

1 **Characterization of accessions of ‘Reine Claude Verte’ plum using**

2 ***Prunus* SRR and phenotypic traits**

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16 **ABSTRACT**

17 European plum (*Prunus domestica* L.) cv 'Reine Claude Verte' is highly
18 appreciated for its excellent organoleptic qualities. Despite its increasing demand, this
19 cultivar is in the process of being eradicated in many commercial orchards because of
20 its generally erratic fruit setting. This situation led us to explore the behaviour of
21 particular accessions with the aim of evaluating if some of them can crop more
22 regularly. For this purpose, we initially evaluated the putative diversity of 24 European
23 plum accessions of 'Reine Claude Verte' collected in different areas of Spain. For the
24 molecular characterization, 16 *Prunus* SSRs were used. Eight of them were selected
25 from previous works of SSRs transferability within the genus and the other eight were
26 selected from a transferability screening of another 75 *Prunus* SSRs, in which 65 SSRs
27 were conserved in the species. Morphological characterization was performed by
28 evaluating 33 qualitative characters of leaves and fruits. Results allowed differentiating
29 the accessions by their genotypic profile and their phenotype. The accessions were
30 grouped in six genotypic profiles according their genetic similarity and in seven clusters
31 according their fruit and leaf traits. High similarity was found between the 'Reine
32 Claude Verte' commercial cultivars. Some of the prospected accessions sharing the
33 same genotypic profile showed some morphological differences, whereas some
34 accessions with different genotypic profiles presented fruit traits similar to 'Reine
35 Claude Verte'. The combined use of molecular and morphological characterization
36 allowed identifying different clones of the cultivar that were selected for further
37 agronomic evaluation.

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39 **Keywords:** fruit; leaf; microsatellite; *Prunus domestica*; SSR; transferability.

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41 **1. Introduction**

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43 European plum (*Prunus domestica* L., $2n = 6x = 48$) belongs to the *Prunus* genus
44 in the Rosaceae as other fruit tree species such as almond [*Prunus dulcis* (Miller) D. A.
45 Webb], apricot (*Prunus armeniaca* L.), sweet (*Prunus avium* L.) and sour cherry
46 (*Prunus cerasus* L.), or peach [*Prunus persica* (L.) Batsch.], and it is the fruit tree
47 species with the greatest number of cultivars in Europe ([Esmenjaud and Dirlewanger,](#)
48 [2007](#)). Within European plum cultivars, 'Reine Claude' comprises an important group
49 of plums, characterized by a roundish-oval fruit of small to medium size and yellowish-
50 green skin ([Hedrick, 1911](#)). 'Reine Claude' plums are used for both fresh-consumption
51 and canning because their high amount of sugar ([Rieger, 2006](#)). In this group of plums,
52 'Reine Claude Verte' is the most popular cultivar. It was introduced in Europe probably
53 from Armenia through Greece and Italy, where it was named 'Verdacchio rotondo',
54 'Mammola' or 'Susina Regina' ([Hedrick, 1911](#)). In the early sixteenth century, the
55 French botanist Pierre Belon introduced it in France ([Lespinasse and Leterme, 2005](#)),
56 where it was named 'Reine Claude' for the Duchess of Brittany Claude (1499-1524),
57 queen consort of King François I of France (1494-1547). In 1724, it was introduced in
58 England by the Gage family, from whom the cultivar got its English name 'Green
59 Gage'. During the 18th century, it was also cultivated in the American colonies
60 ([Hedrick, 1911](#)).

61 Nowadays, 'Reine Claude Verte' is grown worldwide, in particular in
62 Western Europe, where more than 70 synonymies have been detected ([Tabuenca and](#)
63 [Iturrioz, 1991b](#)). More than 500 years of cultivation in Europe suggest that different
64 clones may be growing under the same cultivar name as result of mutations or local
65 selections of seedlings. Furthermore, new commercial cultivars have been selected from

66 seedlings of 'Reine Claude Verte', like 'Reine Claude of Oullins' ([Hedrick, 1911](#)) and
67 'Reine Claude of Bavay' ([Tabuenca and Iturrioz, 1991a](#)), but some of them often are
68 erroneously cultivated as 'Reine Claude Verte' ([Tabuenca and Iturrioz, 1991b](#)). 'Reine
69 Claude Verte' is a highly appreciated plum cultivar for its excellent organoleptic
70 qualities and in many regions of Europe is the most grown European plum cultivar.
71 Despite of its increasing commercial demand, this cultivar is in the process of being
72 eradicated in many commercial orchards because of its generally erratic fruit setting.

