Characterization of accessions of ‘Reine Claude Verte’ plum using *Prunus* SRR and phenotypic traits

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ABSTRACT

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

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

1. Introduction

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

Nowadays, 'Reine Claude Verte' is grown worldwide, in particular in Western Europe, where more than 70 synonymies have been detected (Tabuenca and Iturrioz, 1991b). More than 500 years of cultivation in Europe suggest that different clones may be growing under the same cultivar name as result of mutations or local selections of seedlings. Furthermore, new commercial cultivars have been selected from seedlings of 'Reine Claude Verte', like ‘Reine Claude of Oullins’ (Hedrick, 1911) and ‘Reine Claude of Bavay’ (Tabuenca and Iturrioz, 1991a), but some of them often are erroneously cultivated as 'Reine Claude Verte' (Tabuenca and Iturrioz, 1991b). 'Reine Claude Verte' is a highly appreciated plum cultivar for its excellent organoleptic qualities and in many regions of Europe is the most grown European plum cultivar. Despite of its increasing commercial demand, this cultivar is in the process of being eradicated in many commercial orchards because of its generally erratic fruit setting.

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

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

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

2. Material and methods

2.1. Plant material

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

2.2. Morphological characterization

Morphological characterization was performed on leaves and fruits. Leaves and fruits were collected from the grafted trees placed in the experimental orchard. Since the young trees still did not produce flowers or fruits, fruit characterization was initially done on fruits collected from the original trees. A total of 33 fruit and leaf characters were evaluated in each accession according to the descriptors proposed by the International Union for the Protection of New Varieties of Plants (UPOV, 2002) (Table 2, 3, 4 and 5). For classification analysis, a binomial variable was created for each of the classes of the categorical variables, where the values 0 or 1 indicate absence or presence of the observed trait in each cultivar (Kaufman and Rosseeuw, 2005). Thus, the 33 categorical variables were split up into 106 binomial variables, which were used for cluster analysis. A dendrogram was generated from Jaccard distance matrices using an unweighted pair-group means analysis (UPGMA) performed with SPSS statistical software (Version 15.0; SPSS, Chicago, USA).

2.3. Molecular characterization

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

3. Results

3.1. Morphological traits

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

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

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

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

3.2. Molecular characterization

For the molecular characterization, 13 microsatellites previously reported as conserved and polymorphic in the genus *Prunus* (Wunsch, 2009) were analyzed in the 25 European plum accessions. Four of these SSRs were monomorphic in the genotypes studied (Table 6) and were not used for the characterization. The remaining nine were polymorphic in the sample (Table 6) and eight of them produced clear and easy scoring banding patterns. These eight SSRs were selected for the molecular characterization (Table 7). In order to obtain a better differentiation of the accessions and to identify a greater number of conserved and polymorphic microsatellites in the species, another 75 microsatellite loci developed in different species of *Prunus* were analyzed for transferability (Table 6). To perform this screening, the microsatellites were analyzed in four selected accessions from different origins, 'Reine Claude Verte' (collection AFRUCAS), 'Domingo', 'Claudia del bosque' and 'Reine Claude Fraga' (collection EEAD) (Table 1). From the 75 SSRs screened, ten (13%) failed to amplify or generated non-specific amplification and the remaining 65 (87%) produced amplification products. From these 65 SSRs, that seem conserved in European plum, 38 were monomorphic in the sample, while 27 seemed polymorphic in the sample (Table 6). Eighth of these polymorphic SSRs were selected for molecular characterization and the analysis of all accessions (Table 7).

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

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

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

4. Discussion

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

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

The use of the 16 selected microsatellites in the 25 accessions analyzed resulted in a total of 73 alleles, ranging from 3 to 7 per SSR, with an average of 4.6 alleles per SSR. This average value is within the range observed in similar studies using SSRs in this species, although it depends in the size and diversity of the sample. Decroocq et al. (2004) obtained an average of 9 alleles per locus following the analysis of 10 SSRs in 15 genotypes, Mnejja et al. (2010) observed an average of 3.3 alleles per locus in eight European plum genotypes, and Horvarth et al. (2011) detected up to 29 alleles per locus in a larger sample (58) of European plum accessions. The number of alleles per genotype ranged from 1 to 4, with 4 alleles per genotype detected in the 'Reine Claude Verte' accessions with the microsatellite CPPCT-29, indicating certain level of heterozygosity at this locus. Because European plum is hexaploid, it would be necessary to estimate allele dosage in each locus to be able to estimate allele frequencies and genetic variability. A further study using families would be necessary for this purpose. On the other side, in other loci like BPPCT-002 only one allele per genotype was detected, revealing homozygosity in other loci.

