S-genotype identification, genetic diversity and structure analysis of Italian sweet cherry germplasm

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Abstract

In this study, 186 local sweet cherry accessions from 12 Italian regions, plus eight reference accessions, were analysed for the first time, using 13 microsatellite markers. Moreover, their *S*-incompatibility genotypes were identified with consensus primers for the *S-RNase* and SFB genes. A total of 161 unique genotypes were found; 18 groups of synonyms, along with the discovery of cases of misidentification. The average number of alleles per locus was 9.7, the mean expected heterozygosity (*He*) was 0.63, the mean observed heterozygosity (*Ho*) was 0.65 and the mean polymorphic information content (PIC) was 0.58. The structure analysis revealed the presence of six populations, which reflected in some cases geographical areas, the exchange of material among regions and introduction of material from abroad. A total of 17 different *S*-alleles were found, combined in 24 incompatibility groups of the 47 reported so far. Furthermore, 10 new incompatibility groups, from XLVII to LVI, were identified. Seven genotypes with unique S-allele combinations were included in the pollen donor group 0. The mutant allele of the pollen SFB_5' was found in early ripening genotypes from Sicily and Sardinia. The variability of SSRs present in both introns of the allele S_{13} was also explored; new combinations of variants were found and some accessions presented *SSR* variants typical of wild cherry. It is evident that the Italian sweet cherry germplasm collection represents a relevant source of genetic diversity that needs to be preserved for future breeding programmes.

Keywords Prunus avium, Genetic variability, Germplasm resources, Self(in)compatibility, *S*-alleles, Conservation

Introduction

Europe produces 36.7% of the world's sweet cherry (*Prunus avium* L.) crop (FAOSTAT 2013 http://www.faostat.fao), with an average production of about 842,000 t per year. As reported by Pliny the Elder in his Naturalis Historia, sweet cherry cultivation was first established in Europe by the Romans (Zohary and Hopf, 2000), who imported the cherries from Turkey. Multiple domestication events and introgression from the wild form after domestication were recently postulated by Mariette *et al*. (2010). Italy is the leading sweet cherry producer in Europe (FAOSTAT 2013 http://www.faostat.fao). Hundreds of local sweet cherry landraces have been raised in various environmentally diversified Italian territories; the result of centuries of natural and human selection. Most of these traditional varieties have been cultivated marginally, for local or familial consumption. However, in some areas such as Emilia-Romagna (Giovannini *et al.* 2013), Tuscany (Roselli and Mariotti 1999) and Piedmont (Regione Piemonte 2008), a notable cherry industry based on local varieties has been documented since the beginning of the twentieth century. From the second half of the twentieth

century onwards, the traditional varieties progressively lost their importance as modern cultivars gained commercial favour (CNR 1994). However, local varieties with superior fruit traits and stronger historical links to their territory are still in demand on the niche market. The richness of Italian traditional sweet cherry germplasm was first highlighted in the census carried out by Baldini (1973) and the following ones coordinated by the CNR at the beginning of the 1980s (1988, 1994) and the CREA (Grassi *et al.* 1996), which highlighted the high risk of genetic erosion, with many landraces existing as *in situ* single exemplars (Giovannini and Engel 2006). Several initiatives at the national and regional levels have been put in place since, aimed to track, preserve, document and encourage the use of the existing landraces. The genetic richness of Italian germplasm is still rather unexplored, despite the large variability in terms of agro-pomological features, hence their potential as a source of useful traits in breeding programmes. Moreover, the origin of many landraces is undocumented and their naming confusing, due to the frequent occurrence of homonyms and synonyms. Thus, accurate germplasm identification is urgently needed. For identification and/or genetic discrimination, complementing morphological descriptions with molecular markers has proven a highly useful method for solving uncertainties. In particular, microsatellite markers or simple sequence repeats (SSRs) have been largely employed for cherry genetic diversity analysis (e.g. Dirlewanger *et al.* 2002; Schueler *et al*. 2003; Vaughan and Russell 2004; Marchese *et al*. 2007a; Gisbert *et al*. 2008; Clarke and Tobutt 2009; Lacis *et al*. 2009; Frei *et al*. 2010; Stanys *et al*. 2012; De Rogatis *et al*. 2013) and also used in a few studies for the detection of genetic structure in sweet and wild cherry populations (e.g. Mariette *et al*. 2010; De Rogatis *et al*. 2013). Sweet cherry displays gametophytic self-incompatibility (GSI), controlled by the multi-allelic *S* locus, encoding two linked genes, the *S-RNase* (Bošković and Tobutt 1996; Tao *et al*. 1999) and the SFB (Yamane *et al.* 2003). Most sweet cherries are self-incompatible, and many are cross-incompatible. In sweet cherry, only pollen tubes carrying an *S*-haplotype differing from the two stylar *S*-haplotypes can fertilize the egg cell. Cultivars having the same *S*-genotype are cross-incompatible and are included in the same incompatibility group (IG). S_1–S_{32} alleles are known in sweet and wild cherries (Matthews and Dow 1969; Bošković *et al*. 1997; Bošković and Tobutt 2001; Tobutt *et al*. 2004; De Cuyper *et al.* 2005; Vaughan *et al*. 2008; Wunsch and Hormaza 2004; Schuster 2012). The allele S_{34}, typical of the sour cherry, was also found in sweet cherry (Szikriszt *et al*. 2013), and a new S_{37} allele has recently been identified in sweet cherry from the Black Sea area, for a total of 47 incompatibility groups reported so far (Tobutt *et al.* 2004; Schuster 2012; Lisek *et al*. 2015) and 15 genotypes with unique S-allele combinations belonging to the pollen donor group 0 (Schuster 2012). Knowledge of sweet cherry S-alleles and cross-incompatibility groups is important for growers, permitting the choice of pollinator cultivars that best ensure fruit set, and for breeders, to plan successful crosses (Marchese *et al*. 2017). Most European, Turkish and North American sweet cherry cultivars and wild genotypes have been S-genotyped (e.g. Struss et al. 2003; De Cuyper *et al*. 2005; Vaughan *et al*. 2006, 2007; Schuster *et al*. 2007; Lacis *et al.* 2008; Schuster 2012; Ipek *et al*. 2011; Ercisli *et al*. 2012; Szikriszt *et al*. 2013; Cachi and Wünsch 2014; Lisek *et al*. 2015), whilst partial information is available on the Italian germplasm (Marchese *et al*. 2017), except for a pool of Sicilian landraces showing a wealth of genetic diversity and the occurrence of natural selfcompatible cultivars, carrying the S_5' allele (Marchese *et al*. 2007a, b). Length polymorphism of the microsatellite in the second intron of S_5-*RNase* associated with S_5' was exploited to predict the occurrence of non-defective SFB_5 or the presence of the mutant one (Marchese *et al*. 2007b, 2017). Also, the polymorphism of the microsatellites in the two introns of S_{13}- *RNase* was used to study relationships among cherry populations, cultivars and species, for parentage analysis in sweet and sour cherries and for identification of Duke cherries (hybrid between sweet and sour cherries), since the S_{13} allele is also present in the sour cherry, but SSR variants are quite distinct among species and between cultivated and wild cherries (Marchese *et al*. 2010). In this work, we analysed the genetic diversity of a large set of sweet cherry landraces which originated from geographically diverse Italian territories using microsatellite markers and characterized for the first time their S-genotypes and cross(in)compatibility groups, along with their genetic structure. Knowledge of *S*-genotypes is crucial for planning future breeding programmes including valuable genetic resources. Identification of synonyms and homonyms allows rationalization of germplasm collections, either by reducing redundancy or by unravelling unknown diversity. Findings of the present work will be important for choosing genotypes to include in core collections representing as much as possible the local or national diversity and covering a wide range of phenotypic variability, which will be further studied at the genetic level and eventually employed in breeding programmes.