73 Traditionally cultivar identification in European plum has been based on
74 morphological and phenological characterization ([UPOV, 2002](#)). In the last decades,
75 some studies have reported the characterization of European plum genotypes using
76 different types of molecular markers like RAPDs ([Gregor et al., 1994](#); [Heinkel et al.,
77 2000](#); [Liu et al., 2007](#); [Liu et al., 2006](#)) and AFLPs or ISSRs ([Aradhya et al., 2004](#);
78 [Goulao et al., 2001](#); [Liu et al., 2007](#)). Recently, nuclear and chloroplast microsatellite
79 markers from apricot have been used to study lineage and diversity in European plum
80 accessions ([Decroocq et al., 2004](#)). Likewise, genetic diversity and genetic structure of
81 three plum species (*Prunus domestica* L., *Prunus cerasifera* Ehrh. and *Prunus spinosa*
82 L.) have recently been studied using microsatellites and chloroplast DNA markers
83 ([Horvath et al., 2011](#)). Microsatellites (SSRs) are considered a very suitable tool for
84 molecular characterization of cultivars ([Testolin and Cipriani, 2010](#)) and its use has
85 been very broad in fruit trees. However, studies in European plum using microsatellites
86 markers are still scarce.

87 The erratic yields reported in many orchards of 'Reine Claude Verte' led us to
88 explore the behaviour of particular accessions with the aim of evaluating if some of
89 them can crop more regularly. For this purpose we initially evaluated the putative
90 diversity of 24 accessions of 'Reine Claude Verte' collected in different areas of Spain

91 (Zaragoza, Guadalajara, Navarra y Teruel) in which this cultivar has been cultivated
92 traditionally. The accessions were selected by their good agronomic behaviour or from
93 specific trees of 'Reine Claude Verte' sited in abandoned orchards in risk of
94 disappearing. The objective of this work was to characterise these local accessions of
95 'Reine Claude Verte' in order to select the most interesting accessions for further
96 agronomic evaluation. Characterisation was performed by analysing morphological
97 traits of fruit and leaf and by using microsatellite markers to estimate the presence of
98 homonyms. For the molecular characterization, the transferability of published *Prunus*
99 SSR to *Prunus domestica* was previously explored.

100

101 **2. Material and methods**

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103 2.1. Plant material

104

105 Twenty five European plum accessions were analysed, these include 24
106 accessions of ‘Reine Claude Verte’ and the cultivar ‘Stanley’, used as outgroup. The
107 accessions of ‘Reine Claude Verte’ include commercial cultivars from different
108 collections and accessions collected from different areas of Aragón (Spain) and
109 surrounding regions (Table 1). Collected accessions were grafted on ‘Adesoto’
110 rootstock and planted in an experimental orchard at the CITA in Montañana, Zaragoza
111 (Spain) at 41° 43’ 09” N, 0° 49’ 18” W and 217 m altitude.

112

113 2.2. Morphological characterization

114

115 Morphological characterization was performed on leaves and fruits. Leaves and
116 fruits were collected from the grafted trees placed in the experimental orchard. Since the
117 young trees still did not produce flowers or fruits, fruit characterization was initially
118 done on fruits collected from the original trees. A total of 33 fruit and leaf characters
119 were evaluated in each accession according to the descriptors proposed by the
120 International Union for the Protection of New Varieties of Plants ([UPOV, 2002](#)) (Table
121 2, 3, 4 and 5). For classification analysis, a binomial variable was created for each of the
122 classes of the categorical variables, where the values 0 or 1 indicate absence or presence
123 of the observed trait in each cultivar (Kaufman and Rosseeuw, 2005). Thus, the 33
124 categorical variables were split up into 106 binomial variables, which were used for
125 cluster analysis. A dendrogram was generated from Jaccard distance matrices using an

126 unweighted pair-group means analysis (UPGMA) performed with SPSS statistical
127 software (Version 15.0; SPSS, Chicago, USA).

128

129 2.3. Molecular characterization

130

131 Genomic DNA was extracted from young leaves of each accession following the
132 protocol described by Hormaza ([2002](#)). A total of 88 SSRs previously developed in
133 cherry, peach and cherry by different research groups were analysed for transferability
134 and polymorphism in the European plum accessions studied (Table 6). PCR reactions
135 were carried out in a total volume of 20 µl containing 20 mM Tris-HCl, pH 8.4, 50 mM
136 de KCl, 4 mM MgCl₂, 0.1 mM of each dNTP, 0.2 µM of each primer, 0.45 units of Taq
137 Polymerase and 40 ng of genomic DNA. Amplifications were performed in a GeneAmp
138 PCR System 9700 thermocycler (Applied Biosystems, Norwalk C. T., USA). PCR
139 cycling conditions were: an initial step of 3 min at 94 °C followed by 35 cycles of 45 s
140 at 94 °C, 45 s at different annealing temperatures varying from 40 to 60 °C (according to
141 SSRs references, Table 6), 1 min at 72 °C, and a final step of 7 min at 72 °C. The DNA
142 amplification products were separated by electrophoresis in 3% Metaphor agarose gels
143 (Lonza, Rockland, USA) at 150 V for 2 h in 5X TBE buffer, stained with ethidium
144 bromide, and visualized under UV light. The SSR fragment sizes were estimated by
145 comparison with a 10 bp ladder (Invitrogen, Carlsbad, USA). PCRs and marker
146 analyses were repeated at least twice for each sample. Genetic similarity between
147 accessions from the microsatellite data was estimated with the program NTSYS-pc 2.1
148 (Exeter software, Stauket, New York), using Nei and Li (1979) coefficient of similarity
149 and the unweighted pair-group method (UPGMA) cluster analysis. The robustness of
150 the grouping was assessed with WinBoot ([Yap and Nelson, 1996](#)).

