (Fabbri et al., 1995) or plum (Ortiz et al., 1997) cultivars. Other studies have addressed the estimation of genetic diversity within peach (Warburton & Bliss, 1996) or almond (Bartolozzi et al., 1998) cultivars. However, they are not directly comparable with the results from the present study, as they used a different algorithm to calculate genetic similarities, and there was just one rootstock in common.

Intragroup variation

One of the objectives of this study was to characterize the genetic diversity among clones according to a previous distribution of rootstocks in three groups (Table 1). After doing the combined analysis, a larger number of primers was used to evaluate polymorphisms within groups, i.e. 12 primers for peach-based rootstocks, 13 primers for Myrobalan-Marianna plums and 14 primers for slow growing plums (Table 2). Most of the materials evaluated in this study are clonally propagated for commercialization (all but ‘Nemaguard’ and ‘Nemared’), and thus its genetic constitution is constant. Other studies (Lu et al., 1996) analyzed some peach rootstocks,

but their results are only applicable to their mother trees, and not to commercial material derived from them (peach seedlings), which come from open pollinated seeds.

Peach-based rootstocks

A total of 88 polymorphic, and 19 monomorphic fragments were found across 14 clones. Genetic similarities ranged from 0.584 (‘Albatarrech’ vs ‘Nemared’) to 0.899 (‘Nemaguard’ vs ‘Nemared’), resulting in an average genetic similarity of 0.723. The dendrogram generated by the cluster analysis showed a similar distribution of clones to the one shown in Figure 1, separating the *P. persica* x *P. davidiana* hybrids. We identified one product (a combination of AB10-08 complementary bands of 1100 and 1150 bp) which clearly separated *P. amygdalo-persica* materials from *P. persica* x *P. davidiana* hybrids. ‘Adarcias’ was the most distinct among the *P. amygdalo-persica* materials (Figure 1). This agreed with expectations, because this clone has some differential phenotypic characteristics, like the leaf shape (closer to peach-shape), lack of vigor, and low fertility. Four primers (OPF-17, AB7-14, AB10-06, and AB10-08) completely and reliably separated all rootstocks belonging to this group.

Using the set of 7 primers from the intergroup analysis, the cophenetic correlation coefficient was 0.785 (poor fit, according to Rohlf, 1995), and this value increased to 0.872 with 12 primers. It seems that a larger number of markers was necessary to obtain a dendrogram which accurately reflected the similarity matrix for this group. In a previous study, Lu et al. (1996) classified 18 peach rootstocks, most of them *P. persica*, including ‘Nemaguard’ and ‘Nemared’. A large similarity between these two cultivars was found, as in our study. This was expected, as ‘Nemaguard’ is one of the parents of ‘Nemared’.

Myrobalan-Marianna plums

This was the most diverse group of rootstocks analyzed, including 12 clones of different species (Table 1). Thirteen primers were screened and produced 117 polymorphic and 25 monomorphic bands, with an average genetic similarity of 0.678. The most similar clones, 'Marianna GF 8-1', 'Marianna 2624', and 'Myrobalan 29C', formed a compact cluster (genetic similarities higher than 0.88); another cluster was formed by the Myrobalan plums (*P. cerasifera*). The only difference of the new dendrogram (not shown) with the dendrogram in Figure 1 was the aggregation level of 'Miral 3278 AD'. Using 13 primers, it clustered together with the other *P. cerasifera* (though it is still the most distinct of them). At a higher aggregation level, 'Ishtara' formed a group of its own. Four primers (AB9-14, AB9-20, AB10-07, and AB10-10) completely and reliably separated all rootstocks belonging to this group.