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

For other accessions the morphological data confirmed the molecular characterization. The two accessions of ‘Reine Claude Fraga’ (EEAD and AFRUCAS) were very similar to each other and coincided with the fruit description reported previously (Tabuenca and Iturrioz, 1991a), and thus having the same genotype, they can be considered the same cultivar. Similarly, the two accessions ‘Alcor-1’ y ‘Alcor-2’ were phenotypically very similar to each other, and thus also having the same genotype can also be considered the same clone . The rest of accessions, ‘Río ribazo’, ‘Parcela río ribazo’ and ‘Stanley’, were different from each other morphologically and genotypically from the rest of accessions and can not be considered ‘Reine Claude Verde’.

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

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References

Aradhya, M.K., Weeks, C., Simon, C.J., 2004. Molecular characterization of variability and relationships among seven cultivated and selected wild species of *Prunus* L. using amplified fragment length polymorphism. Sci. Hort. 103, 131-144.

Cipriani, G., Lot, G., Huang, W.G., Marrazzo, M.T., Peterlunger, E., Testolin, R., 1999. AC/GT and AG/CT microsatellite repeats in peach *Prunus persica* (L) Batsch : isolation, characterisation and cross-species amplification in *Prunus*. Theor. Appl. Genet. 99, 65-72.

Decroocq, V., Hagen, L.S., Fave, M.G., Eyquard, J.P., Pierronnet, A., 2004. Microsatellite markers in the hexaploid *Prunus domestica* species and parentage lineage of three European plum cultivars using nuclear and chloroplast simple-sequence repeats. Mol. Breeding 13, 135-142.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M.J., Poizat, C., Zanetto, A., Arus, P., Laigret, F., 2002. Development of microsatellite markers in peach [*Prunus Persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor. Appl. Genet. 105, 127-138.

Esmenjaud, D., Dirlewanger, E., 2007. Plum, In: Kole, C. (Ed.), Genome Mapping & Molecular Breeding. Springer, Heidelberg, pp. 119-135.

Goulao, L., Monte-Corvo, L., Oliveira, C.M., 2001. Phenetic characterization of plum cultivars by high multiplex ratio markers: amplified fragment length polymorphisms and inter-simple sequence repeats. J. Am. Soc. Hortic. Sci. 126, 72-77.

Gregor, D., Hartmann, W., Stösser, R., 1994. Cultivar identification in *Prunus domestica* using random amplified polymorphic DNA markers. Acta Hort. 359, 33-40.

Hedrick, U.P., 1911. The Plums of New York. JB Lyon, Albany.

Heinkel, R., Hartmann, W., Stosser, R., 2000. On the origin of the plum cultivars 'Cacaks Beauty', 'Cacaks Best', 'Cacaks Early' and 'Cacaks Fruitful' as investigated by the inheritance of random amplified polymorphic DNA (RAPD) fragments. Sci. Hort. 83, 149-155.

Hormaza, J.I., 2002. Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. Theor. Appl. Genet. 104, 321-328.

Horvath, A., Balsemin, E., Barbot, J.C., Christmann, H., Manzano, G., Reynet, P., Laigret, F., Mariette, S., 2011. Phenotypic variability and genetic structure in plum (*Prunus domestica* L.), cherry plum (*P. cerasifera* Ehrh.) and sloe (*P. spinosa* L.). Sci. Hort. 129, 283-293.

Kaufman, L., Rosseeuw, P.J. 2005. Finding groups in data. An introduction to cluster analysis. John Wiley & Sons, Hoboken, New Jersey, USA.

Lespinasse, J.M., Leterme, E., 2005. De la taille à la conduite des arbres fruitiers. Editions du Rouergue, Paris.

Liu, W., Li, S., Zhang, A., Liu, D., 2007. Genetic diversity revealed by RAPD markers in plum collection of china. Acta Hort., 287-294.

Liu, W.S., Liu, D.C., Feng, C.J., Zhang, A.M., Li, S.H., 2006. Genetic diversity and phylogenetic relationships in plum germplasm resources revealed by RAPD markers. J. Hort. Sci. Biotechnol. 81, 242-250.