151

152 3. Results

153

154 3.1. Morphological traits

155

156 Morphological characterization of fruits (Tables 2 and 3) and leaves (Tables 4 and
157 5) allowed classifying the 25 accessions into seven main clusters (Fig. 1, Jaccard
158 distance = 17), depending mainly on the similarities or differences in fruit traits
159 compared to 'Reine Claude Verte'. One cluster contained accessions with fruit and leaf
160 characteristics similar to the description of 'Reine Claude Verte': A fruit of small to
161 medium size, roundish shape, asymmetric, greenish skin colour and with an excellent
162 flavour, and elliptical leaves with a right or obtuse apex angle, a rounded tip, a base
163 generally obtuse, and with the incisions of margin serrated ([Tabuenca and Iturrioz,](#)
164 [1991b](#)). This cluster includes the commercial cultivars of 'Reine Claude Verte', 'Reine
165 Claude', 'Claudia Conde' and 'CI-050', (Tables 2, 3, 4 and 5, Fig. 1).

166 Another cluster of 6 accessions differed in some fruit traits to 'Reine Claude
167 Verte', these are 'Claudia del bosque', 'F-4', 'F-9', 'Parcela río', and 'Río ribazo'(Fig.
168 1, Tables 2 and 3). 'Claudia del bosque' showed the same leaf characteristics of 'Reine
169 Claude Verte', but different fruit traits, mainly fruit size, skin colour, flesh colour and
170 flesh firmness (Table 2). The accessions 'F-4', 'F-9', and 'Parcela río' showed a
171 symmetrical fruit, slightly smaller than 'Reine Claude Verte', with orange yellow skin
172 colour and orange flesh colour (Table 2). 'Río ribazo' expressed intermediate characters
173 between the set of 'Reine Claude Verte' and the other accessions of the cluster (Tables
174 2 and 3).

175 Another group included accessions showing some different characteristics of leaf
176 or fruit to 'Reine Claude Verte': 'Alcor-1', 'Alcor-2', 'Arenal', 'Parcela rio ribazo',

177 'Domingo' and 'Tobed'. The leaves and fruits of 'Alcor-1' and 'Alcor-2' were
178 indistinguishable from each other, with fruits and leaves smaller than those of 'Reine
179 Claude Verte', with a shallow stalk cavity, orange flesh colour and hammered lateral
180 surfaces of stone, and with less pubescence in the upper side of petiole. 'Domingo',
181 'Arenal' and 'Tobed' showed the same morphological characteristics of the fruit but
182 different leaf characteristics to 'Reine Claude Verte'. Finally, 'Parcela Rio Ribazo'
183 showed some differences in both leaf and fruit (Tables 2, 3, 4 and 5).

184 'Puente ave' produced very similar fruits like 'F-4', 'F-9' and 'Parcela Río'
185 (Table 2) but different leaves (Tables 4 and 5). No differences were observed between
186 the two 'Reine Claude Fraga' accessions (EEAD and AFRUCAS) of another cluster
187 (Fig. 1). The morphological characters that most discriminated these two accessions
188 were mainly the characters of the leaf (Tables 4 and 5). The fruit presented the same
189 fruit shape as 'Reine Claude Verte', but with a larger size, a greenish yellow colour and
190 an adherent stone to the flesh (Table 2). Finally, 'Stanley' and 'CI-051' showed very
191 different characteristics from the other accessions, 'Stanley' mainly in the form and
192 colour of the fruit (Table 2), and 'CI-051' mainly in the form of leaves and stones
193 (Tables 3, 4 and 5) .

194

195 3.2. Molecular characterization

196

197 For the molecular characterization, 13 microsatellites previously reported as
198 conserved and polymorphic in the genus *Prunus* ([Wunsch, 2009](#)) were analyzed in the
199 25 European plum accessions. Four of these SSRs were monomorphic in the genotypes
200 studied (Table 6) and were not used for the characterization. The remaining nine were
201 polymorphic in the sample (Table 6) and eight of them produced clear and easy scoring

202 banding patterns. These eight SSRs were selected for the molecular characterization
203 (Table 7). In order to obtain a better differentiation of the accessions and to identify a
204 greater number of conserved and polymorphic microsatellites in the species, another 75
205 microsatellite loci developed in different species of *Prunus* were analyzed for
206 transferability (Table 6). To perform this screening, the microsatellites were analyzed in
207 four selected accessions from different origins, 'Reine Claude Verte' (collection
208 AFRUCAS), 'Domingo', 'Claudia del bosque' and 'Reine Claude Fraga' (collection
209 EEAD) (Table 1). From the 75 SSRs screened, ten (13%) failed to amplify or generated
210 non-specific amplification and the remaining 65 (87%) produced amplification
211 products. From these 65 SSRs, that seem conserved in European plum, 38 were
212 monomorphic in the sample, while 27 seemed polymorphic in the sample (Table 6).
213 Eighth of these polymorphic SSRs were selected for molecular characterization and the
214 analysis of all accessions (Table 7).

