MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY OF *PRUNUS* ROOTSTOCKS

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ABSTRACT

Twenty microsatellite primer pairs, previously developed in peach, were used to characterize and to explore genetic relationships among 44 clones, representing three groups of rootstocks defined as: (1) Peach-based rootstocks (*Prunus dulcis x P. persica, P. persica* x *P. davidiana*); (2) Myrobalan - Marianna plums (*P. cerasifera*, and interspecific hybrids having *P. cerasifera* as a parent); and (3) Slow growing plums (*P. insititia*, *P. domestica*, and *P. domestica* x *P. spinosa*). Eighteen SSR markers, from the 20 initially used, were able to amplify polymorphic products for the Peach-based rootstocks and 13 common markers gave also polymorphism for the Myrobalan-Marianna and Slow growing plums groups. The Dice coefficient of similarity was calculated between all pairs of accessions and their genetic similarity represented by a principal coordinate analysis. The genetic diversity detected among the 44 clones studied divided them in three groups, which are in agreement with their current taxonomic classification and their morphological characteristics. A set of three microsatellites (BPPCT001, CPPCT022 and UDP98-407) can distinguish between all the clones analyzed. The analysis within groups reveal another two sets of three SSR to distinguish between the clones from the peach based rootstocks and the myrobalan-Marianna plums respectively and only a single SSR is needed to distinguish within the clones from the Slow growing plums group. These results demonstrate the high potential of the SSR analysis for peach rootstock identification and studies of diversity in *Prunus* species.

Key words: Cultivar identification; Genetic relationship; Peach-based rootstocks; Plums; SSR markers.

INTRODUCTION

Prunus is a large diverse genus of woody plants which belongs to the subfamily *Prunoideae* of the family *Rosaceae* (Rehder, 1940). Many species of this genus are economically important as sources of edible fruits (e.g., apricots, cherries, nuts (almonds), peaches, and plums), oil, timber and ornamentals (Lee and Wen, 2001). In addition, several species of *Prunus* (*P. dulcis* D.A. Webb; *P. persica* (L.) Batsch; *P. cerasifera* Ehrh; *P. domestica* L.; *P. insititia* L.; and their hybrids, etc.) are used as rootstocks.

Rootstocks are responsible for water and nutrient uptake, resistance to soil-borne pathogens, tolerance to environmental stresses, etc. (Cummins and Aldwinckle, 1983; Layne, 1987). Many of the most important agricultural attributes of the trees as a biotic unit, such as vigour, blossom initiation, nutritional status, fruit set, fruit size, and fruit flavour, may be substantially influenced by the rootstock (Dozier et al., 1984; Zarrouk et al., 2005; Jiménez et al., 2007). Therefore, a good rootstock should be compatible with a broad range of scion cultivars, should be disease free, especially virus free, and adapted to a wide range of soil types, soil reaction, soil fertility, and soil moisture (Layne, 1987). It is unlikely that any single rootstock for *Prunus* will have all of these attributes. Nevertheless, it is highly desirable to incorporate as many of these traits as possible to increase usefulness and broaden areas of adaptation of the new *Prunus* rootstocks. There are many different types of rootstocks being used for *Prunus* species on a worldwide basis (Rom, 1982; 1984). Each one has a particular set of advantages and limitations for adaptation to different geographic regions. Studies to improve *Prunus* rootstocks are underway at Aula Dei Experimental Station for obtaining new stone fruit rootstocks, with specific adaptation to Mediterranean environments (Moreno, 2004). Effective control and utilisation of *Prunus* rootstocks in breeding programs, and *Prunus* germplasm management, depends upon accurate and unambiguous characterization. Classical methods of identification and characterization of cultivars in fruit trees are based on morphological, cytological or phytochemical traits, which present some disadvantages like high

susceptibility to environmental factors and low degree of polymorphism. Rootstocks are very difficult to identify using these traditional methods. Also, once grafted, any characteristic leaf, floral or fruit traits of the rootstocks will not be visible (Serrano et al., 2002; Liu et al., 2007). The genetic background of some rootstocks in our breeding program makes more laborious the classical classification requiring more accurate tools to allow an unquestionable characterization of them. Furthermore, rootstock identification is important for peach breeders and growers. It provides evidence to protect plant variety patents for breeders, and growers can be more confident in their purchases since there is a method to identify and confirm rootstocks in their orchards (Serrano et al., 2002; Liu et al., 2007). The use of molecular markers based on DNA results in a consistent and robust method to identify plant material based on their stability in different environmental conditions or different tissues. Molecular analyses have been previously performed in *Prunus* genus using different markers as isozymes (Mowrey and Werner, 1990), RFLPs (Kaneko et al., 1986; Uematsu et al., 1991), RAPDs (Gogorcena and Parfitt, 1994; Lu et al., 1996; Casas et al., 1999), AFLPs (Aradhya et al., 2004; Fang et al., 2006), PCR-RFLPs (Badenes and Parfitt, 1995; Bouhadida et al., 2007b) and SNPs (Fang et al., 2006). These methods were widely used to characterize and classify commercial cultivars or to estimate relationship between members of the *Prunus* genus. However, there are some discrepancies in the characterization of rootstocks because their complex genetic background. For that, the use of microsatellites markers come out as a useful tool for genotyping, germplasm characterization and fingerprinting, because of their high level of polymorphism, co-dominant inheritance, abundance in the genome, transferability and high reproducibility. Several authors have revealed the potential of SSRs to differentiate between cultivars (Sosinski et al., 2000; Aranzana et al., 2002; Aranzana et al., 2003a; Romero et al., 2003) and their transferability between *Prunus* species (Downey and Iezzoni, 2000; Wang et al., 2000; Dirlewanger et al., 2002; Romero et al., 2003). Moreover, SSR markers were used successfully to fingerprint peach rootstocks (Serrano et al., 2002; Liu et al., 2007).