Material and methods

Plant material

One hundred eighty-six sweet cherry accessions, originating from 12 Italian regions, were analysed together with eight reference genotypes— "Goodnestone Black", "Napoleon", "Noble", "Noire de Meched", "F12/1", "E621", "SL64" and "F1292"—included as standards, following the recommendation of the European Collaborative Programme for Genetic Resources (ECPGR) in order to harmonize SSR allele scores among laboratories and databases (Clarke and Tobutt 2009) (Supplementary Table S1). *Ex situ* collection sites where most of these sweet cherry resources are maintained are listed in Supplementary Table S1, and information relating to the genetic material studied is available through the Genome Database for Rosaceae (www.rosaceae.org).

DNA extraction, SSR analysis and S-allele identification

Genomic DNA was extracted from young leaves according to the protocol of Doyle and Doyle (1987). Polymerase chain reaction (PCR) amplifications were carried out with 13 fluorescent SSR primer pairs, 11 of which belonging to the set of SSRs selected by the ECPGR Prunus working group for their high power of discrimination and polymorphism (Clarke and Tobutt 2009) —CPPCT: 06, 22 (Aranzana *et al*. 2002); BPPCT037 (Dirlewanger *et al*. 2002); EMPA: 002, 003, 017 (Clarke and Tobutt 2003); EMPaS: 01, 02, 06, 11, 12 (Vaughan and Russell 2004); UCD-CH14 (Struss et al. 2003), UDP98-412 (Testolin *et al*. 2000) (Table 1). Multiplex reactions (MR) were developed (MR1 Vic-EMPA003, 6-Fam-EMPA017, Pet-EMPaS12; MR2 6-Fam-EMPA002, Hex-CPPCT22; MR3 6-FamUDP98-412, primers for the S-locus; MR4 6-FamEMPaS02, Hex-EMPaS06; MP5 Pet-EMPaS01, 6-FamEMPaS11), whilst the remaining primers (6-Fam-CPPCT6; 6-Fam UCD-CH14; 6-Fam BPPCT037) were used in single reaction. The SSRs used in this study are distributed across the eight *P. avium* linkage groups in the maps developed by Olmstead *et al*. (2008) and Clarke *et al*. (2009). The *SFB* alleles were amplified with primer pairs 6-FAM-FBOX50A/ F-BOXintronR (Vaughan *et al*. 2006) and the *S-RNase* alleles with the primer pairs VIC-PaConsI-F/PaConsIR2 (Sonneveld *et al*. 2003, 2006) to identify the *S*-genotypes of the landrace pool analysed. The *S*-locus is located on LG6 in the *P. avium* map "Emperor Francis" × "New York 54" (cM 72) reported by Olmstead *et al*. (2008) and in the interspecific map "Napoleon" × *P. nipponica* (cM 86.1) reported by Clarke *et al.* (2009). PCRs were performed in a final volume of 8 μL as described by Vaughan and Russell (2004) and Marchese *et al*. (2007a) and following the PCR cycles reported by Vaughan and Russell (2004). Amplicon analysis was performed using an ABI 3130 Genetic Analyzer. Fragment sizes were scored with GeneMapper® v4.1 software. To confirm alleles S_2, S_7 and S_{12}, which have similar product sizes, allele specific primers were also used following the methods described by Sonneveld *et al.* (2003).

S_5' detection

The primer pairs S_5-2SSR-F 6-FAMTGTTATTATCGTGC AGACGTTATG and S_5-2SSR-R TTTGACTTGAAGCT TTCATTTAGG reported by Marchese *et al*. (2007b) were used to find variants of *S_5-RNase*, differing with respect to a microsatellite present in the second intron, indirectly associated with the presence of the pollen part mutation *S_5-SFB*. PCR conditions and screening of allelic variants were as described by Marchese *et al*. (2007b).

S_{13} variants

The primer pair S_{13}-1SSR-F-ATT ATG AGC ACT GGT GGG TTG C; S_{13}-1SSR-R-ACC AAA GAA ACC ATG CAG AAA TGT, flanking the microsatellite present in the first intron of the *S_{13}-RNase* allele, and the primer pairs S_{13}-2SSR-F-TTT GAT GTT GGT TTT CTG TTA GG; S_{13}-2SSR-R TTTGAG AAA ACA GAT

AGA TAG ACA G, flanking the second microsatellite in the second intron of the same allele, were used to detect intra-allelic variants as described by Marchese *et al*. (2010), which can be useful for analysis of relationship among genotypes, population and species carrying the S13 allele, for discovering Duke cherries or genotypes derived from them, considering that S_{13} is also present in sour cherry. In addition, relative occurrences of *S*-alleles in 153 Italian sweet cherry accessions were calculated, excluding genotypes sharing the same genetic profiles and individuals not having diploid *S*-genotypes (Table 5).

Molecular data analysis and paternity inference analysis

The number of alleles per locus (*Na*), the observed and expected heterozygosity (*Ho* and *He*, respectively), the frequency of null alleles (F-*null*), the polymorphic information content (PIC) value, the deviation from Hardy-Weinberg equilibrium (HWE), inferred by sequential Bonferroni correction, and combined non-exclusion probabilities (for the first parent NE-1P and second parent NE-2P; parent pair NE-PP; identity NE-I and siblings identity NE-SI) were computed using the CERVUS 3.0 software package (Marshall *et al*. 1998; Kalinowski *et al*. 2007) in 161 sweet cherry accessions, showing unique profiles, excluding the non *P. avium* genotypes. The non-exclusion probability of unrelated individuals is a parameter that indicates the usefulness of markers in relatedness, identity and parentage analyses. The probability that genotypes at a single locus do not differ between two randomly chosen individuals was computed by mean of the non-exclusion probability between two unrelated individuals (NE-I) and two hypothetical full siblings (NE-SI) as reported by Marra *et al*. (2013). In parentage analysis, the nonexclusion probability parameter represents the probability of not excluding any unrelated candidate parent or parent pair from parentage of a certain offspring at one locus (Jung and Jo 2012). Paternity inference analysis, based on SSRs and the S-locus, was accomplished as well with CERVUS 3.0, using the Blikelihood^ approach of Thompson (1975, 1976) and Meagher (1986), by choosing settings for "both parents being unknown" and "incomplete parental sampling". Internal simulations were performed (set to 10,000 runs) in order to calculate the significance of LOD scores (the logarithm of the likelihood ratio). Thresholds of 95 and 99% were chosen as relaxed and strict confidence levels, respectively, with the proportion of loci mistyped equal to 0.005.

Cluster analysis based on SSRs and *S*-alleles

The SSR profiles of 186 Italian accessions were scored, excluding the eight reference accessions. Four local genotypes that amplified three or four S-alleles, whilst diploid at the SSR loci, were also included in the analysis without entering their S-alleles. Genotypes presenting a single peak were considered to be homozygous for that specific locus; the data were converted in a frequency matrix in which, for each locus, the presence of an allele was indicated by 0.5, putative homozygous condition was indicated by 1 and the absence of an allele was indicated with 0. Then, a similarity matrix was generated using Nei (1973) coefficient, with the software Power Marker (Liu and Muse 2005). From the matrix, an unweighted pair group method with arithmetic average (UPGMA) dendrogram was constructed based on the similarity data of SSRs and the S-locus to reveal relationships among accessions, and to visually depict possible synonyms or homonyms (Supplementary Fig. S1).

Structure analysis

In order to study the genetic relationships among 186 sweet cherry accessions from the 12 Italian regions and to identify the genetic structure of the Italian germplasm and the degree of intermixing, the software package *Structure* 2.3.1 (Pritchard *et al*. 2000; Falush *et al.* 2003, 2007; Evanno *et al*. 2005; Hubisz *et al.* 2009) was used. The eight reference genotypes were excluded from the analysis, whilst the four genotypes amplifying more than two *S*-alleles were included, not entering Sallele data. The"admixture" model, postulating 1 to 10 populations (*K*), a burn-in length of 30,000, and 100,000 runs at each *K*, with 20 replicates for every *K*, was used. No prior information was utilized to define the clusters. The log likelihood for each *K* (L(*K*)), following the plateau criterion employed by Rosenberg *et al*. (2002), and the ΔK method (Evanno *et al*. 2005), implemented in *Structure Harvester* (Earl and Vonholdt 2012), were adopted to detect the most significant

number of populations (*K*). Cultivars showing membership probabilities equal to or above 0.80 were taken to group together and to share a close genetic background.