Mnejja, M., Garcia-Mas, J., Audergon, J.M., Arus, P., 2010. Prunus microsatellite marker transferability across rosaceous crops. Tree Genet. Genomes 6, 689-700.

Nei, M., Li, W.H., 1979. Mathematical-model for studying genetic-variation in terms of restriction endonucleases. P. Natl. Acad. Sci. USA 76, 5269-5273.

Rieger, M., 2006. Introduction to Fruit Crops. Haworth Press, New York

Tabuenca, M.C., Iturrioz, M., 1991a. Description of European plum varieties. I. Native varieties. An. Aula Dei 20, 119-152.

Tabuenca, M.C., Iturrioz, M., 1991b. Description of European plum varieties. II. Reina Claudia Verde. An. Aula Dei 20, 153-163.

Testolin, R., Cipriani, G., 2010. Molecular markers for germplasm identification and characterization. Acta Hort. 859, 59-72.

UPOV, 2002. Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability of European Plum. International Union for the Protection of New Varieties of Plants, Geneve, Switzerland.

Wunsch, A., 2009. Cross-transferable polymorphic SSR loci in Prunus species. Sci. Hort. 120, 348-352.

Yap, I.V., Nelson, R.J., 1996. WinBoot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. International Rice Research Institute, Manila, Philippines.

Figure legends

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

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

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

Name of the accesions	Collection site (Location, Province)	Coordinates		
		Latitude N	Longitude W	altitude (m)
Reine Claude Verte	Collection AFRUCAS[a] , Zaragoza	41º14′10″	0º02′34″	150
Reine Claude Verte	Collection CITA[b], Zaragoza	41º43′09″	0º49′18″	217
Reine Claude Verte	Collection EEAD[c], Zaragoza	41º43′30″	0º48′58″	220
Reine Claude Verte	Collection ITG, Navarra	42º51′25″	1º36′25″	630
Reine Claude Verte (clone 1119)	Collection ITG[d], Navarra	42º51′25″	1º36′25″	630
Reine Claude Verte (clone 1330)	Collection ITG, Navarra	42º51′25″	1º36′25″	630
Alcor-1	Alcorisa, Teruel	40º52′59″	0º22′00″	775
Alcor-2	Alcorisa, Teruel	40º52′59″	0º22′00″	775
Arenal	Cifuentes, Guadalajara	40º45′00″	2º31′59″	875
CI-050	Daroca, Zaragoza	41º06′55″	1º24′50″	790
CI-051	Daroca, Zaragoza	41º06′55″	1º24′50″	790
Claudia Conde	Zuera, Zaragoza	41º51′44″	0º45′09″	300
Claudia del bosque	Ricla, Zaragoza	41º30′00″	1º24′00″	390
Domingo	Cifuentes, Guadalajara	40º45′00″	2º31′59″	875
F-4	Paracuellos de Jiloca, Zaragoza	41º18′00″	1º37′59″	564
F-9	Paracuellos de Jiloca, Zaragoza	41º18′00″	1º37′59″	564
Parcela río	Paracuellos de Jiloca, Zaragoza	41º18′00″	1º37′59″	564
Parcela río ribazo	Paracuellos de Jiloca, Zaragoza	41º18′00″	1º37′59″	564
Puente ave	Paracuellos de Jiloca, Zaragoza	41º18′00″	1º37′59″	564
Reine Claude	Aniñón, Zaragoza	41º25′59″	1º46′56″	725
Reine Claude Fraga	Collection AFRUCAS, Zaragoza	41º14′10″	0º02′34″	150
Reine Claude Fraga	Collection EEAD, Zaragoza	41º43′30″	0º48′58″	220
Río ribazo	Paracuellos de Jiloca, Zaragoza	41º18′00″	1º37′59″	564
Tobed	Tobed, Zaragoza	41º20′00″	1º24′00″	637

[a]AFRUCAS: Asociación Profesional de Fruticultores de la Comarca de Caspe, Gobierno de Aragón, and Ayuntamiento de Caspe.
[b]CITA: Centro de Investigación y Tecnología Agroalimentaria de Aragón.
[c]EEAD: Estacion Experimental Aula Dei.
[c]ITG: Instituto Técnico y de Gestión Agraria de Navarra.