93 Hence, the main objectives of this work are (1) to perform a molecular characterization of
94 commercial and selected *Prunus* rootstocks from the breeding program at Aula Dei Experimental
95 Station using SSR markers and (2) to analyze the genetic diversity among the different interspecific
96 hybrids and species of *Prunus* at the germplasm collection of Aula Dei for their conservation,
97 management and utilization in future rootstock breeding programs.

MATERIALS AND METHODS

Plant material

The forty-four genotypes used in this study were obtained from the germplasm collection maintained at Aula Dei Experimental Station (Zaragoza, Spain). For practical purposes, the rootstocks and accessions were divided into three groups, as shown in Table 1. This classification was based on previous knowledge of taxonomic and morphologic similarity among plant material. The groups were defined as: 1) Peach-based rootstocks, including twelve almond x peach hybrids (*P. dulcis* D.A. Webb x *P. persica* (L.) Batsch.); three *P. persica* (L.) Batsch. x *P. davidiana* (Carr.) Franch hybrids, and one [*P. persica* (L.) Batsch. x *P. davidiana* (Carr.) Franch] x *P. persica* (L.) Batsch; 2) Myrobalan-Marianna plums which included six *P. cerasifera* Ehrh. rootstocks, and five interspecific hybrids having *P. cerasifera* as a parent; and 3) Slow growing plums (after the denomination proposed by Bernhard and Renaud (1990), which included ten *P. insititia* L., five *P. domestica* L. rootstocks and one interspecific *P. domestica* L. x *P. spinosa* L. hybrid. In this group, we have included the Spanish "Pollizo" plums, apparently *P. insititia*, and traditionally utilized in the region of Murcia (Spain) as rootstock for peaches, almonds and apricots (Kester and Graselly, 1987). With the criteria stated above, it was not possible to assign 'Fereley-Jaspi' (*P. japonica* Thunb. x *P. spinosa* L.) to any of the mentioned groups. It was included in the second group because it shared some morphological characteristics similar with this group.

Genomic DNA extraction and amplification

Genomic DNA was extracted from leaf samples according to the protocol described in Casas et al. (1999). Twenty SSR markers were studied, using primer pairs previously developed in peach (Table 2). The selection of these markers was based on the information available from studies performed by several authors (Testolin et al., 2000; Aranzana et al., 2002; Dirlewanger et al., 2002) as their high power of discrimination. Nine of the SSR were part of the proposed 24 SSR marker 'genotyping set' for *Prunus* (Aranzana et al., 2003b). The 20 markers are randomly located in the

Table 1

Table 2

123 *Prunus* genome with at least one by linkage group. PCR amplification was performed according to
124 the protocol cited by Bouhadida et al. (2007a) on a Gene Amp 2700 thermocycler (Applied
125 Biosystems) using the following temperature cycles: 1 cycle of 3 min at 95ºC, 35 cycles of 1 min at
126 94ºC, 45 s at the corresponding annealing temperature (Table 2) and 1 min at 72ºC. The DNA
127 amplification products were loaded on denaturing 5% polyacrilamide gels. Gels were run for 2h at
128 65 W and silver-stained according to the protocol described by Bassam et al. (1983). Fragment sizes
129 were estimated with the 30-330 bp AFLP ladder DNA sizing markers (Invitrogen, Carlsbad, Calif.),
130 and analysed by the Quantity One software (Bio Rad, Hercules, CA).

131 **Data analysis**

132 The following parameters were calculated: number of alleles per locus, observed heterozygosity
133 (H_o calculated as the number of heterozygous genotypes divided by the total number of genotypes)
134 and expected heterozygosity ($H_e = 1 - \Sigma p_i^{2)}$, where p_i is the frequency of i^{th} allele (Nei, 1973). The
135 power of discrimination was calculated as $PD = 1 - \Sigma g_i^2$, where g_i is the frequency of i^{th} genotype
136 (Kloosterman et al., 1993). The H_o, H_e and the PD were calculated only for the Peach-based
137 rootstocks group, which represented diploid genotypes. The ploidy level of the second group as far
138 as we know is diploid for all (although different ploidy levels are cited for *P. cerasifera* and
139 Marianna (Okie, 1987)). Marianna GF8-1 is triploid according to Salesses (cited in Okie, 1987) and
140 there are not exact descriptions for Marianna 2624. The third group was composed by hexaploid
141 *Prunus* species, but Damas GF 1869 that is pentaploid.