215 The 16 microsatellites selected for the molecular characterization of the 25
216 accessions of European plums produced 73 allele fragments (Table 7). The number of
217 alleles per locus for all the accessions ranged from 3 (UDP96-008, EMPaS01 y BPPCT-
218 026) to 7 (pchcms5), with an average of 4.6 alleles per locus (Table 7). The number of
219 alleles per genotype ranged from 1 to 4, with a maximum of 4 alleles for the locus
220 CPPCT-29 in 21 of the 25 accessions (Table 7).

221 The analysis of the 25 accessions using the 16 selected SSR markers allowed
222 distinguishing 7 different genotypic profiles (Fig. 2). The accessions clustered by
223 UPGMA based on the Nei and Li similarity index, yielded a cophenetic correlation
224 coefficient of $r = 0.99$, indicating a good representation of the similarity by the
225 dendrogram (Fig. 2). Additionally, the bootstrap analysis revealed values above 50% for
226 all the nodes (Fig. 2).

227 The same genotypic profile was identified in 17 accessions including the ‘Reine
228 Claude Verte’ commercial clones from the collections EEAD, AFRUCAS, CITA and
229 ITG, and accessions collected from the different locations (Fig. 2). Another genotypic
230 profile corresponded to two accessions, ‘Alcor-1’ and ‘Alcor-2’, collected in Alcorisa
231 (Teruel), that was differentiated from the previous profile with a single microsatellite,
232 BPPCT-014. The five remaining genotypic profiles corresponded to ‘CI-051’,
233 prospected in Daroca (Zaragoza), two accessions from Paracuellos de Jiloca (Zaragoza),
234 the accessions of ‘Reine Claude Fraga’ from the collections of CITA and AFRUCAS,
235 and, as expected, the cultivar ‘Stanley’ (Fig. 2).

236

237 4. Discussion

238

239 In this work molecular and morphological characterization of 24 European plum
240 accessions of 'Reine Claude Verte' was carried out using morphological descriptors of
241 leaf and fruit, and *Prunus* microsatellite markers. The results have allowed
242 differentiating some accessions by defining their genotypic profile and their phenotype,
243 and to group them according their genetic similarity and their fruit and leaf traits.

244 For the molecular characterization, a set of 16 *Prunus* microsatellite loci were
245 selected based in their polymorphism, clarity and reproducibility in the species. Eight
246 selected SSRs belong to the set of SSRs previously reported to be transferable and
247 polymorphic in several *Prunus* species ([Wunsch, 2009](#)), and two of them, BPPCT-007
248 and -014, were also recently used for *Prunus domestica* genotyping ([Horvath et al.,](#)
249 [2011](#)), but the remaining 8 SSRs have been selected for the characterization of this
250 species in this work (Table 6). From the *Prunus* SSRs evaluated for transferability 65 of
251 them resulted conserved in the species producing clear and reproducible amplification
252 products and may be of interest for further works in the species. As in previous works,
253 the results confirm the high transferability of SSR loci within the *Prunus* genus and
254 specifically between peach and European plum. Cipriani et al. ([1999](#)) observed a rate of
255 transferability of 71% between these two species, while Dirlewanger et al. ([2002](#))
256 obtained amplification products in plum in 82% of 41 SSR markers developed in peach.
257 Likewise, high rates of transferability in European plum have been found using SSRs
258 from Japanese plum (100%) and almond (88%) ([Mnejja et al., 2010](#)).

259 The use of the 16 selected microsatellites in the 25 accessions analyzed resulted in
260 a total of 73 alleles, ranging from 3 to 7 per SSR, with an average of 4.6 alleles per
261 SSR. This average value is within the range observed in similar studies using SSRs in

262 this species, although it depends in the size and diversity of the sample. Decroocq et al.
263 ([2004](#)) obtained an average of 9 alleles per locus following the analysis of 10 SSRs in
264 15 genotypes, Mnejja et al. ([2010](#)) observed an average of 3.3 alleles per locus in eight
265 European plum genotypes, and Horvarth et al. ([2011](#)) detected up to 29 alleles per locus
266 in a larger sample (58) of European plum accessions. The number of alleles per
267 genotype ranged from 1 to 4, with 4 alleles per genotype detected in the 'Reine Claude
268 Verte' accessions with the microsatellite CPPCT-29, indicating certain level of
269 heterozygosity at this locus. Because European plum is hexaploid, it would be necessary
270 to estimate allele dosage in each locus to be able to estimate allele frequencies and
271 genetic variability. A further study using families would be necessary for this purpose.
272 On the other side, in other loci like BPPCT-002 only one allele per genotype was
273 detected, revealing homozygosity in other loci.

274 The molecular analysis allowed distinguishing 7 different genotypic profiles.
275 Seventeen accessions, including the six commercial 'Reine Claude Verte', resulted in a
276 single genotypic profile. However, phenotypic characterization allowed the
277 differentiation of some of these 17 accessions. The 4 commercial cultivars 'Reine
278 Claude Verte' and accessions 'Reine Claude', 'Claudia Conde' and 'CI-050' coincided
279 with the description reported for 'Reine Claude Verte' ([Tabuenca and Iturrioz, 1991b](#)).
280 However, another five, 'Puente ave', 'Parcela río', 'F9', 'F4' and 'Claudia del Bosque'
281 showed some morphological differences. Morphological differences among accessions
282 with the same genotype could be due to environmental influence, inducing changes in
283 the phenotypic expression of some characters. Alternatively, the genetic variability
284 between these accessions may have not been detected. Additional morphological and
285 phenological characterization of these accessions in the next years will allow clarifying
286 if these accessions are clones of the same genotype.