Results

SSR diversity

The SSR markers successfully amplified 126 distinct alleles across 191 accessions (Table 1), counting "Goodnestone Black", "Napoleon", "Noble", "Noire de Meched" and "F12/1", but excluding the three non-*P. avium* genotypes E621, F1292 and SL64. A unique profile was obtained for 161 of them and a further 18 identity groups were found (Supplementary Table S1; Table 2). The average number of alleles per locus was 9.7, ranging from three with EMPA002 to 14 with BPPCT037 (Table 1). The mean *He* was 0.63, ranging from 0.11 for the locus EMPA002 to 0.82 for EMPAS02, the mean *Ho* was 0.65, ranging from 0.06 for EMPA002 to 1 for the locus EMPA003, whilst the mean PIC was 0.58. Null allele frequency values varied from −0.23 (EMPA003) to +0.25 (EMPA002) (Table 1). EMPA003, EMPAS12, BPPCT037 and CPPCT22 deviated from Hardy-Weinberg equilibrium (HWE) after Bonferroni's correction (*P value* < 0.0000001). The value of the total probability of identity for the 13 SSR surveyed and the *S*-locus was 2.508E−0012. The non-exclusion probability between two unrelated individuals (NE-I) ranged from 0.058 with EMPaS02 to 0.8 with EMPA002; the non-exclusion probability between two hypothetical full siblings (NE-SI) ranged from 0.36 with EMPaS02 to 0.9 with EMPA002 (Table 1). Concerning the simulation of parentage, performed to discover putative parentage relationships, combined non-exclusion probabilities for the first parent (NE-1P), second parent (NE-2P) and parent pairs (NEPP) were $7.38 * 10^{-3}$, $1.795 * 10^{-4}$ and $4.7 * 10^{-7}$, respectively, including the *S*-locus (Table 1).

S-locus diversity

A total of 17 *S*-alleles were found in the germplasm surveyed, and the following parameters were found in 153 genotypes (excluding all the identical genotypes, the four polyploids at the S-locus and ECPGR reference genotypes): 1 (*Ho*); 0.85 (*He*); 0.83 (PIC); −0.089 (*F-null*), 0.040 (NE-I); 0.34 (NE-SI); 0.46 NE-1P; 0.3 NE-2P; and 0.13 NE-PP. The *S*-locus significantly deviated from Hardy-Weinberg equilibrium (*P value* < 0.0000001).

Simulation of parentage

Parent simulation assignment, based on SSRs and *S*-alleles, built on LOD scores and confidence level values, identified possible parents for 22 cultivars with significant or positive pair confidence values (Table 3). Furthermore, "Bombardune" (S_6S_{16}) could be derived from the cross "Carrammendula 2" (S_6S_{16}) × "Della Recca" ($S_{13}S_{16}$) (Trio LOD score 1.86E + 01, Trio loci mismatching 0); whils" BDella Recca" ($S_{13}S_{16}$) could have originated from the cross "Bombardune" (S_6S_{16}) × "Montenero" (S_3S_{13}) (Trio LOD score 1.97E + 01, Trio loci mismatching 0).

Cluster and structure analysis

Four accessions "Graffione" (Lazio), "Gambolungo di Garbagna" (Piedmont), "Gemella CI 512" (Emilia Romagna), "Quarantana" (Sicily) and "Di Nello"/"Papale" (Tuscany) grouped quite apart from the other Italian germplasm (Supplementary Fig. S1). A total of 18 groups of probable synonyms were found (Table 2; Supplementary Fig. S1), and six landraces shared identical SSR profiles with cultivars of the ECPGR reference set. In general, there was not a robust clustering based on regions of origin; however, some subclusters were in agreement with regions of provenance (e.g. Sicily, Calabria, Emilia-Romagna, Tuscany), and in some cases, landraces of geographically neighbouring regions displayed a similar genetic background (Emilia-Romagna/ Piedmont/Tuscany/Veneto; Apulia/Calabria/Campania/ Sicily). The software Structure assigned the 186 Italian landraces to six presumed populations (*K*), supported by the plateau criterion employed by Rosenberg

et al. (2002) and the Δ*K* criterion by Evanno *et al*. (2005). A graphical representations generated by the software Structure harvester confirmed that the most likely number of populations (*K*) was 6 (Supplementary Fig. S2). The six populations were indicated in Fig. 1 by the following colours: black, dark grey, grey, light grey, grey white and white. The dark population contained 12 landraces, mainly originated in the two main Italian islands Sardinia (6) and Sicily (3). The dark grey group included a total of 27 landraces, mostly from Southern Italy: Calabria (9), Sicily (4), Apulia (5), and Campania (9). The grey population included seven Italian landraces from various geographical locations, some of which fall into the 18 groups of identity reported in Table 2. Among them "Furticchiara", "Limone" and "Sardinia 1" were indistinguishable from "Napoleon"; "Ferrovia" and "Maiatica di Taurasi" were indistinguishable from "Noire de Meched". The light grey population was composed mostly of landraces from Tuscany, including also one accession from Piedmont ("Bella di Pistoia B", whose name suggests Tuscan origin) and four landraces from Calabria. The grey white group contained a total of 24 accessions, if we exclude those sharing identical SSR profiles, most of which were from regions of Northern and Central Italy— Emilia-Romagna (12), Veneto (4), Tuscany (3), Trentino (1) and Friuli Venezia-Giulia (1)—although occasionally landraces from Southern regions also grouped in this population. The white population included 16 landraces mainly from Piedmont (9), the remaining were from Tuscany (3), Emilia (1), Apulia (1), Campania (1), Lazio (1) and Sicily (1). Tuscan accession "Siso" was identical to the Apulian "Francia"/ "Francesina"

SI groups and relative occurrence of *S*-alleles

A total of 17 different S-alleles (S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_9, S_{10}, S_{12}, S_{13}, S_{14}, S_{16}, S_{19}, S_{21}, S_{22} and S_5') were found combined in 24 out of the 47 incompatibility groups (Table 4) described in the literature so far (Schuster 2012; Szikriszt *et al*. 2013; Lisek *et al.* 2015). Furthermore, 10 new combinations of *S*-alleles were found, which were considered as new incompatibility groups, and named XLVII to LVI. Seven cultivars fell in the universal pollen donor group 0, two of which "Moscatella" and "Nucigliara" were already included in the update table published by Schuster (2012) which included data from various published sources. The three accessions "Maiolina Santa Lucia del Mela" (Sicily), "Sardinia 11" and "Nera di Nuchis" (Sardinia) may have the mutant allele of the pollen SFB_5' discovered in the Sicilian cultivar "Kronio" by Marchese *et al*. (2007b), and therefore, they were taken to be self-compatible (Table 4). As concern "Maiolina Santa Lucia del Mela", the putative presence of SFB_5' was reported by Marchese *et al*. (2017) in a preliminary work on sweet cherry diversity in a restricted number of Italian regions. We decided to supplement Table 4 with the *S*-genotypes of 28 Sicilian sweet cherry accessions previously reported by Marchese et al. (2007a) to provide a complete picture of *S*-allele diversity in the Italian germplasm. Four accessions presented three *S*-alleles: "Cantona" ($S_{12}S_{13}S_{21}$); "Marchiana" ($S_3S_{13}S_{14}$) and "Molfetta" ($S_6S_{10}S_{13}$), and one accession four *S*-alleles "Bianca di Piemonte" ($S_3S_6S_{13}S_{16}$) (Table 4). The most frequent *S*-alleles in 158 Italian sweet cherry accessions (excluding all the identical genotypes, and including the four polyploids at the *S*-locus) were S_3 (25%), S_{13} (20%), S_6 (19%) and S_{16} (7%) followed by S_{10} (5%), S_9 (4%), S_{22} (3.4%), S_{12} and S_{14} (3%) (Table 5). The rarest alleles were S_2, S_4, S_{17}, S_{19} and S_{21} (Table 5).