142 The presence (1) or absence (0) of amplified fragments was recorded for each cultivar. A
143 similarity matrix was generated using the Dice coefficient (Nei and Li, 1979). A principal coordinate
144 analysis (Gower, 1966) on the similarity matrix was performed. Also, similarity data were processed
145 through the unweighted pair group method (UPGMA) cluster analysis using the NTSYS program
146 (Rohlf, 2000) and finally depicted in one dendrogram for the Peach-based rootstocks group.

RESULTS AND DISCUSSION

Twenty SSR markers have been tested on 44 genotypes of *Prunus*, belonging to three taxonomic groups and two subgenera (*Amygdalus* and *Prunophora*). The SSR primer pairs used were previously developed for peach (Table 2). Of the 20 primer pairs investigated, 18 generated good amplifications in the first group of rootstocks (Peach-based rootstocks, section *Euamygdalus,* subgenus *Amygdalus*), while only 13 detected amplification products for the second and the third group belonging to the subgenus *Prunophora* (Table 2). Although the markers, BPPCT008 and CPPCT029, had been reported by Dirlewanger et al. (2002) and Aranzana et al. (2002) respectively as good markers to amplify different species of *Prunus*, they gave faint bands difficult to resolve or did not amplify in our genotypes. Thus, these SSRs were not included in the analysis. Our results illustrate the high transferability of the SSR used among *Prunus* species albeit they were develop for peach and the origin of the analyzed rootstocks is in many cases derived from interspecific crosses. This transportability across *Prunus* species was already confirmed by different authors (Dirlewanger et al., 2002; Wünsch and Hormaza, 2002; Decroocq et al., 2003; Zhebentyayeva et al., 2003; Decroocq et al., 2004). The level of polymorphism and the degree of amplification varied for each species.

Two types of comparisons were carried out to assess the genetic diversity for the three groups of rootstocks studied: (1) comparison among groups, and (2) comparison within groups.

Genetic diversity among groups

Thirteen SSR primer pairs gave polymorphic bands for all the 44 clones studied, and were used for the analysis of diversity among rootstock groups, while the remaining SSR primers were discarded. The 13 selected primer pairs generated distinctive products in the range of 92-306 bp in the three different taxonomic groups (Table 2). It is relevant to notice that three markers across groups (BPPCT001, CPPCT022 and UDP98-407) (Table 3) allowed the unambiguous differentiation of all the clones studied. Previously, seven RAPD markers were selected by Casas et al. (1999) to

separate among the same three rootstock groups. The results obtained were not enough accurate to differentiate all the genotypes, since they did not allow the separation between 'Alguazas' and 'Adesoto 101' clones. Two of the three selected SSR markers (BPPCT001 and UDP98-407) allow the differentiation of these two clones (Table 3). Thus, the present study reveals the power of SSR markers with respect to RAPDs, and we can consequently propose the use of the three selected SSRs (BPPCT001, CPPCT022 and UDP98-407) in future programs for identification of different *Prunus* rootstocks.

Table 3

The principal coordinate analysis (PCA) performed from the similarity matrix showed two significant axes, which explain 15% and 11% of the total variance respectively (Figure 1). The PCA clearly establishes the distribution of rootstocks in three groups, which coincide with the Peach-based, Myrobalan-Marianna and Slow growing group rootstocks defined in this experiment. The most different clones in this study were 'Ishtara', 'Miral 3278 AD'and 'Fereley-Jaspi'. The PCA analysis confirmed the possible presence of *P. persica* in the 'Ishtara' pedigree. Its position in the PCA (Figure 1), between the two groups of Peach-based rootstocks and Myrobalan-Marianna plums, but nearest to the second group, agrees with the double dose of *P. cerasifera* and the single dose of *P. persica* in the pedigree of 'Ishtara' (Table 1). 'Miral 3278 AD' was localized close to 'Ishtara' but nearest of the Myrobalan-Marianna plums group. This clone morphology resembles Myrobalans, but it has some almond-like characteristics. Thus, a *P. cerasifera* x *P. dulcis* origin was postulated for this clone (Casas et al., 1999). In the present study, this clone showed common alleles with the Peach-based rootstocks that are also present in the Myrobalan-Marianna plums (data not shown). Our results support the hypothesis that the pedigree of 'Miral 3278 AD' includes other species besides *P. cerasifera*, but does not provide further clarification on their identity. The PCA (Figure 1) also showed the position of 'Fereley-Jaspi' between the two groups of plums. According to its pedigree, 'Fereley-Jaspi' is a hybrid between plum species belonging to the *Prunophora* subgenus, which could explain its relationship with other plum species of this study.

Figure 1

Figure 2

Additionally, it was possible to detect specific alleles for each group (data not showed). The detection of specific alleles was also reported by other authors in previous works (Serrano et al., 2002) and can be used for characterization and identification of *Prunus* rootstocks or *Prunus* species. The higher number of common alleles was observed between Myrobalan-Marianna and Slow growing plums, which can be explained by the close relationship among clones of these two groups. It is widely believed that the hexaploid European plums, *P. domestica* and *P. insititia,* have arisen from a cross between a diploid (2n=2x=16) cherry plum or myrobalan, *P. cerasifera*, and a tetraploid (2n=4x=32) sloe or blackthorn, *P. spinosa* (Crane and Lawrence 1952). Nevertheless, another hypothesis based on RFLP variation in cpDNA genes suggested that European plum may have originated from polyploid forms of myrobalan plum (Reynders and Salesses, 1991). Both hypotheses agree with our findings and the high levels of genetic similarities among diploid, triploid, and hexaploid plums.