287 For other accessions the morphological data confirmed the molecular
288 characterization. The two accessions of 'Reine Claude Fraga' (EEAD and AFRUCAS)
289 were very similar to each other and coincided with the fruit description reported
290 previously ([Tabuenca and Iturrioz, 1991a](#)), and thus having the same genotype, they can
291 be considered the same cultivar. Similarly, the two accessions 'Alcor-1' y 'Alcor-2'
292 were phenotypically very similar to each other, and thus also having the same genotype
293 can also be considered the same clone . The rest of accessions, 'Río ribazo', 'Parcela río
294 ribazo' and 'Stanley', were different from each other morphologically and genotypically
295 from the rest of accessions and can not be considered 'Reine Claude Verte'.

296 The combined use of molecular and morphological characterization of the
297 accessions of 'Reine Claude Verte' allowed detecting differences and similarities that
298 would not have been detected with a single method, highlighting that both
299 methodologies are complementary. Some accessions sharing the same genotypic profile
300 showed some morphological differences. On the other side, some accessions with
301 different genotypic profile or differing in leaf traits showed fruit traits similar to 'Reine
302 Claude Verte'. These results indicate that 'Arenal', 'Domingo', 'Tobed', and 'CI-050'
303 are probably different clones of 'Reine Claude Verte'. These accessions are
304 genotypically or phenotypically different from 'Reine Claude Verte' but have the same
305 characteristics of fruit, and thus were selected for further agronomical evaluation.

306

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318

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385
- 386

387 **Figure legends**

388 Figure 1. UPGMA cluster analysis of 22 European plum accessions based on 33 fruit
389 and leaf characters using Jaccard distances. RCV: Reine Claude Verte, RCF:
390 Reine Claude Fraga.

391

392 Figure 2. UPGMA grouping of 25 European plum accessions based on Nei and Li
393 (1979) similarity from 16 SSR loci. RCV: Reine Claude Verte, RCF: Reine
394 Claude Fraga.

Table 1. Name and origin of ‘Reine Claude Verte’ accessions included in this study.

Name of the accessions	Collection site (Location, Province)	Coordinates		
		Latitude N	Longitude W	altitude (m)
Reine Claude Verte	Collection AFRUCAS ^a , Zaragoza	41°14'10"	0°02'34"	150
Reine Claude Verte	Collection CITA ^b , Zaragoza	41°43'09"	0°49'18"	217
Reine Claude Verte	Collection EEAD ^c , Zaragoza	41°43'30"	0°48'58"	220
Reine Claude Verte	Collection ITG, Navarra	42°51'25"	1°36'25"	630
Reine Claude Verte (clone 1119)	Collection ITG ^d , Navarra	42°51'25"	1°36'25"	630
Reine Claude Verte (clone 1330)	Collection ITG, Navarra	42°51'25"	1°36'25"	630
Alcor-1	Alcorisa, Teruel	40°52'59"	0°22'00"	775
Alcor-2	Alcorisa, Teruel	40°52'59"	0°22'00"	775
Arenal	Cifuentes, Guadalajara	40°45'00"	2°31'59"	875
CI-050	Daroca, Zaragoza	41°06'55"	1°24'50"	790
CI-051	Daroca, Zaragoza	41°06'55"	1°24'50"	790
Claudia Conde	Zuera, Zaragoza	41°51'44"	0°45'09"	300
Claudia del bosque	Ricla, Zaragoza	41°30'00"	1°24'00"	390
Domingo	Cifuentes, Guadalajara	40°45'00"	2°31'59"	875
F-4	Paracuellos de Jiloca, Zaragoza	41°18'00"	1°37'59"	564
F-9	Paracuellos de Jiloca, Zaragoza	41°18'00"	1°37'59"	564
Parcela río	Paracuellos de Jiloca, Zaragoza	41°18'00"	1°37'59"	564
Parcela río ribazo	Paracuellos de Jiloca, Zaragoza	41°18'00"	1°37'59"	564
Puente ave	Paracuellos de Jiloca, Zaragoza	41°18'00"	1°37'59"	564
Reine Claude	Aniñón, Zaragoza	41°25'59"	1°46'56"	725
Reine Claude Fraga	Collection AFRUCAS, Zaragoza	41°14'10"	0°02'34"	150
Reine Claude Fraga	Collection EEAD, Zaragoza	41°43'30"	0°48'58"	220
Río ribazo	Paracuellos de Jiloca, Zaragoza	41°18'00"	1°37'59"	564
Tobed	Tobed, Zaragoza	41°20'00"	1°24'00"	637

^aAFRUCAS: Asociación Profesional de Fruticultores de la Comarca de Caspe, Gobierno de Aragón, and Ayuntamiento de Caspe.