S_{13} SSR variants

Intra-allelic variations of the two microsatellites located in both introns of the cherry S_{13}-*RNase* gene can enable the distinction of sweet, wild and sour cherries (Marchese *et al*. 2010). In the present work, a total of 85 accessions having the S_{13} allele were screened to check both SSR variants of this allele (Table 6). Variants from 263 bp to 273 bp were found for the SSR in the first intron of the S_{13} allele; variants from 312 bp to 320 bp were found for the SSR in the second intron. "Ceresa Montecastello", "Del Monte", "Galucio", "Niredda Laconi" and the polyploid "Bianca di Piemonte" ($S_3S_6S_{13}S_{16}$) and "Molfetta" ($S_6S_{10}S_{13}$) showed the trace of an extra allele of 249 bp for the first intron SSR, but not for the second intron SSR.

Discussion

The SSR markers used were revealed to be appropriate for genotyping and for depicting genetic relatedness among the accessions surveyed. The average number of alleles per locus was 9.7, ranging from three with

EMPA002 to 14 with BPPCT037, higher than in previous studies of sweet cherry diversity, indicating the richness of the Italian material surveyed. Marchese *et al*. (2007a) obtained 7.2 alleles per locus in 39 Sicilian cultivars with 13 SSR loci; Stanys *et al*. (2012) found an average number of alleles of 5.29 using 14 primers, 12 of which were in common with our work, in 31 Lithuanian sweet cherry cultivars; Frei *et al.* (2010) found 8.1 alleles per loci in 441 sweet cherry from the national Swiss collection using 16 SSR primers, nine of which in common with our study. In general, SSR allelic frequency fitted with frequency expected under Hardy-Weinberg equilibrium, indicating that genotypes surveyed performed like a random mating collection, but four SSR loci deviated from it, EMPA003 and EmPaS12 showing an excess of heterozygotes and BPPCT037 and CPPCT22 presenting an excess of homozygotes. Human or natural selection acting in genes associated to these SSR as well as relatedness among some cultivars may explain these phenomena. In the present study, several cases of synonyms were found which were possibly due to the fact that many landraces were named according to their features (traits related to fruit size, firmness, taste, ripening period or geographical origin), to the exchange of material among regions and to the introduction of foreign cultivars. Cultivars like "Napoleon" and "Burlat" were introduced in Italy in the fifties, and in the same period, "Durone nero 1, 2 and 3", "Ferrovia", "Mora di Cazzano", "Mora di Vignola", and "Napoleona" started to be cultivated in many regions. "Ferrovia" and "Maiatica di Taurasi" were undistinguishable from the Iranian cultivar "Noire de Meched", with whom also share the same *S*-genotype (S_3S_{12}). The identity between "Ferrovia" and "Noire de Meched" has been already reported in the literature (Boritzki *et al*. 2000; Palasciano *et al*. 2009; Campoy *et al*. 2016), whilst we suppose that "Maiatica di Taurus" was mislabelled in the Campanian collection since on the basis of the descriptions provided by Baldini (1973) and Albertini and Della Strada (1996), the two cultivars appear to be morphologically and phenologically distinct. Accurate morphological characterization of "Maiatica di Taurasi" at the site of origin is needed to confirm or reject that putative identity. The SSR profile and the *S*- genotype (S_3S_4) of "Furticchiara", "Limone" and "Sardinia 1" resulted identical to that of "Napoleon" (Tables 2 and 4; Fig. 1), and "Bella di Pistoia A" profile was identical to "Noble", an ancient English variety of unknown origin (Brooks and Olmo 1972). "Noble" is also known with the synonym "Tradescant Heart" (Brooks and Olmo 1972), where Tradescant is the name of two British naturalists, who introduced many new plants and trees to England after expeditions around Europe, between the 16th and 17th centuries. "Noble" showed also a close relationship with "Durone di Cesena", a traditional cultivar of Emilia-Romagna, sharing 26 out of 30 putative alleles (similarity coefficient of 0.94). Regarding the remaining possible cases of identity reported in this study, a meticulous morphological characterization will be started in each collection of origin and if possible material will be transferred in a unique collection to check their phenotypes under the same environmental conditions. The low combined non exclusion probabilities values (NE1P, $1.57 * 10^{-2}$; NE-2P, $5.87 * 10^{-4}$; NE-PP, $3.52 * 10^{-7}$; NE-I 5.984E−0011; NE-SI, $6.1 * 10^{-6}$) indicated that this set of 13 microsatellites was reliable for performing identity and parentage analyses. For instance, NE-1P ($1.57 * 10^{-2}$) indicated that there was a 1.6% probability that these SSR loci combined would not exclude an unrelated candidate parent from parentage of a random offspring when none of the parents were known. NE-2P ($5.87 * 10^{-4}$) revealed that under the assumption that one parent was known, more than 99.9% of the candidate parents could be excluded from paternity. The offspring/parent relationship, built on LOD scores and confidence level values, between "Della Recca" from Campania and the Calabrian "Bombardune" could be explained by the exchange of genetic material between these two regions. "Bombardune" might have originated from the cross between "Carrammendula 2" (Calabria) and "Della Recca". The exchange of material between growers of neighbouring regions may also explain relationships between the Calabrian "Carrammendula 2" with the Apulian "Colafemmina" and the Campanian "Del Monte", as well as relationships between "Mulegnana riccia", "Carrammendula 2" and "Abenavoli bianco". The UPGMA dendrogram showed that landraces from different regions were scattered in different clusters nevertheless, in some cases sub-clustering reflected the area of origin and cultivation (e.g. Sicily, Calabria and Emilia-Romagna) (Supplementary Fig. S1). This was also supported by the structure analysis (Fig. 1) that reflected in some cases geographical areas and indicated possible exchange of material among regions, the hybrid origin of some accessions, and the introduction of material from abroad. In the structure analysis, the plateau criterion employed by Rosenberg *et al*. (2002) and the ΔK criterion by Evanno *et al*. (2005) (Supplementary Fig. S2) allowed the identification of six populations that in most cases reflected geographic area having long tradition of sweet cherry cultivation. The grey white group contained mostly accessions from Northern Italian regions, and, in a few cases, landraces from Southern