The origin of Marianna rootstocks is not known. It has been postulated (Crossa-Raynaud & Audergon, 1987) that they come from a natural hybrid between *P. cerasifera* and an American diploid species of *Prunus* that is thought to be *P. munsoniana.* Therefore, their relatively close association with Myrobalans is not unexpected. The position of 'Myrobalan 29C' in the dendrograms (together with the *P. cerasifera x P. munsoniana* hybrids) may seem as a misclassification, according to its accepted denomination (*P. cerasifera*). Nevertheless, the term Myrobalan used to designate Myrobalan hybrids, is particularly confusing, as it has been used to name natural and artificial hybrids of diverse origins (for instance, 'Myrobalan GF 3-1' is actually a *P. cerasifera* x *P. salicina* hybrid). The morphology of 'Myrobalan 29C' resembles Marianna rootstocks and, according to Grasselly (cited in Crossa-Raynaud & Audergon, 1987), is also a Marianna seedling. There were 14 RAPDs shared by the two Marianna rootstocks, which were absent in all Myrobalans, including 'Myrobalan GF 3-1' and 'Miral 3278 AD'. 'Myrobalan 29 C' had 11 of these bands in common with the Mariannas, and only lacked 3 of

them. These results support the proposal cited above, in favor of considering this clone as a Marianna rootstock.

Slow growing plums

As mentioned before, fifteen clones were studied in this group, using 14 primers that generated 89 polymorphic and 32 monomorphic bands, with an average genetic similarity of 0.704. Using the set of 75 amplified bands from the intergroup analysis, the cophenetic correlation coefficient between the dendrogram and the original similarity matrix was low (0.771). A higher number of markers gave rise to a different dendrogram (Figure 3) that reflected better the similarity matrix (cophenetic correlation coefficient of 0.820), although the distribution of the clones appeared shuffled compared to Figure 1. Four primers (OPF-20, AB7-11, AB10-10, and AB10-11) completely and reliably separated all rootstocks belonging to this group.

In this analysis *P. insititia* and *P. domestica* rootstocks did not form different clusters. This is not surprising, because both species have evolved in the same geographical area (Ramming & Cociu, 1990), and are inter-fertile (Crane & Lawrence, 1952). Natural hybridization between *P. domestica* and *P. insititia* may have been favored by the existence of pollen-style self-incompatibility genes in old plum cultivars (Bernhard et al., 1951). Therefore, their proximity may have been caused by intensive recombination among genotypes, in nature and during breeding activities. Also, this lack of differentiation between the two species may be partly caused by the fact that ‘PM 105AD’, ‘PM 137AD’, ‘PM 150AD’, ‘Montizo’ and ‘Monpol’ come from open pollinated populations of ‘Pollizo’ (local Spanish *P. insititia* plum) and, though they have been described as *P. insititia*, their male parent is not known. ‘Adesoto 101’, ‘Puebla de Soto 67’, and ‘Alguazas’ were originally identified as ’Pollizos’ when

collected. These ‘Pollizos’ were collected in the same location, and thus its proximity in the dendrograms was not surprising. ‘PM 105AD’, ‘PM 137AD’, and ‘PM 150AD’ were selected from a bulk of open-pollinated ‘Pollizo’ seeds, and based on their classification (Figure3), it seems likely that they received pollen from other slow growing plums. Ortiz et al. (1997) evaluated 31 plum cultivars, most of them *P. domestica*, and were able to differentiate all the genoytpes using only 3 RAPD primers, one of them common with ours (AB7-14). In their report, they also found diffuse clusters and no main group formation among the *P. domestica* cultivars.

Finally, it is questionable whether the sets of primers used were large enough to give a representative picture of the genetic diversity present in each group of materials. A comparison of the similarity matrices obtained using 7 or more primers resulted in Mantel test statistics of 0.866, 0.922, and 0.836 for the three groups, respectively. These values denote a good fit among pairs of matrices (Rolhf, 1995). Accordingly, the dendrograms produced for both analyses were very similar for the first two groups though, as mentioned before, the cophenetic coefficient for the 7 primer dendrogram in the peach-based group was fairly low. The dendrograms obtained for the slow growing plums, however, were somewhat different (Figs. 1 and 3). Therefore, a set of seven primers seems large enough to represent phylogenetic relationships in the Myrobalan-Marianna rootstock group. For the peach-based rootstocks, and the slow growing plums, however, it seems sensible to recommend the use of a large number of primers to classify germplasm putatively belonging to these groups.

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Table 1. List and description of rootstocks used in this study.