^bCITA: Centro de Investigación y Tecnología Agroalimentaria de Aragón.

^cEEAD: Estacion Experimental Aula Dei.

^dITG: Instituto Técnico y de Gestión Agraria de Navarra.

Table 2. Morphological characterization of European plum accessions using qualitative descriptors of fruit according to UPOV (2002).

Accession	Size	Shape	Symmetry	Depth of suture	Depression at apex	Pubescence at apex	Depth of stalk cavity	Ground colour of skin	Colour of flesh	Firmness of flesh	Juiciness	Degree of adherence stone-flesh
R.C.V. (AFRUCAS)	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	med-firm	high	semi-adh
R.C.V. (CITA)	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	med-firm	high	semi-adh
R.C.V. (EEAD)	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	med-firm	high	semi-adh
R.C.V. (ITG)	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	med-firm	high	semi-adh
R.C.V. (clone 1119)	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	med-firm	high	semi-adh
R.C.V. (clone 1330)	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	med-firm	high	semi-adh
Alcor-1	small	cir	asy	med	abs/weak	abs	sha	yel green	orange	med	high	adh
Alcor-2	small	cir	asy	med	abs/weak	abs	sha	yel green	orange	med	high	adh
Arenal	med	cir	asy	med	abs/weak	abs	med	yel green	orange	med	high	adherent
CI-050	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	firm	med-high	adh
CI-051	med-large	cir	sym	sha	abs/weak	abs	med-deep	green	yel green	med	med	semi-adh
Claudia Conde	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	firm	med-high	semi-adh
Claudia del bosque	small	cir	asy	sha	abs/weak	abs	sha	orange yel	orange	soft-med	med-high	semi-adh
Domingo	med	cir	asy	med	abs/weak	abs	med	green	yel green	med	high	semi-adh
F-4	small-med	cir	sym	sha	abs/weak	abs	sha	orange yel	orange	med	med-high	semi-adh
F-9	small-med	cir	sym	sha	abs/weak	abs	sha	orange yel	orange	med	med-high	semi-adh
Parcela río	small-med	cir	sym	sha	abs/weak	abs	sha-med	orange yel	orange	med	high	semi-adh
Parcela río ribazo	small	cir	asy	shallow	abs/weak	abs	sha	orange yel	orange	med	high	adh
Puente ave	small-med	cir	sym	sha	abs/weak	abs	sha	orange yel	orange	med	med-high	semi-adh
Reine Claude	med	cir	asy	sha-med	abs/weak	abs	med	green	yel green	firm	med-high	semi-adh
R.C.Fraga (AFRUCAS)	med-large	cir	asy	sha-med	weak-int	abs	med-deep	yel green	yel green	med	med-high	adh
R.C.Fraga (EEAD)	med-large	cir	asy	sha-med	weak-int	abs	med-deep	yel green	yel green	med	med-high	adh
Río ribazo	small-med	cir	asy	med	abs/weak	abs	sha-med	yel green	orange	med-firm	med	semi-adh
Stanley	med	elliptic	asy	med	abs/weak	abs	med	dark blue	orange	firm	med	adh
Tobed	med	cir	asy	med	abs/weak	abs	med	green	yel green	med	high	semi-adh

R.C.V: Reine Claude Verte, R.C.: Reine Claude, asy: asymmetric, sym: symmetric, med: medium, abs: absent, int: intermediate, yel: yellowish, cir: circular, adh: adherent, sha: shallow

Table 3. Morphological characterization of European plum accessions using qualitative descriptors of stone according to UPOV (2002).

Accession	General shape	Shape in ventral view	Development of keel	Texture of lateral surfaces	Width at base	Shape of apex
R.C.V. (AFRUCAS)	elliptic	broad elliptic	medium	grained	narrow	obtuse
R.C.V. (CITA)	elliptic	broad elliptic	medium	grained	narrow	obtuse
R.C.V. (EEAD)	elliptic	broad elliptic	medium	grained	narrow	obtuse
R.C.V. (ITG)	elliptic	broad elliptic	medium	grained	narrow	obtuse
R.C.V. (clone 1119)	elliptic	broad elliptic	medium	grained	narrow	obtuse
R.C.V. (clone 1330)	elliptic	broad elliptic	medium	grained	narrow	obtuse
Alcor-1	elliptic	broad elliptic	medium	hammered	narrow	rounded
Alcor-2	elliptic	broad elliptic	medium	hammered	narrow	rounded
Arenal	elliptic	broad elliptic	medium	hammered	narrow	obtuse
CI-050	elliptic	broad elliptic	medium	grained	narrow	obtuse
CI-051	narrow elliptic-elliptic	broad elliptic	strong	grained	medium	acute-obtuse
Claudia Conde	elliptic	elliptic	medium	grained	narrow	obtuse
Claudia del bosque	elliptic	elliptic	medium	grained	narrow	obtuse
Domingo	elliptic	broad elliptic	medium	hammered	narrow	rounded
F-4	elliptic	broad elliptic	medium	grained	narrow	obtuse-rounded
F-9	elliptic	broad elliptic	medium	grained	narrow	obtuse
Parcela río	elliptic	elliptic	medium	grained	narrow	obtuse
Parcela río ribazo	elliptic	broad elliptic	medium	hammered	narrow	rounded
Puente ave	elliptic	broad elliptic	medium	grained	narrow	obtuse
Reine Claude	elliptic	broad elliptic	medium	grained	narrow	obtuse
R.C. Fraga (EEAD)	elliptic	elliptic-broad	strong	grained	narrow-medium	rounded
R.C. Fraga (AFRUCAS)	elliptic	elliptic-broad	strong	grained	narrow-medium	rounded
Río ribazo	narrow elliptic-elliptic	elliptic-broad	medium	grained	narrow	obtuse
Stanley	narrow elliptic	cuneate	weak-medium	hammered	narrow	obtuse
Tobed	elliptic	broad elliptic	medium	hammered	narrow	rounded