Genetic diversity within groups

Genetic diversity was detected among clones of each group of rootstocks. As mentioned before, 13 SSR markers gave polymorphic bands in all rootstocks studied. In addition, other five SSRs were polymorphic for only the Peach-based rootstocks group. We will describe first the diversity found within the two plum groups, amplified with 13 SSR markers, followed by the analysis of genetic diversity within Peach-based rootstocks, analysed with the 18 polymorphic SSRs.

Myrobalan-Marianna plum variation

The group of Myrobalan-Marianna plums included 12 clones of different species (Table 1). Most of them are diploid (*P. cerasifera*) or triploid plums (*P. cerasifera x P. munsoniana* e.g. Marianna GF 8-1). Thirteen SSR primers were used for the screening of this group and produced a [illegible] alleles, ranging from 2 (CPPCT017 and UDP98-408) to 10 (CPPCT005 and CPPCT006), with a mean value of 6.31 per locus (Table 4). All the clones of this group could be distinguished with only three of the SSR tested. The selection of the two most polymorphic loci which revealed the highest

Table 4

number of alleles for this group, CPPCT005 (10) and CPPCT006 (10), allowed us to distinguish unambiguously all the 12 clones of rootstocks group with the exception of 'Myrobalan 29C' and 'Marianna GF 8-1' which gave the same genetic profile. To identify these two cultivars, an additional CPPCT028 marker was selected which revealed distinct alleles between them. Hence, we can propose the use of three selected SSRs (CPPCT005, CPPCT006 and CPPCT028) to distinguish between the rootstocks studied in this group.

The position in the PCA (Figure 1) of the 'Myrobolan 29C' is close to the Marianna clones confirmed by their similar morphology. Day (1953) already reported that Marianna resembles 'Myrobalan 29C' group and the same author mentioned some doubt as to its relationship with the American wild plums (*P. munsoniana*). Grasselly (cited in Crossa-Raynaud and Audergon 1987), reported that 'Myrobalan 29C' was a Marianna seedling. Moreover, 'Myrobalan 29C', 'Marianna GF 8-1' and 'Marianna 2624' shared 4 alleles through 4 loci which were absent in the rest of the Myrobalan-Marianna plums studied, which support the high similarity found among these clones. Casas et al. (1999) and Serrano et al. (2002), also reported a very close relationship among 'Myrobalan 29C' and Marianna clones using RAPD and SSR markers respectively. This agrees with our findings and we could support the hypothesis that 'Myrobalan 29C' could be considered as a Marianna rootstock, although more genetic work with more SSR have to be done to try to determine its true relationships and ploidy levels.

Slow growing plums variation

This group included 15 hexaploid *Prunus* (*P. insititia* and *P. domestica*), and a pentaploid clone 'Damas GF 1869' (*P. domestica* x *P. spinosa*) (Table 1), and each primer used was able to detect from one to three loci (Table 4). Thirteen SSR primer pairs, from the 20 initially used, amplified a total of 133 polymorphic alleles through 28 loci detected in this group (Table 4) with a mean value of 4.75 alleles per locus. The primer BPPCT001 amplified a total of 19 alleles through the three loci detected in this case and allowed the unambiguous separation of all the 16 clones.

247 All the slow-growing plums were in the same axe in the PCA analysis (Figure 1). 'Adesoto 101',
248 'Puebla de Soto 67', and 'Alguazas' are very closely related in the analysis, which is explained by
249 the fact that they are 'Pollizo' collected in the same geographic area (South of Spain).

250 ***Peach-based rootstocks***

251 ***Microsatellite diversity***

252 In our set of Peach-based rootstocks, peach-derived SSR markers detected considerable
253 polymorphism. Eighteen of the 20 SSR markers used in this study produced a total of 124
254 polymorphic bands over the 16 screened clones of the Peach-based rootstocks group (Table 5). As
255 mentioned before, five microsatellites (BPPCT015, BPPCT017, BPPCT038, CPP
256 UDP98-022) were specific of this group of rootstocks (Table 2). The PD of these SSRs was very
257 high varying between 0.73 and 0.89 (Table 5)

Table 5

258 The number of alleles observed at each locus ranged from 2 (CPPCT005) to 12 (UDP98-025),
259 with an average of 6.90 alleles per locus (Table 5). This value obtained for only 16 clones is
260 relatively high compare with the values reported for other *Prunus* species considering the number of
261 accessions studied (Serrano et al., 2002; Aranzana et al., 2003a). In our study, observed and
262 expected heterozygosities averaged over the 18 SSR loci were 0.46 and 0.66, respectively (Table 5).
263 These parameters are higher than the mean values reported for SSRs in peach (Aranzana et al.,
264 2003a; Bouhadida et al., 2007a). High allele number and high heterozygosity obtained in the present
265 study reflect the ability of SSR markers to provide unique genetic profile for individual plant
266 genotypes.