Italy, as a result of material exchange followed by local renaming. Landraces from Piedmont (9) mostly belonged to the white population, apart from other accessions of Northern Italy. The production of cherries in Piedmont was considerable over the '30 and '60 and was based on the employment of locally selected clones ("Galucio", "Martini", "Vigevano", "Graffione" and "Vittona") and varieties originating from Southern France (Regione Piemonte 2008). The light grey population was composed of accessions predominantly of Tuscan origin. Tuscany has a long tradition in sweet cherry production especially in the hills around Pisa (Basso and Natali 1959), particularly in the Municipality of Lari (e.g. "Cuore", "Usignano") and around Florence ("Poponcina"). It is difficult to explain why four Calabrian landraces "Cuore Aspromonte", "Vallescura 1", "Vallescura 2" and "Maiatica" present in the same area of cultivation, in the southern Tyrrhenian coast of Aspromonte, fall also into this population; however, they could have been brought a long time ago to Calabria from Tuscany or may have originated from seeds of foreign cultivars. The dark grey group included landraces from Southern Italy Apulia, Calabria, Campania and Sicily. Campania has a long tradition of cherry cultivation, based in particular on the accessions "Del Monte" and "Della Recca", considered the best typical sweet cherry in the whole region (Di Vaio *et al*. 2015). We found in our parentage analysis that some Campanian accessions like "Della Recca", "Del Monte" and "Mulegnana riccia" shared offspring/parent relationship with Calabrian and Apulian landraces, indicating exchange of material among neighbouring regions. The dark group contained landraces from Sicily and Sardinia. We do not know the reason of relationship among genotypes of these geographically distant islands, but it is possible to speculate that they may share a common ancestral genetic pool or that they may derived from few cultivars introduced from abroad from which local ecotypes were subsequently derived. Interestingly, the natural pollen part mutation SFB_5', found for the first time in Sicilian sweet cherries (Marchese *et a*l. 2007b, 2017), may be also present in Sardinian genotypes. The grey population included seven Italian landraces from various geographical locations four of which are possible synonyms of the foreign cultivars BNapoleon^ and BNoire de Meched^ (Table 2), confirming the introduction of material from abroad. The Tuscan accession "Di Nello"/"Papale", "Graffione" from Lazio, the Sicilian "Quarantana" and "Furticchiara" and "Gambolungo di Garbagna" from Piedmont, fell into the grey group, probably as the result of exchange of material among regions. Their close grouping in the cluster analysis, distant from the rest of the germplasm (Supplementary Fig. S1), can be due to possible relationship with international cultivars, considering the identity between "Furticchiara" and "Napoleon". Different considerations should be made for cultivars "Benedetta", "Carlotta" and "Vittoria", which presented a mixed genetic structure (Fig. 1). These cultivars were obtained from controlled crosses, using local genotypes as parental material, around the 1970s ("Vittoria"; Bargioni 1970) and the 1980s ("Benedetta" and "Carlotta"; Roselli *et al*. 1983). In recent work reporting the structure analysis of 210 modern cultivars and landraces from
16 countries based on SNP markers, two main populations were found divided in nine subgroups corresponding to regions of landrace distribution (Campoy *et al*. 2016). In the present work, the Italian germplasm clustered in six populations and thus displayed a considerably high level of diversity. Knowledge of clusters depicted by structure analysis could assist the choice of parental genotypes in breeding programmes in order to increase genetic diversity (Campoy *et al*. 2016). A total of 24 diverse incompatibility groups were found (Table 4) from the 47 groups reported in the literature (Tobutt *et al.* 2004; Schuster 2012; Szikriszt *et al.* 2013; Lisek *et al*. 2015) and 10 new incompatibility groups, from XLVII to LVI (Table 4), confirming the high diversity of the Italian sweet cherry germplasm. The seven Italian landraces possessing unique *S*- genotypes have been placed in the pollen donor group 0, which contained, in literature, 15 genotypes with unique *S*-allele combinations up to the harmonization table by Schuster (2012), that was supplemented with data from various published sources, including the *S*-genotypes of the Sicilian cultivars "Moscatella Chiusa" and "Nucigliara", reported by Marchese *et al*. (2007a) and here included in the SSR characterization. However, the accession "Giorgia", which shared the *S*-genotypes S_1S_{13} with "Durella di Cesena" and "Durona San Giovanni CI 504" was removed from the group O and constituted the new S-group LI. Thus, in total, 14 genotypes so far belong to the pollen donor group O. Twenty-eight Sicilian accessions previously *S*-genotyped by Marchese *et al.* (2007a) were included in Table 4 to provide complete information on *S*-incompatibility groups useful to growers and breeders. Two Sardinian genotypes "Sardinia11" and "Nera Nuchis" and the Sicilian "Maiolina Santa Lucia del Mela", that was recently *S*-genotyped by Marchese *et al.* (2017), may have the mutant allele of the pollen SFB_5', since they showed the SSR variant of 154 bp of the allele S_5-*RNase*, which correlates with SFB_5', discovered in the Sicilian cultivars "Kronio" and "Maiolina a rappu" by Marchese

et al. (2007b), and therefore, they may be selfcompatible. However, controlled self-pollination tests are needed to confirm these data. Two discrepancies were found in *S*-genotype identification between our study and the updated table of Schuster (2012), reporting the *S*-genotypes of 734 sweet cherries; for the cultivars "Grossa di Pistoia" and "Vittoria". "Grossa di Pistoia" had the genotype S_1S_3 instead of S_3S_6, whilst "Vittoria" was shown in this study to have the S_3S_5 genotype, instead of S_3S_{14}. In addition, a discrepancy was found in the *S* genotype of "Turca", between our work and the harmonization table of Tobutt *et al*. (2004). "Turca" seemed to have S_3S_{13}, whilst it was reported to have S_6S_{13}. Therefore, it is possible that some confusion exists on these cultivars, and further analysis including morphological interrogation is needed to compare them. It is also possible that some errors occurred in the collection or labelling of the material; hence, this work represents an opportunity to eliminate mistakes in the original collections. The most frequent *S*-alleles in the Italian germplasm were, in descending order, S_3, S_{13}, S_6 and S_{16} (Table 5). The considerations previously reported by Marchese *et al*. (2007a, 2017) studying the relative occurrence of *S* alleles in the Sicilian sweet cherry germplasm and in local landraces from a smaller number of Italian regions were confirmed in this study, in particular the rarity of S_1, S_2 and S_4 that in contrast are very common in other sweet cherry pools (Bošković and Tobutt 2001; Lacis *et al*. 2008; Ipek *et al*. 2011; Schuster 2012). S_1 occurrence (3%) was similar to that reported in Turkish germplasm (2.5%) by Ipek *et al*. (2011) and in Croatian germplasm (3%) by Ercisli *et al*. (2012). The S_4 allele was extremely rare in the Italian landraces (1%), since it was identified only in three accessions "Furticchiara"/"Limone"/"Sardinia 1" (synonyms of "Napoleon", S_3S_4), and in "Pagliarella" (S_4S_{13}) and "Calusetto Tumà" (S_4S_6). The low frequency (3%) of the S_4 allele was also found in the Croatian germplasm (Ercisli *et al*. 2012), where S_3 and S_{12} were the most frequent (39 and 19%, respectively). Regarding S_{16}, occurring at a frequency of 7% in the Italian germplasm, this allele was found in wild cherry from Belgium (DeCuyper *et al*. 2005), France (Mariette *et al.* 2010) and UK (Vaughan *et al*. 2008) as well as in European sweet cherry cultivars (Sonneveld *et al*. 2003; Tobutt *et al*. 2004; Marchese *et al*. 2007a; Stanys *et al*. 2012; Cachi and Wünsch 2014). The S_{13} allele, occurring at a frequency of 20% in the Italian sweet cherry landraces, is not common in sweet cherry, being found in less than 2% of the 734 cultivars studied by Schuster (2012). S_9 (4%) was less frequent than reported in the study of Tobutt *et al.* (2004) whilst S_{10} (5%) had an occurrence similar to that of local Turkish sweet cherry genotypes investigated by Ipek *et al*. (2011). Interestingly, the S_{22} allele found in 3.4% of the Italian germplasm investigated has been previously found in wild cherry genotypes from Belgium (De Cuyper *et al*. 2005), in the Hungarian cultivar "Rita" (Bekefi *et al.* 2003) and in two German cultivars "Danners Sp te" and "Kiechelsberger Kracher" (Schuster 2012). We do not know why S_3, S_6, S_{16} and S_{13} are frequent in the Italian germplasm whilst other *S*-alleles are rare; however, we can speculate that some alleles may be linked with traits of adaptation to Italian environmental conditions (Marchese *et al*. 2007a, 2017) or could be the result of a founder effect and selection events (Kato and Mukai 2004). A considerable high number of accessions carried the S_{13} allele, which also occur in sour cherry (Marchese *et al*. 2010). Length polymorphism of the microsatellites in the two introns of *S_{13}-RNase* was investigated since it has been demonstrated that variants differ among species and between wild and cultivated sweet cherry, and thus, their analysis can be useful to clarify relationships and gene flow among cherry populations, cultivars and species, carrying the S_{13} allele, and for identification of Duke cherries (hybrid between sweet and sour cherries) (Marchese *et al.* 2010). In the 85 Italian sweet cherry accessions carrying S_{13} allelic variants of the SSR in the first intron ranged from 263 to 273 bp (Table 6). In Marchese *et al.* (2010), variants 271 and 273 bp were restricted to wild cherry. Therefore, "Mora Piacentina" may be derived from a wild genotype with a peculiar combination of SSR variants (273/320). Regarding the SSR variants of the second intron, they ranged from 316 to 320 bp, but the accessions "Molfetta" and "Fuciletta nostrale" presented the variant 312 bp, previously reported in sour cherry by Marchese *et al.* (2010). "Molfetta" (variants 267/312) had the genotype $S_6S_{10}S_{13}$ and presented the trace of an extra peak of 249 bp, previously detected in the sour cherry cultivar "Ferracida", which had S_{13} SSR variants 249 and 312 bp. The accession "Fuciletta nostrale" showed very peculiar variants (272/312); the variant 272 was found in two Italian sour cherries "Marasca del Lavena" (272/308) and "Marasca Savena" (272/308) and the variant 312 bp reported in sour cherry accessions (Marchese *et al*. 2010). The accessions "Camponica", "Ciliegie bianche Marosticane", "Palermina", "Sotto l'acquavite" and "Sardinia5"/"Tempio Bonannaro" presented the variant 318 bp; "Marfatana", "Mora Piacentina" and "Niredda Laconi" (the latter showing traces of an extra peak of 249 bp) the variant of 320 bp. In Marchese *et al*. (2010), sour cherries presented seven variants, from 308 to 320 bp of which those from 308 to 314 bp were restricted