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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	Pubes-cence 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	Pubes-cence 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

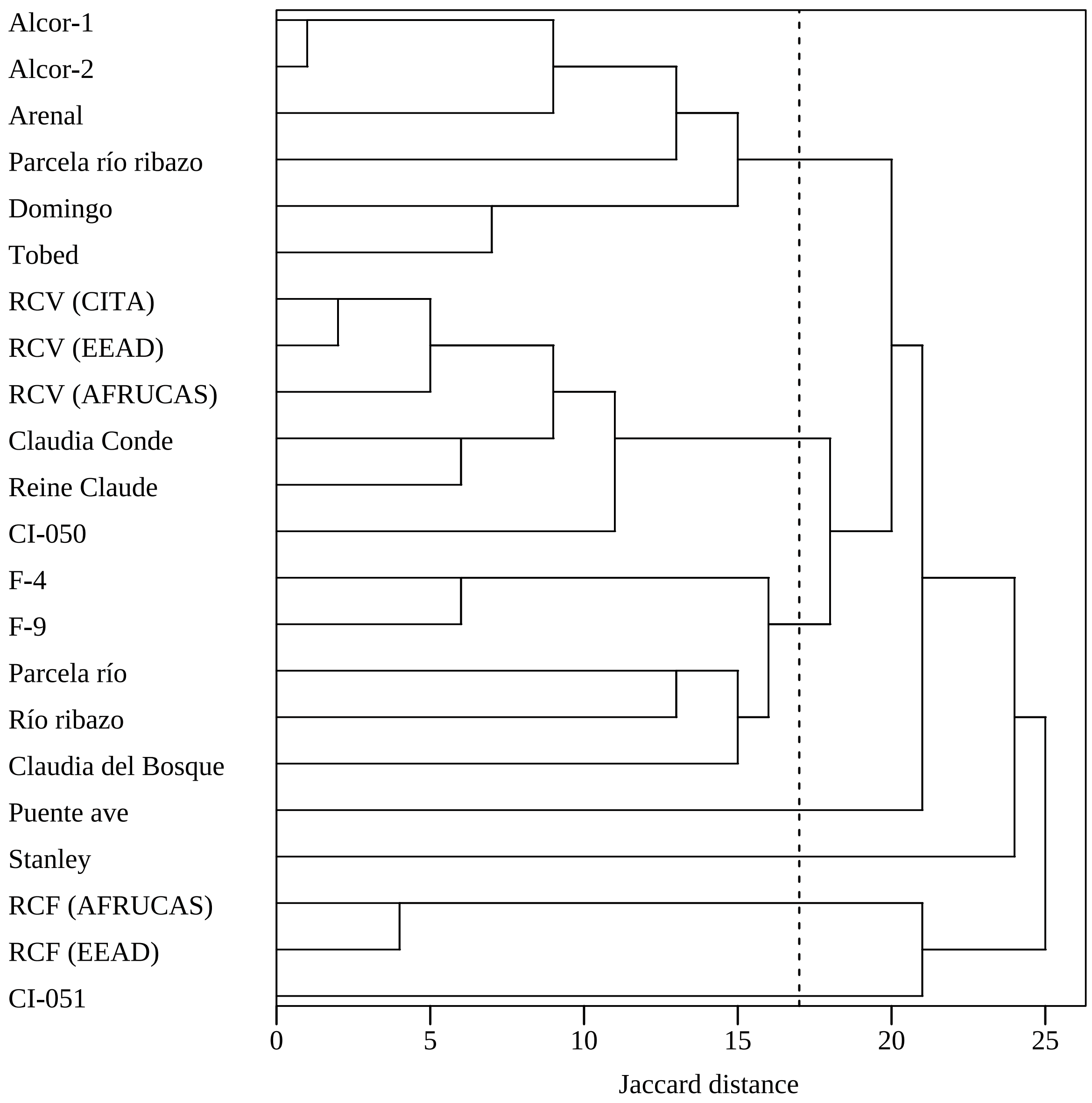
Table 6. List of *Prunus* SSRs analysed in this work.