No.	Rootstock	Species	Origin	Reference
Peach-based rootstocks				
1	Adafuel	*Prunus amygdalo-persica*	Spain	Cambra (1990)
2	Adarcias	*P. amygdalo-persica*	Spain	Moreno & Cambra (1994)
3	GF 677	*P. amygdalo-persica*	France	Bernhard & Grasselly (1981)
4	GF 557	*P. amygdalo-persica*	France	Bernhard & Grasselly (1981)
5	Cadaman	*P. persica* x *P. davidiana*	France-Hungary	Edin & Garcin (1994)
6	Barrier	*P. persica* x *P. davidiana*	Italy	De Salvador et al. (1991)
7	Nemaguard	*P. persica* x *P. davidiana*	U.S.A.	Layne (1987)
8	Nemared	*(P. persica* x *P. davidiana)* x *P. persica*	U.S.A.	Ramming & Tanner (1983)
9	Albatarrech	*P. amygdalo-persica*	Spain	EEAD *
10	Calanda	*P. amygdalo-persica*	Spain	EEAD
11	Alcañiz	*P. amygdalo-persica*	Spain	EEAD
12	Herce	*P. amygdalo-persica*	Spain	EEAD
13	Tauste	*P. amygdalo-persica*	Spain	EEAD
14	Caspe	*P. amygdalo-persica*	Spain	EEAD
Myrobalan-Marianna plums				
15	Adara	*P. cerasifera*	Spain	Moreno et al. (1995b)
16	Ademir	*P. cerasifera*	Spain	Moreno et al. (1995c)
17	Myrobalan 713 AD	*P. cerasifera*	Spain	EEAD
18	Miral 3278 AD	*P. cerasifera* x *P. amygdalus* ?	Spain	EEAD
19	Myrobalan B	*P. cerasifera*	U.K.	Okie (1987)
20	Myrobalan 29C	*P. cerasifera*	U.S.A.	Okie (1987)
21	Myrocal	*P. cerasifera*	France	Bernhard & Renaud (1990)
22	Myrobalan GF 3-1	*P. cerasifera* x *P. salicina*	France	Bernhard & Renaud (1990)
23	Ishtara	*(P. cerasifera* x *P. salicina)* x *(P. cerasifera* x *P. persica)*	France	Renaud et al. (1988)
24	Fereley-Jaspi	*P. japonica* x *P. spinosa*	France	Bernhard & Renaud (1990)
25	Marianna GF 8-1	*P. cerasifera* x *P. munsoniana*	France	Salesses (1977)
26	Marianna 2624	*P. cerasifera* x *P. munsoniana*	U.S.A.	Okie (1987)
Slow growing plums				
27	Adesoto 101	*P. insititia*	Spain	Moreno et al. (1995a)
28	PM 105 AD	*P. insititia*	Spain	Moreno (1990)
29	PM 137 AD	*P. insititia*	Spain	EEAD
30	PM 150 AD	*P. insititia*	Spain	EEAD
31	Puebla de Soto 67	*P. insititia*	Spain	Cambra (1970)
32	Alguazas	*P. insititia*	Spain	Cambra (1970)
33	Montizo	*P. insititia*	Spain	Felipe (1989)
34	Monpol	*P. insititia*	Spain	Felipe (1989)
35	St. Julien A	*P. insititia*	France	Okie (1987)
36	GF 655/2	*P. insititia*	France	Bernhard & Grasselly (1959)
37	Constanti	*P. domestica*	Spain	Cambra et al. (1989)
38	Brompton	*P. domestica*	U.K.	Okie (1987)
39	Torinel	*P. domestica*	France	Anonymous (1992)
40	Tetra	*P. domestica*	Italy	J. Pinochet, pers. comm.
41	Damas GF 1869	*P. domestica* x *P. spinosa*	France	Salesses (1987)