to this species; wild cherries had variants of 318 bp or longer, and the 322 bp variant was restricted to wild cherry. In this study, eight accessions had variants typical of wild cherry. None of the Italian accessions had variants in the range 247/308 to 261/318 typical of sour cherry; whilst it has been reported that one wild sweet cherry accession of the Black Sea regions presented a combination of variants (251/310) overlapping with sour cherry (Szikriszt *et al*. 2013). In Italy, it is likely that some landraces are close to wild sweet cherry genotypes, which may present a broad variability of S_{13} SSR variants, still unexplored; however, further study is needed to test the validity of this hypothesis, which might involve extending the current analysis to wild sweet cherry genotypes collected in different regions. Wild sweet cherry diversity analysis carried out by De Rogatis *et al*. (2013) showed that the Italian germplasm had very high level of genetic diversity and that wild cherry trees were composed from such a high number of groups that the entire gene pool could be considered an unstructured population, maybe also because there are many areas in which wild cherry traditionally cultivated for wood production coexist with sweet cherry for fruit production, leading an exchange of genetic material between the two groups.

Conclusion

The conservation and characterization of heritage germplasm is a cultural strategy for the valorisation of the genetic resources linked with the history and the traditions of a territory. This study represents the first comprehensive Sgenotype identification of the Italian sweet cherry germplasm useful for choosing pollinators, designing crosses, population studies and conservation of *S*-alleles. It expands our knowledge on the existence of 10 new incompatibility groups, from XLVII to LVI, new universal pollen donor cultivars and on differences of *S*-allele occurrence in the European sweet cherry germplasm. Furthermore, it is the first report on the diversity of a large assortment of Italian sweet cherry landraces, and it represents the first fundamental stage of characterization of accessions. This study will thus be useful for the rationalization of field collections and the establishment of core collections of genetic resources at risk of disappearance, which have yet to be exploited for breeding. A spread of genetic diversity was found in the landraces selected in the environmentally diversified Italian regions for their adaptation to the local growing conditions. This material, being phenologically and morphologically characterized and evaluated in the collections of origin, is featured with traits that may be of interest for breeding programmes, such as the extremely early ripening period of "Maiatica rossa" and "Kronio" (the latter belonging to the "Maiolina" group from Sicily endowed with low chilling requirement, in some cases coupled with selfcompatibility (Marchese *et al.* 2007b) or the very late "Lombardune". 'Interestingly, some Sicilian and Sardinian genotypes may have the mutant allele of the pollen SFB_5'. "Zuccaredda" from Calabria and "Corniola" from EmiliaRomagna are featured with traits such as high flesh firmness coupled with high sugar content and excellent taste. *Ex situ* and on-farm collections represent invaluable resources for allelic diversity and therefore should be further supported for long-term preservation of germplasm, for further improvement by breeders and farmers, and for direct use by farmers for production and marketing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Data archiving statement Sweet cherry SSR data are available in the Genome Database for Rosaceae.

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Table 1 Parameters for 13 microsatellite markers analysed in 161 sweet cherry accessions, showing unique profiles

Locus name	Map location and locus position in centiMorgan (cM)	N_A	*Ho*	H_E	F *(null)*	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI
EmPaS01	LG6 2.4 cM Olmstead et al. (2008); 28 cM Clarke et al. (2009)	11	0.73	0.71	-0.02	0.65	0.71	0.54	0.36	0.14	0.43
EmPaS02	LG3 73.8 cM Olmstead et al. (2008);77.9 cM Clarke et al. (2009)	11	0.79	0.82	+0.02	0.79	0.54	0.36	0.18	0.06	0.36
EmPaS06	LG4 24.9 cM Clarke et al. (2009)	12	0.83	0.78	-0.03	0.75	0.60	0.42	0.23	0.08	0.38
EmPaS11	LG5 46 cM Olmstead et al. (2008); 27.9 cM Clarke et al. (2009)	12	0.60	0.56	-0.05	0.53	0.82	0.64	0.44	0.22	0.52
EmPaS12	LG3 37 cM Olmstead et al. (2008); 38.5 cM Clarke et al. (2009)	11	0.98	0.79	− 0.12[a]	0.75	0.60	0.42	0.23	0.08	0.38
EMPA002	LG1 46.3 cM Clarke et al. (2009)	3	0.06	0.11	+0.25	0.10	0.99	0.95	0.91	0.80	0.90
EMPA003	LG1 114.8 cM Clarke et al. (2009)	5	1.00	0.66	− 0.23[a]	0.60	0.77	0.61	0.44	0.18	0.46
EMPA017	LG2 Olmstead et al. (2008)	6	0.53	0.49	-0.07	0.45	0.88	0.67	0.55	0.30	0.58
BPPCT037	LG5 30.8 cM Clarke et al. (2009)	14	0.67	0.80	+ 0.09[a]	0.77	0.57	0.40	0.21	0.07	0.37
UCDCH14	LG7 54.8 cM Clarke et al. (2009)	12	0.67	0.72	+0.04	0.67	0.69	0.52	0.33	0.13	0.42
CPPCT06	LG8 31.0 cM Clarke et al. (2009)	13	0.58	0.62	+0.03	0.56	0.79	0.64	0.47	0.21	0.49
CPPCT22	LG7 36.4 cM Olmstead et al. (2008); 49 cM Clarke et al. (2009)	6	0.43	0.52	+ 0.10[a]	0.42	0.87	0.77	0.65	0.63	0.57
UDP98-412	LG6 76.9 cM Clarke et al. (2009)	10	0.55	0.62	+ 0.06	0.55	0.79	0.65	0.48	0.22	0.49
Mean		9.69	0.65	0.63	0.01	0.58	0.74	0.58	0.42	0.24	0.49
Total		126									
Combined							$1.57 * 10^{-2}$	$5.87 * 10^{-4}$	$3.52 * 10^{-7}$	5.984E−0011	$6.1 * 10^{-6}$
Combined (including *S*-alleles)							$7.38 * 10^{-3}$	$1.8 * 10^{-4}$	$4.7 * 10^{-7}$	2.508E−0012	$2.1 * 10^{-6}$