267 A total of 136 genotypes were observed for all loci, with an average of 7.56 genotypes per locus.
268 The selection of the two most polymorphic loci, BPPCT017 and UDP98-025, allowed to distinguish
269 unambiguously all the 16 clones of Peach-based rootstocks group, with the exception of
270 'Albatarrech' and 'Alcañiz' which gave the same genetic profile. To differentiate these two
271 cultivars, an additional marker (BPPCT007) was selected, which revealed distinct alleles between

272 them. A set of three SSR markers (BPPCT007, BPPCT017 and UDP98-025) can be proposed to
273 distinguish between the peach rootstocks studied.

274 The PD of the SSRs tested was in all the case superior to 0.50 except for CPPCT005 with the
275 lower number of polymorphic loci and PD= 0.12. The average power of discrimination (PD=0.75)
276 observed for Peach-based rootstocks is high and is comparable with the 0.64 mean value reported for
277 peach by Aranzana et al. (2003a). Our findings indicate that peach SSR markers are very efficient to
278 identify genetic variability among these Peach based-rootstocks.

279 To elucidate genetic relationships among Peach-based rootstock clones, a dendrogram was
280 produced using UPGMA cluster analysis and the Dice coefficient over 18 SSR loci (Figure 2). The
281 genotypes studied can be divided into two main groups. The first group contained the four genotypes
282 presenting the pedigree *P. persica* x *P. davidiana*, whereas the second group included the peach
283 hybrids of the type *P. persica* x *P. dulcis*. All non-released rootstocks of EEAD collection (but
284 'Herce 5') were clustered together in a subgroup (Figure 2). This separation of all the Peach-based
285 rootstocks was in good agreement with their botanical classification. This suggests that a larger
286 number of markers probably lead to a more accurate genetic relationship among clones which
287 respond truly to the morphological characteristics and the botanical descriptions. The cophenetic
288 correlation coefficient was 0.80 suggesting a good fit of the dendrogram of the Figure 2 with the
289 similarity matrix.

Figure 2

290 'Albatarrech' and 'Alcañiz' showed a very close relationship, as Casas et al. (1999) reported
291 using RAPD markers, due to their origin from the same region and the morphological characteristics.
292 A high genetic similarity was also detected between 'Nemared' and 'Nemaguard' with a similarity
293 value of 0.71. This was expected, as 'Nemaguard' is one of the parents of 'Nemared' (Ramming and
294 Tanner, 1983). A close relationship was also mentioned by Lu et al. (1996) and Casas et al. (1999)
295 between these two cultivars. 'Nemaguard' originated from a commercial seedlot labelled *P.*
296 *davidiana*, but Okie (1998) refers that probably is a pure peach. However, it has a close relationship

297 with Barrier and Cadaman, other rootstocks reported as having *P. davidiana* in their genetic
298 background. In future works, the search of any specific alleles shared by this group of rootstocks and
299 not present in pure peaches and almonds, could help to elucidate the genetic identity of these group
300 of rootstocks.

301 In summary, the analysis of genetic diversity among groups of *Prunus* rootstocks using peach-
302 derived SSR markers, allowed us to cluster successfully the clones according to their morphological
303 characteristics, and their botanical classification.

304 Molecular characterization of *Prunus* rootstocks is of great interest for breeding propagation
305 process, avoiding environmental factors that may limit or influence phenotypic characterization.
306 Also, once grafted, any phenotipic traits of the rootstock will not be visible. Therefore, DNA
307 fingerprinting could provide evidence to demonstrate the genetic identity of the rootstocks. This
308 approach is very useful to choose parental genotypes for crosses, and to optimize germplasm
309 conservation and management of diversity. In the present study, polymorphism observed among the
310 rootstocks is large, since we have been able to distinguish the 44 clones analyzed unambiguously
311 using only a set of three SSR markers (BPPCT001, CPPCT022 and UDP98-407). The analysis of the
312 genetic diversity within groups allows us to define another set of three markers to differentiate
313 between all Myrobalan-Marianna plums clones (CPPCT005, CPPCT006 and CPPCT028), **t**hree SSR
314 markers (BPPCT007, BPPCT017 and UDP98-025) can be proposed to distinguish between the
315 clones included in the peach-based rootstocks group and only a single marker BPPCT001 was
316 needed to distinguish the clones from the Slow growing plums group. The combination of few
317 selected markers can distinguish each of the 44 rootstocks analyzed indicating the robustness of SSR
318 markers to be use in the rootstocks characterization.

319

320 **AKNOWLEDGEMENT**

This research was funded by CICYT (Comisión Interministerial de Ciencia y Tecnología AGL 2005-05533), INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, RF03-014-C2 and RF2007-00026-C02-01), DGA (Diputación General de Aragón, A44). M. Bouhadida was supported by a fellowship from the AECI (Agencia Española de Cooperación Internacional) of the Spanish Ministry of Foreign Affairs and an I3P-PC2006 contract from the CSIC-FSE for M.J. Gonzalo.

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476 **Table 1**. Characteristics of rootstocks used in the study.