* non-released clones from the Aula Dei breeding program

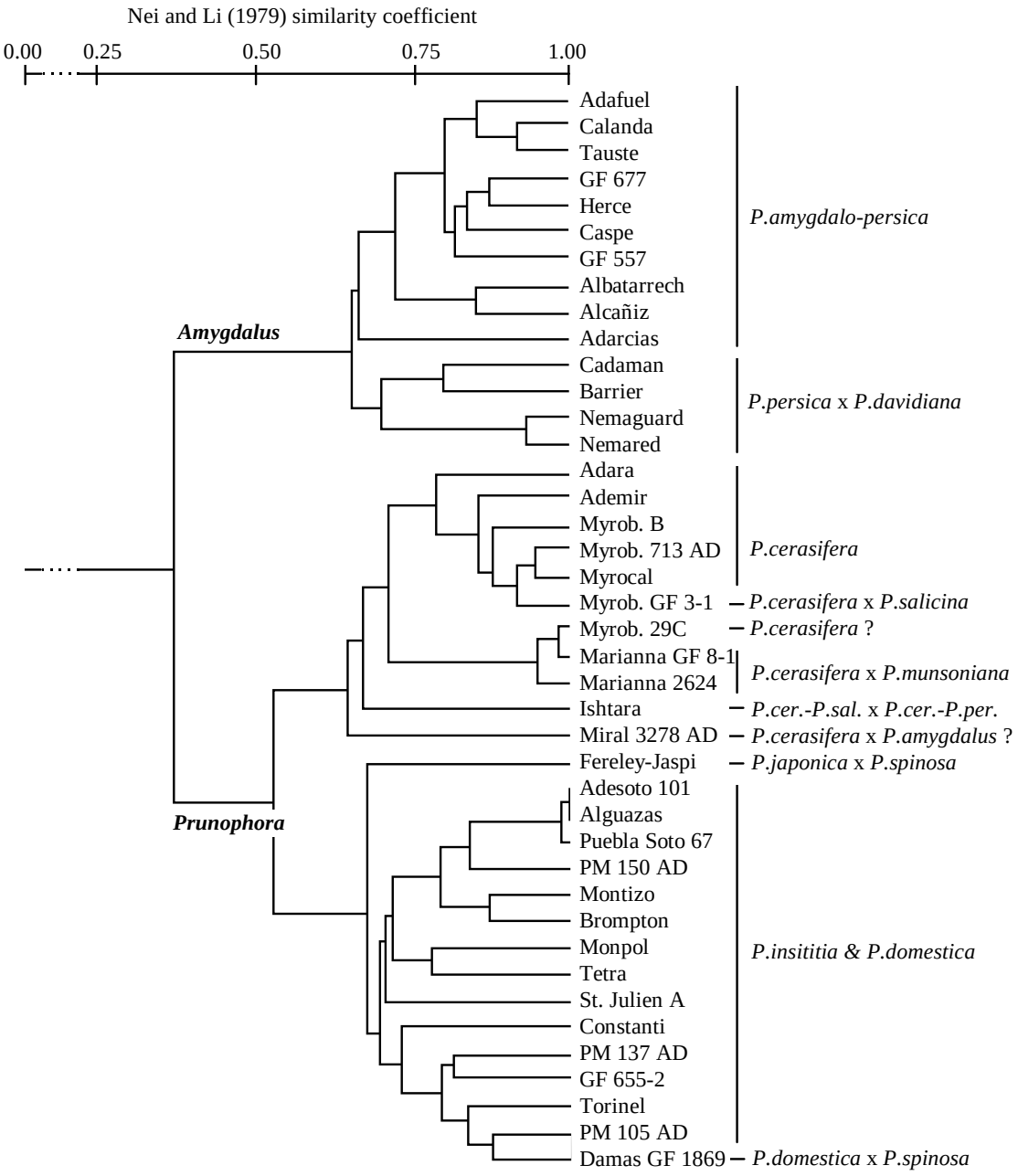
Table 2. Description of RAPD primers used, and number of bands scored at each one.