Map location, N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, *F (null)* null allele frequency, *PIC* polymorphic information content, *NE-1P* non-exclusion probability first parent, *NE-2P* non-exclusion probability second parent, *NE-PP* non-exclusion probability parent pair, *NE-I* non-exclusion probability (identity), *NE-SI* non-exclusion probability (sib identity), [a] Significant and diverging from Hardy–Weinberg equilibrium

Table 2 List of genotypes assumed identical at the molecular level

Group of identity	Cultivars with identical S-alleles and SSR profile
1	"Avorio"/"Capo di serpe"/"Corittu"/"Durona di Matterello"/"Gavorgnana"/"Nerona"/"Ravenna precoce"/"Roma"/"Sardinia 8"
2	"Bella di Pistoia A"/"Noble"
3	"Cappucciarica Cardeto"/"Cappucciarica San Lorenzo"
4	"Ciliegie bianche Marostegane"/"Zambana"
5	"Corniola CI 79"/"Corniola CI 503"/"Corniola CI 508"
6	"Crevalcore"/"Lingua de fori"
7	"Cuore Aspromonte"/"Di Guglielmo"/"Usignano"
8	"Di Nello"/"Papale"
9	"Durona milanese"/"Mora di Cazzano 40"
10	"Ferrovia"/"Maiatica di Taurasi"/"Noire de Meched"
11	"Fiore CI165"/"Fiore CI510"
12	"Francia"/"Francesina"/"Siso"
13	"Graffione bianco"/"Graffioni del Piemonte"
14	"Furticchiara"/"Limone"/"Napoleon"/"Sardinia 1"
15	"Malizia"/"Malizia falsa"/"San Giorgio"
16	"Morena CI 502"/"Morette"
17	"Napoletana"/"Precoce di Cevoli"/"Precoce della Marca"/"Primaticcia"
18	"Tempio Bonannaro"/"Sardinia 5"

Table 3 Parentage assignment of Italian sweet cherry cultivars by means of SSRs and S-locus based on positive LOD scores and pair confidence level values (+ positive; * significant)

Putative offspring	Putative candidate parents	Pair loci number	Pair loci mismatching	Pair LOD scores	Pair confidence	
Abenavoli bianco	Mulegnana riccia	14	0	6.15E + 00	+	
Bianca di Garbagna	Biancona di Garbagna	14	0	1.21E + 01		*
Ciliegie bianche marostegane/Zambana	Marostegana	14	0	7.74E + 00	+	
Colafemmina	Carammendula 2	14	0	6.27E + 00	+	
Della Recca	Bombardune	14	0	9.91E + 00		*
Del Monte	Carramendula 2	14	0	7.10E + 00	+	
Don Vincenzo	Mulegnana Riccia	14	0	6.30E + 00	+	
Morandina	Corniola 79/Corniola CI 503/Corniola CI 508	14		7.10E + 00	+	
Durone nero 1 155	Durone nero 2201	14	0	6.95E + 00	+	
Forlì	Durona San Giovanni CI 504	14	0	7.87E + 00	+	
Grammendula 2	Carraffune bianco	14	0	8.11E + 00		*
Moddacchia	Cappucciarica San Lorenzo	14	0	8.00E + 00		*
Mulegnana nera	Antuono (*I cand.)*	14	0	6.67E + 00	+	
Mulegnana riccia	Carammendula 2 (*I cand.)*	14	0	7.72E + 00	+	
Montenero	Imperiale di Caserta (*I cand.)*	14	0	6.20E + 00	+	
Niredda	Carraffune bianco (*I cand.)*	14	0	8.14E + 00		*
Paesanella	Antuono	14	0	6.60E + 00	+	
S.Anna	Bella di Firenze	14	0	7.07E + 00	+	
Raffiuna S. Mela	S. Angelo S. Mela	14	0	8.95E + 00		*
Vittona	Galuciu	14	0	1.09E + 01		*
Vittoria	Limone/Furticchiara/Napoleon/Sardinia 1	14	0	8.85E + 00		*
Zambana	Marostegana	14	0	7.74E + 00	+	

Table 4 *S*- genotypes and SI groups of the Italian sweet cherry germplasm

Cultivar name	*S*-genotype	SI group
"Puntalazzese"[a]	S_1S_2	I
"Durone di Coredo"	S_1S_3	II
"Del cuore"		
"Grossa di Pistoia"		
"Vigevano"		
"Furticchiara"[a] /"Limone"/"Napoleon"/"Sardinia 1"	S_3S_4	III
"Quarantana"[a]	S_2S_3	IV
"Corniola CI 508"/"Corniola CI 523"/"Corniola CI 79"	S_3S_6	VI
"Benedetta"		
"Durone di Cesena CI 255"		
["Ginuisa"[b]]		
"Grammendula 1"		
"Grammendula 2"		
"Iancuzza napoletana"		
["Maiolina Messina"[b]]		
"Niredda"		
"Cappuccia Castelbuono"		
"Dura del reddito"		
"Este Brognolico"		
["Napoleona precoce"] [b]		
["Muddisa"[b]]		
"Patanara"		
"Petrocca"		
["Toscana"[b]]		
"Vittoria"	S_3S_5	VII
["Maiolina a rappu"[b]]		
"Maiolina Etna"[a]	S_6S_9	X
"Francesina"/"Francia"/"Siso"		
["Napoleona Virifica"[b] /"Raffiuna Etna"[b]]		
"Durone di Cesena CI 514"/"Durone nero II CI201"	S_6S_{13}	XII
"Maiatica rossa"		
"Duroncino di Cesena CI 500"		
"Durone compatto Vignola"		
"Martini Roma"		
"Imperiale di Caserta"		
"Niedda Laconi"		
"Ciliegia di Udine"		
"Fuciletta nostrale"		
"Bella do Piastoia A"/"**Noble**"		
"Morellona"		
"Bella di Garbagna"		
"Cornaiola"		
"Del Monte"		
"Galucio"		
"Marfatana"		
"Martini Piemonte"		
"Mulegnana riccia"		
["Sampitrisa B"[b]]		
"S. Anna"		
"Gemella' CI 512"	S_1S_5	XIV

Table 4 (*continued*)

Cultivar name	S-genotype	SI group
[“Dura succosa”[b]] “Cristallina” “Di Giardino”/”Vallescura 1”/”Vallescura 2” [“Furistera Napoleona”[b]] “Maiatica” [“Moretta”[b]] [BNapoletana Castelbuono^b] “Nero inferno”	S_3S_9	XVI
“Calusetto Tumà”	S_4S_6	XVII
“Cuore Aspromonte”	S_3S_{13}	XIX
[“Agostina”[b]] “Avorio”/”Capo di serpe”/”Corittu”/”Durona di Matterello”/”Gavorgnana”/ “Nerona”/”Ravenna precoce”/”Roma”/Sardinia8 “Bella di Pistoia B” “Biancona di Garbagna” “Ceresa Colombè” “Ceresa Montecastello” “Corniola di Montecatini” “Don Vincenzo” “Durella di Cesena CI 506” “Duroncino di Costasavina” “Durone nero 1 CI 155” “Fiore CI165”/”Fiore CI510” “Forlì” “Graffioni del Piemonte”/”Graffione bianco” “Malizia”/”Malizia falsa”/”San Giorgio” “Marostegana” “Montenero” “Morandina_CI_506” “Mora piacentina” “Morena CI 502”/”Morette” “Papalona” “Popocina” “Ravenna tardiva” “Roana” “Turca”		
“Maggiolina”	S_1S_6	XX
“Di Nello”	S_3S_{12}	XXII
“Ferrovia”/”Maiatica di Taurasi”/”**Noire de Meched**” “Gambolungo di Garbagna” “Graffione” (Lazio) “San Nicola” “Napoletana”/”Precoce della Marca”/”Precoce di Cevoli”/Primaticcia “Papale”		
[“Durona”[b] /”Napoletana Etna”[b]] [“Minnulara”[b]] “Mulegnana nera” “Pomella” [“Sampitrisa A”[b]]	S_3S_{16}	XXIII