No.	Rootstock	Species	Origin	References
Peach-based rootstocks (Subgenus *Amygdalus*)				
1	Adafuel	*Prunus dulcis x P. persica*	Spain	Cambra (1990)
2	Adarcias	*P. dulcis x P. persica*	Spain	Moreno and Cambra (1994)
3	Albatarrech	*P. dulcis x P. persica*	Spain	EEAD*
4	Alcañiz	*P. dulcis x P. persica*	Spain	EEAD*
5	Calanda	*P. dulcis x P. persica*	Spain	EEAD*
6	Caspe	*P. dulcis x P. persica*	Spain	EEAD*
7	GF 557	*P. dulcis x P. persica*	France	Bernhard and Grasselly (1981)
8	GF 677	*P. dulcis x P. persica*	France	Bernhard and Grasselly (1981)
9	Herce 5	*P. dulcis x P. persica*	Spain	EEAD*
10	Logroño	*P. dulcis x P. persica*	Spain	EEAD*
11	Tamarite	*P. dulcis x P. persica*	Spain	EEAD*
12	Tauste	*P. dulcis x P. persica*	Spain	EEAD*
13	Barrier	*P. persica* x *P. davidiana*	Italy	De Salvador et al. (1991)
14	Cadaman	*P. persica* x *P. davidiana*	France-Hungary	Edin and Garcin (1994)
15	Nemaguard	*P. persica* x *P. davidiana*	U.S.A.	Layne (1987)
16	Nemared	*(P. persica* x *P. davidiana) x P. persica*	U.S.A.	Ramming and Tanner (1983)
Myrobalan-Marianna plums (Subgenus *Prunophora*)				
17	Adara	*P. cerasifera*	Spain	Moreno et al. (1995a)
18	Ademir	*P. cerasifera*	Spain	Moreno et al. (1995b)
19	Myrobalan 713 AD	*P. cerasifera*	Spain	EEAD*
20	Myrobalan B	*P. cerasifera*	U.K.	Okie (1987)
21	Myrobalan 29C	*P. cerasifera*	U.S.A.	Okie (1987)
22	Myrocal	*P. cerasifera*	France	Bernhard and Renaud (1990)
23	Myrobalan GF 3-1	*P. cerasifera* x *P. salicina*	France	Bernhard and Renaud (1990)
24	Marianna GF 8-1	*P. cerasifera* x *P. munsoniana*	France	Salesses (1977)
25	Marianna 2624	*P. cerasifera* x *P. munsoniana*	U.S.A.	Okie (1987)
26	Miral 3278 AD	*P. cerasifera* x *P. dulcis* ?	Spain	EEAD*
27	Ishtara	*(P. cerasifera* x *P. salicina) x (P. cerasifera x P. persica)*	France	Renaud et al. (1988)
28	Fereley-Jaspi	*P. japonica* x *P. spinosa*	France	Bernhard and Renaud (1990)
Slow growing plums (Subgenus *Prunophora*)				
29	Adesoto 101	*P. insititia*	Spain	Moreno et al. (1995c)
30	Alguazas	*P. insititia*	Spain	Cambra (1970)
31	St Julien GF 655/2	*P. insititia*	France	Bernhard and Grasselly (1981)
32	Montizo	*P. insititia*	Spain	Felipe (1989)
33	Monpol	*P. insititia*	Spain	Felipe (1989)
34	PM 105 AD	*P. insititia*	Spain	Moreno (1990)
35	PM 137 AD	*P. insititia*	Spain	Moreno (1990)
36	PM 150 AD	*P. insititia*	Spain	Moreno (1990)
37	Puebla de Soto 67	*P. insititia*	Spain	Cambra (1970)
38	St. Julien A	*P. insititia*	France	Okie (1987)
39	Brompton	*P. domestica*	U.K.	Okie (1987)
40	Constantí	*P. domestica*	Spain	Cambra et al. (1989)
41	Penta	*P. domestica*	Italy	Nicotra and Moser (1997)
42	Tetra	*P. domestica*	Italy	Nicotra and Moser (1997)
43	Torinel	*P. domestica*	France	Anonymous (1992)
44	Damas GF 1869	*P. domestica* x *P. spinosa*	France	Salesses et al. (1988)

477 * Non-released clones from the Aula Dei breeding program

478 **Table 2**. List of the 20 SSR primers used in this study, size range in base pairs (bp), annealing
479 temperature, and level of amplification for all the groups studied.

480

Locus code	References	Size range (bp)	Ta (ºC)	Peach-based rootstocks	Myrobalan-Marianna plums	Slow growing plums
BPPCT001	Dirlewanger et al. 2002	124-195	60ºC	++	++	++
BPPCT007*	Dirlewanger et al. 2002	123-167	58ºC	++	++	++
BPPCT008*	Dirlewanger et al. 2002	-	59ºC	dr	-	-
BPPCT015*	Dirlewanger et al. 2002	164-258	62ºC	++	-	-
BPPCT017*	Dirlewanger et al. 2002	139-181	60ºC	++	-	-
BPPCT038	Dirlewanger et al. 2002	103-139	62ºC	++	-	-
CPPCT002*	Aranzana et al. 2002	92-108	58ºC	++	++	++
CPPCT004	Aranzana et al. 2002	254-266	56ºC	++	-	-
CPPCT005	Aranzana et al. 2002	122-158	58ºC	++	++	++
CPPCT006	Aranzana et al. 2002	166-220	60ºC	++	++	++
CPPCT017*	Aranzana et al. 2002	180-202	60ºC	++	++	++
CPPCT022*	Aranzana et al. 2002	214-306	58ºC	++	++	++
CPPCT028	Aranzana et al. 2002	120-148	58ºC	++	++	++
CPPCT029	Aranzana et al. 2002	-	58ºC	dr	-	-
CPPCT030	Aranzana et al. 2002	160-190	56ºC	++	++	++
CPPCT033*	Aranzana et al. 2002	135-167	58ºC	++	++	++
UDP98-022	Testolin et al. 2000	113-139	64ºC	++	-	-
UDP98-025*	Testolin et al. 2000	101-159	65ºC	++	++	++
UDP98-407	Testolin et al. 2000	166-240	60ºC	++	++	++
UDP98-408	Testolin et al. 2000	100-106	56ºC	++	++	++