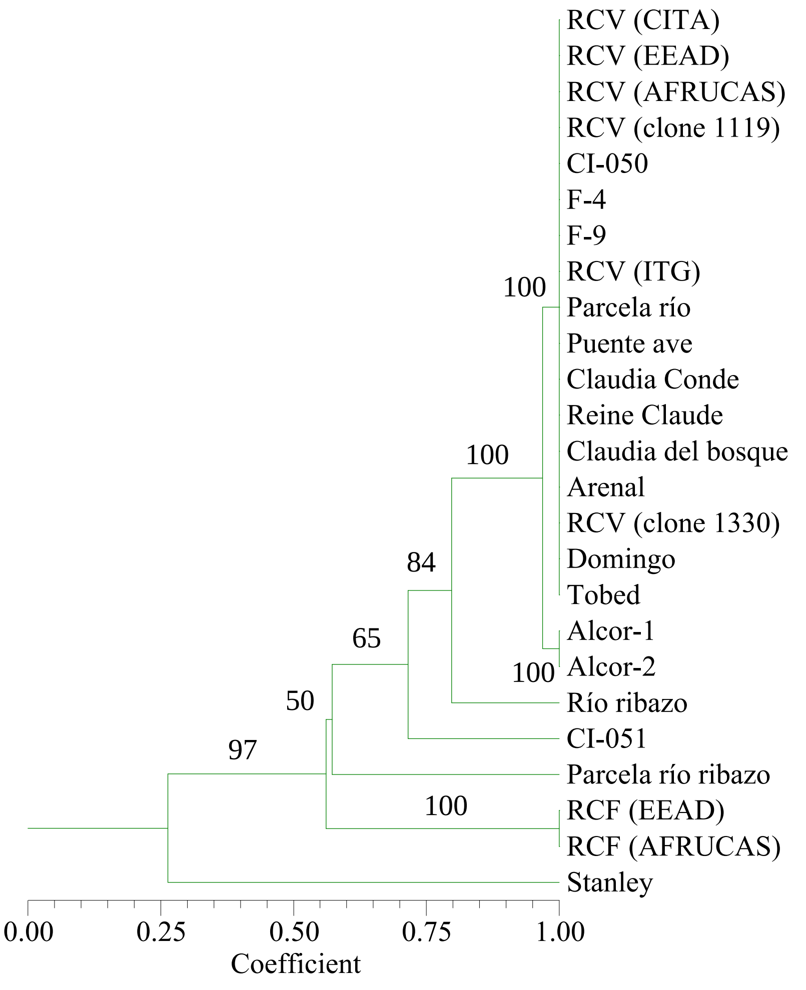
Reference	Original species	SSR loci
Aranzana et al., 2002	Peach	**CPPCT-6**, CPPCT-8[a], CPPCT-13[a], CPPCT-016[a], CPPCT-021[a], CPPCT-22, CPPCT-023[a], CPPCT-29[a], CPPCT-30[a], CPPCT-33, CPPCT-036[a]
Cantini et al., 2001; Struss et al., 2002	Sweet cherry	PceGA59[a], PMS3[b], PMS40[a], PMS49[a], PMS67[a]
Cipriani et al., 1999	Peach	UDP96-001[a], UDP96-008[a], 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**, BPPCT-012[a], BPPCT-013[a], **BPPCT-014**, BPPCT-015, BPPCT-017, BPPCT-019[a], BPPCT-021[a], **BPPCT-026**, BPPCT-028[ab], 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**, UPD98-021[b], UPD98-022, UPD98-024[b], **UPD98-410**, UPD98-411, UPD98-412
Vaughan and Russell, 2004	Sweet cherry	EMPaS01[a], EMPaS02[b], EMPaS05[a], 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


Alcor-1
Alcor-2
Arenal
Parcela río ribazo
Domingo
Tobed
RCV (CITA)
RCV (EEAD)
RCV (AFRUCAS)
Claudia Conde
Reine Claude
CI-050
F-4
F-9
Parcela río
Río ribazo
Claudia del Bosque
Puente ave
Stanley
RCF (AFRUCAS)
RCF (EEAD)
CI-051
0
5
10
15
20
25
Jaccard distance


RCV (CITA)
RCV (EEAD)
RCV (AFRUCAS)
RCV (clone 1119)
CI-050
F-4
F-9
RCV (ITG)
Parcela río
Puente ave
Claudia Conde
Reine Claude
Claudia del bosque
Arenal
RCV (clone 1330)
Domingo
Tobed
Alcor-1
Alcor-2
Río ribazo
CI-051
Parcela río ribazo
RCF (EEAD)
RCF (AFRUCAS)
Stanley
100
100
84
65
50
97
100
100
0.00
0.25
0.50
0.75
1.00
Coefficient