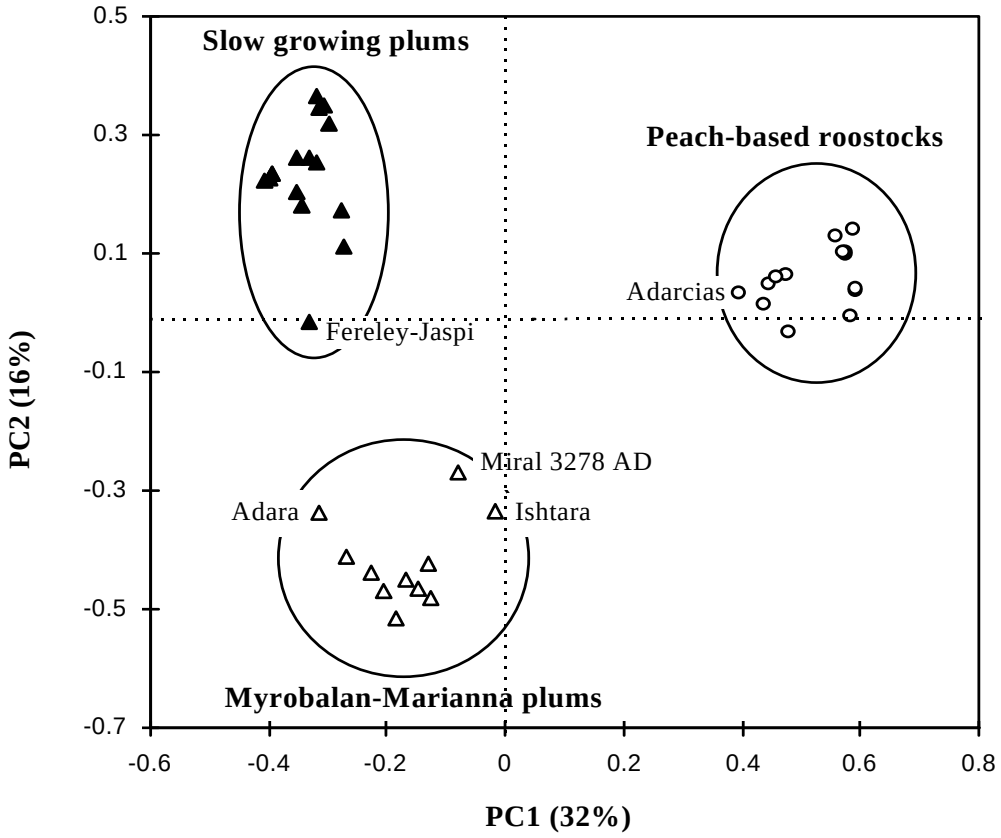
		41 rootstocks		Peach-based rootstocks		Myrobalan-Marianna plums		Slow growing plums	
Primer	Sequence	Range (bp)	Bands	Range (bp)	Bands	Range (bp)	Bands	Range (bp)	Bands
OPF-08	GGGATATCGG	450-2100	13	420-1600	11	600-1600	7	460-2100	10
AB7-14	GAACGAGGGT	260-1800	15	260-1800	13	250-1800	17	360-1800	11
AB9-06	GGGAACCCGT	300-1700	9	300-1700	10	300-1800	6	660-1800	4
AB9-18	TGTCCTGCGT	270-1500	7	260-1500	7	240-2200	13	410-1500	9
AB10-03	ACGGTTCCAC	420-2200	15	430-1650	11	320-2200	14	420-2200	12
AB10-06	GGCGCGTTAG	180-1300	7	240-1380	10	180-1900	14	180-1480	11
AB10-10	CCCGTCTACC	470-1800	9	480-1300	8	470-1900	9	480-1950	8
OPF-03	CCTGATCACC			450-1600	11				
OPF-17	AACCCGGGAA			400-1700	7				
AB10-08	GGCTGCCAGT			620-1150	5				
AB7-03	TCTCGCCTAC			250-800	5	170-1600	11		
AB9-07	TCGCTGCGGA			350-2100	9	360-1850	10		
AB9-14	AGCCGGGTAA					380-1060	7		
AB9-20	GAGTCCTCAC					300-1550	15		
AB10-07	GACGAGCAGG					580-1320	8		
AB10-17	ACTTCCGCGA					430-1800	11		
OPF-20	GGTCTAGAGG							400-1450	6
AB7-11	CAATCGGGTC							260-2000	16
AB9-01	ACTCCACGTC							430-1400	7
AB10-01	CACACCGTGT							450-1450	4
AB10-02	GTCCTCGTGT							280-2400	11
AB10-05	GTCACCTGCT							410-1140	5
AB10-11	ACCGTGCCGT							480-1650	7
Total			75		107		142		122