481 Note: Ta, annealing temperature; ++ good amplification; - no amplification; dr: Bands difficult to
482 resolve; * SSR markers from the 'genotyping set' proposed by Aranzana et al. (2003b) for *Prunus*
483 genome

Table 3. Allele sizes of the SSR markers (BPPCT001, CPPCT022 and UDP98-407) selected by their potential to distinguish unambiguously the 44 rootstocks analyzed. The specific alleles for each clone are in bold.

		Allele size																
No.	**Rootstock**	**BPPCT001**						**CPPCT22**						**UDP98-407**				
Peach-based rootstocks																		
1	Adafuel	160						224	290					180	228			
2	Adarcias	160						224	290					202				
3	Albatarrech	143	163					288						202				
4	Alcañiz	143	158					224	288					180	200			
5	Calanda	136	163					**214**	290					202	228			
6	Caspe	136	160											190	202			
7	GF 557	160	166					290						180	228			
8	GF 677	160						290						202				
9	Herce 5	160						224	290					180	228			
10	Logroño	160						224	288					190	202			
11	Tamarite	160						224	290					180				
12	Tauste	160						224	**292**					202				
13	Barrier	160	166					**248**	288					190	204			
14	Cadaman	160						234	290					182	204			
15	Nemaguard	**124**	156					**228**	290					180	200			
16	Nemared	156	160					234	290					180	190			
Myrobalan-Marianna plums																		
17	Adara	134	144					250						194	**218**	**240**		
18	Ademir	134						250						210	238			
19	Myrobalan 713 AD	156						250						210	224			
20	Myrobalan B	140						224	250					228				
21	Myrobalan 29C	140						250						206	226			
22	Myrocal	134	158					250	262					226				
23	Myrobalan GF 3-1	134	142					250	260					228				
24	Marianna GF 8-1	134	140					250						228				
25	Marianna 2624	156						250	290					228				
26	Miral 3278 AD	142						254	**274**					180	206	238		
27	Ishtara	134	158					250	260					226				
28	Fereley-Jaspi	140	158					250	260					224				
Slow growing plums																		
29	Adesoto 101	142	144	**177**				240	258	278				166	184	202		
30	Alguazas	138	142	179	191			250	258	278				166	200			
31	St Julien GF 655/2	142	152	179										166	182	**192**		
32	Montizo	142	152	185	191			240	260	270				182	194			
33	Monpol	142	185					240	260	278				166	186			
34	PM 105 AD	142	179					240	260	278				166	182	200		
35	PM 137 AD	138	142	179	187									182	204			
36	PM 150 AD	138	142	152				224	244	250	270			**198**				
37	Puebla de Soto 67	126	140	144	**173**			244	260	270				168	178	186		
38	St. Julien A	126	138	140	142	164		224	252	**268**	**280**	**306**		168	178	184		
39	Brompton	138	142	152	187	**195**								166	180	200		
40	Constantí	142	144	171				244	250	262	**276**			170	182			
41	Penta	126	138	142	152	164		252	270	278				**176**				
42	Tetra	140	142	**154**	171			244	270	278				166	186			
43	Torinel	142	144	**146**	156	179	**193**	254	262	270				170	200			
44	Damas GF 1869	138	140	142	144	171		244	262	270	278			180	200			

Table 4. Number of loci and alleles observed for each rootstock group with the 13 SSR polymorphic among all the clones studied. These SSRs were used for the analysis of the diversity among groups.

489

Locus code	Peach-based rootstocks		Myrobalan-Marianna plums		Slow growing plums	
	Loci number	**Alleles number**	**Loci number**	**Alleles number**	**Loci number**	**Alleles number**
BPPCT001	1	8	1	6	3	19
BPPCT007	1	8	1	7	3	12
CPPCT002	1	5	1	4	3	8
CPPCT005	1	2	1	10	2	16
CPPCT006	1	10	1	10	2	11
CPPCT017	1	5	1	2	1	1
CPPCT022	1	7	1	7	3	15
CPPCT028	1	3	1	7	2	10
CPPCT030	1	4	1	4	2	6
CPPCT033	1	9	1	8	2	8
UDP98-025	1	12	1	6	2	10
UDP98-407	1	7	1	9	2	15
UDP98-408	1	4	1	2	1	2
Total	**13**	**84**	**13**	**82**	**28**	**133**
Mean		**6.46**		**6.31**		**4.75**

490 **Table 5**. Allele number and parameters of variability in the Peach-based rootstocks group with the

491 18 polymorphic SSRs.