R.C.V: Reine Claude Verte, R.C.: Reine Claude

Table 4. Morphological characterization of European plum accessions using qualitative descriptors of leaf blade according to UPOV (2002).

Accession	Length	width	Ratio length/width	shape	angle of apex (excluding tip)	shape of base	Green colour of upper side	Glossiness of upper side	Pubescence of lower side	Incisions of margin
R.C.V. (AFRUCAS)	med-long	med	med	elliptic	right-angled	acute-obtuse	med	med	absent	serrate
R.C.V. (CITA)	med	med	med	elliptic	right-angled	acute-obtuse	med	med	absent	serrate
R.C.V. (EEAD)	med	med	small-med	elliptic	right-angled	acute-obtuse	med	med	absent	serrate
R.C.V. (ITG)	-	-	-	-	-	-	-	-	-	-
R.C.V. (clone 1119)	-	-	-	-	-	-	-	-	-	-
R.C.V. (clone 1330)	-	-	-	-	-	-	-	-	-	-
Alcor-1	short-med	narrow-med	med	ovate-elliptic	right-angled	obtuse	med	med	absent	serrate
Alcor-2	short-med	narrow-med	med	ovate-elliptic	right-angled	obtuse	med	med	absent	serrate
Arenal	short-med	narrow-med	med	ovate-elliptic	right-angled	obtuse	light-med	weak-med	absent	serrate
CI-050	med	med	small-med	elliptic	right-angled	acute-obtuse	med	med	present	serrate
CI-051	med-long	broad	small	obovate	obtuse	truncate	med-dark	weak-med	present	crenate
Claudia Conde	med	med	med	elliptic	right-angled	acute	med	med	absent	serrate
Claudia del bosque	med	med	med	elliptic	right-angled	acute	light	strong	absent	serrate
Domingo	med	med	med	ovate-elliptic	right angled-obtuse	obtuse	light-med	weak-med	absent	serrate
F-4	med	narrow-med	med	elliptic	right-angled	acute	light	med-strong	absent	serrate
F-9	short-med	narrow	med	elliptic	right-angled	acute	light	med-strong	absent	serrate
Parcela río	med	med	med	elliptic	right-angled	acute	med	med	absent	serrate
Parcela río ribazo	short-med	narrow-med	med	elliptic	right-angled	acute	light-med	med-strong	absent	serrate
Puente ave	short	narrow-med	small	ovate	obtuse	obtuse	med	med	absent	serrate
Reine Claude	med	med	med	elliptic	right-angled	acute-obtuse	light-med	med	absent	serrate
R.C. Fraga (EEAD)	med	med-broad	small	ovate	obtuse	obtuse-truncate	light-med	weak	present	crenate
R.C. Fraga (AFRUCAS)	med	med-broad	small-med	ovate	obtuse	obtuse-truncate	light-med	weak	present	crenate
Río ribazo	med	med	med	elliptic	right-angled	acute	med	med	absent	serrate
Stanley	short-med	narrow	med	elliptic	right-angled	acute	light	med-strong	present	serrate
Tobed	med	narrow-med	med	ovate-elliptic	right-angled	obtuse	light-med	weak-med	absent	serrate

R.C.V: Reine Claude Verte, R.C.: Reine Claude, med: medium

Table 5. Morphological characterization of European plum accessions using qualitative descriptors of leaf according to UPOV (2002).