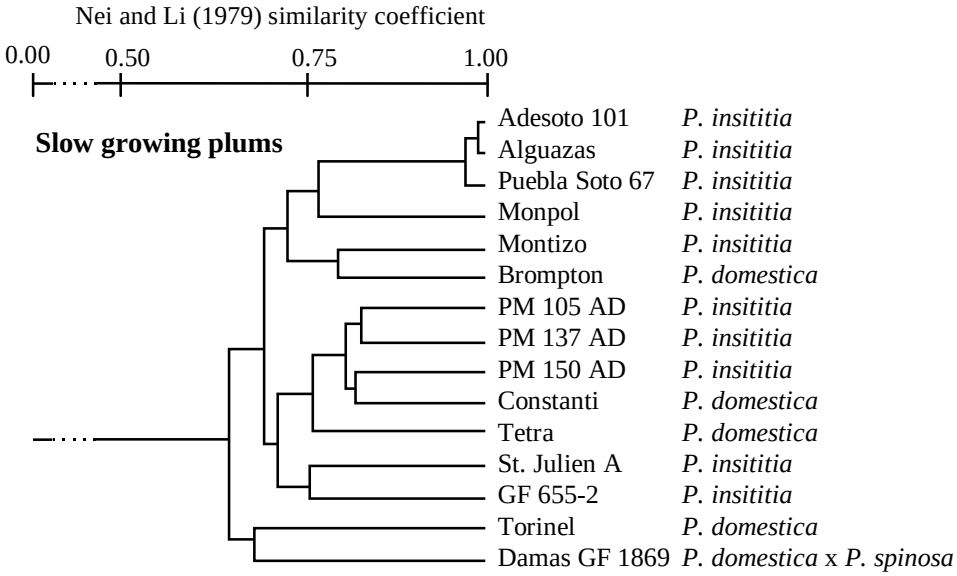
Figure 1. Dendrogram constructed from UPGMA cluster analysis of 41*Prunus* rootstock clones, based on the similarity index of Nei and Li (1979), for RAPDs produced by 7 primers.

Figure 2. Plot of the first two components (PC1 and PC2) of a principal coordinate analysis on the similarity matrix for 41 *Prunus* rootstock clones, based on RAPDs produced by 7 primers. Names of the most relevant clones appear highlighted.

Figure 3. Dendrogram constructed from UPGMA cluster analysis of 14 slow growing plums, based on the similarity index of Nei and Li (1979), for RAPDs produced by 14 primers.


Nei and Li (1979) similarity coefficient
0.00
0.25
0.50
0.75
1.00
Amygdalus
Prunophora
Adafuel
Calanda
Tauste
GF 677
Herce
Caspe
GF 557
Albatarrech
Alcañiz
Adarcias
P.amygdalo-persica
Cadaman
Barrier
Nemaguard
Nemared
P.persica x P.davidiana
Adara
Ademir
Myrob. B
Myrob. 713 AD
Myrocal
P.cerasifera
Myrob. GF 3-1
P.cerasifera x P.salicina
Myrob. 29C
P.cerasifera ?
Marianna GF 8-1
Marianna 2624
P.cerasifera x P.munsoniana
Ishtara
P.cer.-P.sal. x P.cer.-P.per.
Miral 3278 AD
P.cerasifera x P.amygdalus ?
Fereley-Jaspi
P.japonica x P.spinosa
Adesoto 101
Alguazas
Puebla Soto 67
PM 150 AD
Montizo
Brompton
Monpol
Tetra
St. Julien A
Constanti
PM 137 AD
GF 655-2
Torinel
PM 105 AD
P.insititia & P.domestica
Damas GF 1869
P.domestica x P.spinosa


Slow growing plums
Peach-based roostocks
Adarcias
Fereley-Jaspi
Miral 3278 AD
Adara
Ishtara
Myrobalan-Marianna plums
PC2 (16%)
PC1 (32%)
0.5
0.3
0.1
-0.1
-0.3
-0.5
-0.7
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8


Nei and Li (1979) similarity coefficient
0.00
0.50
0.75
1.00
Slow growing plums
Adesoto 101 P. insititia
Alguazas P. insititia
Puebla Soto 67 P. insititia
Monpol P. insititia
Montizo P. insititia
Brompton P. domestica
PM 105 AD P. insititia
PM 137 AD P. insititia
PM 150 AD P. insititia
Constanti P. domestica
Tetra P. domestica
St. Julien A P. insititia
GF 655-2 P. insititia
Torinel P. domestica
Damas GF 1869 P. domestica x P. spinosa