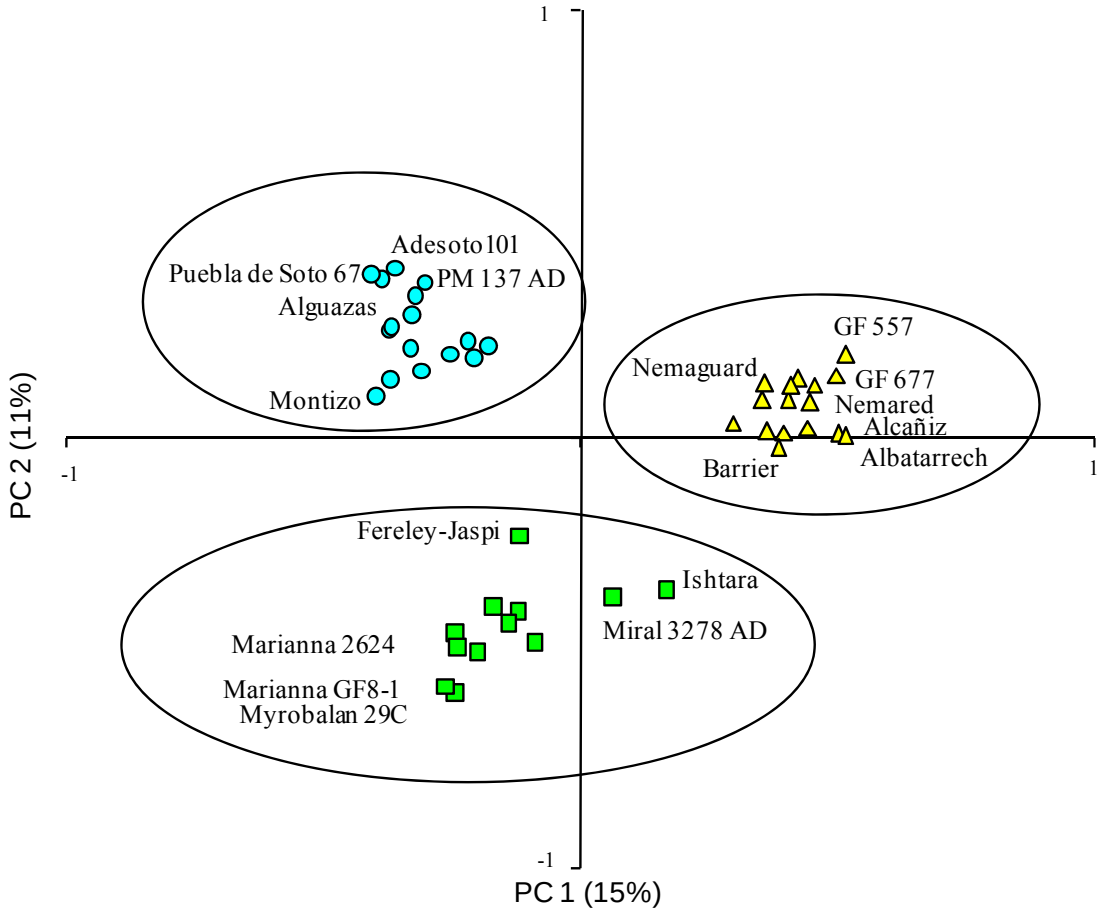
Locus code	Alleles number	Ho	He	#Genotypes	PD
BPPCT001	8	0.50	0.55	8	0.71
BPPCT007	8	0.44	0.77	10	0.84
BPPCT015	11	0.00	0.87	10	0.87
BPPCT017	11	0.81	0.82	11	0.89
BPPCT038	8	0.69	0.64	9	0.84
CPPCT002	5	0.50	0.61	8	0.84
CPPCT004	5	0.00	0.73	4	0.73
CPPCT005	2	0.06	0.06	2	0.12
CPPCT006	10	0.88	0.76	7	0.88
CPPCT017	5	0.63	0.67	6	0.63
CPPCT022	7	0.69	0.76	9	0.87
CPPCT028	3	0.00	0.53	3	0.53
CPPCT030	4	0.38	0.32	4	0.55
CPPCT033	9	0.69	0.78	10	0.86
UDP98-022	5	0.19	0.72	7	0.79
UDP98-025	12	0.62	0.79	12	0.92
UDP98-407	7	0.69	0.77	10	0.87
UDP98-408	4	0.50	0.67	6	0.82
Total	**124**			**136**	
Mean	**6.90**	**0.46**	**0.66**	**7.56**	**0.75**

492 Note: Ho, observed heterozygosity; He, expected heterozygosity; # Genotypes, different genotypes

493 per locus; and PD, power of discrimination.

494 **Figure legends**

495 **Figure 1.** Plot of the two first components (PC1 and PC2) of principal coordinate analysis on the
496 similarity matrix for 44 *Prunus* rootstocks after amplification with 13 SSR primer pairs. Names of
497 some relevant clones are shown in the figure.



498
499

Figure 2. Dendrogram of the 16 peach-based rootstocks obtained from the UPGMA cluster analysis, using the Dice coefficient (Nei and Li, 1979) after amplification with 18 SSR primer pairs.