Accession	Petiole Length	Pubescence of upper side of petiole	Ratio length of leaf blade/length of petiole	Presence of nectaries	Position of nectaries
R.C.V. (AFRUCAS)	medium-long	medium-strong	medium	present	equally on base of blade and petiole
R.C.V. (CITA)	medium	medium-strong	medium	present	equally on base of blade and petiole
R.C.V. (EEAD)	medium	medium-strong	medium	present	equally on base of blade and petiole
R.C.V. (ITG)	-	-	-	-	-
R.C.V. (clone 1119)	-	-	-	-	-
R.C.V. (clone 1330)	-	-	-	-	-
Alcor-1	medium	weak-medium	small-medium	present	equally on base of blade and petiole
Alcor-2	medium	weak-medium	small-medium	present	equally on base of blade and petiole
Arenal	medium	weak-medium	small-medium	present	equally on base of blade and petiole
CI-050	medium	weak-medium	medium	present	Predominantly on base of blade
CI-051	medium	weak	medium	present	Predominantly on base of blade
Claudia Conde	medium	medium	medium	present	equally on base of blade and petiole
Claudia del bosque	medium	medium	small-medium	present	equally on base of blade and petiole
Domingo	medium	weak	small-medium	present	equally on base of blade and petiole
F-4	short-medium	medium	medium	present	Predominantly on base of blade
F-9	short-medium	medium	medium	present	Predominantly on base of blade
Parcela río	medium	medium	medium	present	equally on base of blade and petiole
Parcela río ribazo	medium	medium	small-medium	present	Predominantly on base of blade
Puente ave	short	weak-medium	large	present	Predominantly on base of blade
Reine Claude	medium	medium	medium	present	equally on base of blade and petiole
R.C. Fraga (EEAD)	short-medium	medium	medium	present	Predominantly on base of blade
R.C. Fraga (AFRUCAS)	medium	medium	medium	present	Predominantly on base of blade
Río ribazo	medium	weak-medium	medium	present	equally on base of blade and petiole
Stanley	long	weak	small	present	equally on base of blade and petiole
Tobed	medium	weak-medium	small-medium	present	equally on base of blade and petiole

R.C.V: Reine Claude Verte, R.C.: Reine Claude

Table 6. List of *Prunus* SSRs analysed in this work.

Reference	Original species	SSR loci
Aranzana et al., 2002	Peach	CPPCT-6 , CPPCT-8 ^a , CPPCT-13 ^a , CPPCT-016 ^a , CPPCT-021 ^a , CPPCT-22, CPPCT-023 ^a , <u>CPPCT-29^a</u> , CPPCT-30 ^a , CPPCT-33, CPPCT-036 ^a
Cantini et al., 2001; Struss et al., 2002	Sweet cherry	PceGA59 ^a , <u>PMS3^b</u> , PMS40 ^a , PMS49 ^a , PMS67 ^a
Cipriani et al., 1999	Peach	UDP96-001 ^a , <u>UDP96-008^a</u> , UDP97-403 ^b , UDP98-409 , UPD97-402
Clarke y Tobutt, 2003	Sweet cherry	EMPA-001 ^a , EMPA-002 ^a , EMPA-003 ^a , EMPA-004 ^a , EMPA-005, EMPA-006, EMPA-007 ^b , EMPA-008, EMPA-009 ^a , EMPA-010, EMPA-011, EMPA-012 ^a , EMPA-013, EMPA-014 ^a , EMPA-015 ^a , EMPA-016, EMPA-017 ^a , EMPA-018, EMPA-019
Dirlewanger et al., 2002	Peach	BPPCT-002 , BPPCT-004 ^a , BPPCT-005 ^a , BPPCT-007 , BPPCT-008 ^a , BPPCT-009 ^a , BPPCT-010 , <u>BPPCT-012^a</u> , BPPCT-013 ^a , BPPCT-014 , BPPCT-015, BPPCT-017, BPPCT-019 ^a , BPPCT-021 ^a , BPPCT-026 , <u>BPPCT-028^{ab}</u> , BPPCT-029, BPPCT-034 ^a , BPPCT-035, BPPCT-037 ^a , BPPCT-038, BPPCT-039, BPPCT-040 ^a
Downey and Iezzoni., 2000	Sour cherry, Sweet Cherry	PceGA34, PS12A02
Sosinski et al., 2000	Peach, Sour cherry	pchcms1 ^{ab} , pchcms3, pchcms4 ^b , pchcms5 , pchgms1 ^a , PS08E08
Testolin et al., 2000	Peach	UDP96-005 , <u>UPD98-021^b</u> , UPD98-022, UPD98-024 ^b , UPD98-410 , UPD98-411, UPD98-412
Vaughan and Russell, 2004	Sweet cherry	<u>EMPaS01^a</u> , EMPaS02 ^b , <u>EMPaS05^a</u> , EMPaS06 ^a , EMPaS07 ^a , EMPaS10, EMPaS11 ^a , EMPaS12 ^a , EMPaS13 ^a , EMPaS14 ^b

Bold text: SSRs previously shown to be transferable within the genus (Wünsch, 2009).

Underlined text: SSRs selected for the molecular characterization of the European plum accessions.

^a SSRs (42) amplified in European plum accessions.

^b SSRs (10) polymorphic in European plum accession

Table 7. SSRs used to analyse the European plum accessions, size range, alleles, and maximum number of alleles per genotype, in each locus.

Locus	Size range (bp)	No. alleles	Maximum no. alleles per genotype
BPPCT-002	196-235	4	2
BPPCT-007	118-154	5	2
BPPCT-010	103-143	5	2
BPPCT-012	127-193	6	2
BPPCT-014	178-237	4	3
BPPCT-026	137-168	3	2
BPPCT-028	148-183	5	2
CPPCT-29	160-218	5	4
EMPaS01	210-273	3	3
EMPaS05	150-205	5	3
pchcms5	264-310	7	3
PMS3	129-165	4	2
UDP96-005	110-170	5	2
UDP96-008	142-161	3	2
UDP98-409	119-153	4	2
UPD98-021	96-145	5	3
Total		73	
Average		4.6	2.4