Table 4 (*continued*)

Cultivar name	S-genotype	SI group
"S. Angelo S. Mela"		
"Silvestre"		
"Olpina Desulo"	S_6S_{12}	XXIV
"Passaguai"		
"**Goodnestone Black**^	S_2S_6	XXVI
"Amarena grecanica"	S_3S_{14}	XXXIV
"Carlotta"		
"Crevalcore"/"Lingua de fori"		
"Crognolo"		
"Durona milanese"/"Mora di Cazzano 40"		
["Cavallaro"[b]]	S_5S_9	XXXVII‡
"Zuccaredda"	S_3S_{22}	XXXVIII
"Carrubbedda"[a]		
["Niura dell'Etna"[b]]		
"S. Giovanni"		
["Toscanella"[b]]		
"Bombardune"	S_6S_{16}	XXXIX
"Cappuccia imperiale"[a]		
"Carammendula 2"		
"Caraffune"		
"Durona di Bisceglie"		
["Forma di Cuore"[b] /"Raffiuna Messina"[b]]		
"Montagnola"		
"Raffiuna S Mela"		
"Semenzale morbido"		
["Cappuccia Bivona"[b]]	S_6S_{22}	XL
["Cappuccia doppia"[b]]		
["Cappuccia Etna"[b]]		
"Moddacchia Aspromonte"	S_2S_{10}	XLII
"Pagliarella"	S_4S_{13}	XLV§
"San Pietro San Lorenzo"	$S_{16}S_{22}$	XLVII**
["Don Antoni"[b]]		
"Abenavoli bianco"	S_6S_{10}	XLVIII**
"Bertiello"		
"Cavaliere"		
"Crognolina di Maenza"		
"Graffiona"		
"Zuccarigna"		
"Paesanella"		
"Ciauzara"	S_3S_{10}	XLIX**
"Citra"		
"Cappucciarica Cardeto"/"Cappucciarica San Lorenzo"		
"S. Pietro Cardeto"		
"Lattacci"		
"Zucchero"		
"Zuccarenella"		
"Caraffune bianco"	S_6S_{14}	L**
"Lombardune"		
"Durella di Cesena"	S_1S_{13}	LI**†
"Durona S. Giovanni"		
"S.Pietro S. Lorenzo"	$S_{16}S_{22}$	LII**

Table 4 (*continued*)

Cultivar name	S-genotype	SI group
"Niredda Cappuccia"		
"Cordada Niedda"		
"Caddusa" /["Cappuccia Chiusa Sclafani"[b]]	$S_{13}S_{22}$	LIII**
"Tempio Bonannaro"		
"Vittona"		
"Sardinia 5"		
"Ciliegie Bianche Marostegane"	S_3S_{14}	LIV**
"Zambana"		
"Maggiola2		
"Bella di Firenze"	$S_{13}S_{16}$	LV**
"Camponica"		
"Colafemmina"		
"Della Recca"		
"Cuore"/"Di Guglielmo"/"Usignano"	S_9S_{13}	LVI**
"Durona Misciano"		
"Palermina"		
"Sotto l'acquavite"		
"Antuono"	$S_{10}S_{16}$	O
F12/1	S_7S_{14}	O
"Lauretana"	S_2S_{16}	O
"Moscatella chiusa"[a]	$S_{17}S_{21}$	O
"Nucigliara"[a]	$S_{14}S_{16}$	O
"Meuredda dei Merli"	$S_{12}S_{22}$	O
"Tenalgi Gulza"	$S_{12}S_{16}$	O
"Morella"	S_3S_{19}	O
"Kronio"[a]	$S_5'S_6$	SC
BMaiolina S.Mela^, BSardinia 11^, BNera di Nuchis^	S_3S_5'	SC
Putative polyploid at the S-locus only		
"Bianca di Piemonte"	$S_3S_6S_{13}S_{16}$	
"Cantona"	$S_{12}S_{13}S_{21}$	
"Marchiana"	$S_3S_{13}S_{14}$	
"Molfetta"	$S_6S_{10}S_{13}$	

[a] Sicilian cultivars reported by Marchese et al. (2007a) and genotyped also in the present work with SSR markers

[b] Sicilian cultivars reported by Marchese et al. (2007a), not genotyped with SSR in the present work, included in brackets

In bold reference cultivars

**New group of self-incompatibility

§Including the accession "Bladoròzowa" (Lisek et al. 2015)

†Including the accession "Giorgia" (Schuster et al. 2007)

‡Including the accession "Krupnoplodnaja^" (Bekefi et al. 2003)

/ possible synonyms

O universal pollen donor

SC self-compatible

Table 5 Occurrence of S-alleles in 153 Italian sweet cherry accessions showing unique profiles and diploid S-genotypes

Allele	Occurrence (%)
S_1	0.03
S_2	0.01
S_3	0.25
S_4	0.01
S_5'	0.01
S_5	0.01
S_6	0.19
S_7	0.003
S_9	0.04
S_{10}	0.05
S_{12}	0.03
S_{13}	0.19
S_{14}	0.03
S_{16}	0.07
S_{17}	0.006
S_{19}	0.003
S_{20}	0
S_{21}	0.003
S_{22}	0.003

Table 6 SSR variants present in the first and the second introns of the S_{13} allele analysed in 85 accessions

Accession	S_{13} first intron SSR (bp)	S_{13} second intron SSR (bp)
"A cuore"	263	316
"Avorio"	263	316
"Bella di Garbagna"	265	316
"Bella di Pistoia A"	265	316
"Bella di Pistoia B"	265	316
"Bella di Firenze"	267	316
"Bianca di Piemonte"	269*	316
"Biancona di Garbagna"	265	316
"Caddusa"	269	316
"Camponica"	263	318
"Cantona"	265	316
"Capo di serpe"	265	316
"Ceresa Colombè"	269	316
"Ceresa Montecastello"	265*	316
"Ciliegia Udine"	267	316
"Ciliegie bianche Marostegane"	267	318
"Colafemmina"	263	316
"Cornaiola"	265	316
"Corittu"	265	316
"Corniola di Montecatini"	263	316
"Cuore Aspromonte"	263	316
"Cuore"	263	316
"Del cuore"	263	316
"Del Monte"	263*	316
"Della Recca"	265	316
"Di Guglielmo"	263	316
"Don Vincenzo"	263	316
"Durella di Cesena"	265	316
"Durona di S. Giovanni CI 504"	263	316
"Durona di Mattarello"	265	316
"Durona di Misciano"	263	316
"Duroncino di Costasavina"	267	316
"Duroncina Marcianina CI 500"	265	316
"Durone compatto Vignola"	265	316
"Durone di Cesena CI 514"	265	316
"Durone nero 1 CI 155"	265	316
"Durone nero 2 CI 201"	265	316
"Fiore CI 165"	265	316
"Fiore CI 510"	265	316
"Forlì"	267	316
"Fuciletta nostrale"	272	312
"Galucio"	267*	316
"Gavorgnana"	263	316
"Goodnestone Black"[a]	269	316
"Graffione bianco"	267	316
"Graffioni del Piemonte"	269	316
"Imperiale Caserta"	267	316
"Maggiola"	265	316
"Maiatica rossa"	265	316

Table 6 (continued)

Accession	*S13* first intron SSR (bp)	*S13* second intron SSR (bp)
"Malizia"	263	316
"Malizia falsa"	263	316
"Marchiana"	265	316
"Marfatana"	263	320
"Marostegana"	267	316
"Martini"	267	316
"Martini Piemonte"	265	316
"Mulegnana riccia"	265	316
"Molfetta"	267*	312
"Montenero"	265	316
"Mora piacentina"	273	320
"Morandina CI 506"	265	316
"Morellona"	265	316
"Morena CI 502"	265	316
"Moretta"	265	316
"Nerona"	263	316
"Niredda Laconi"	269*	320
"Noble"[a]	265	316
"Pagliarella"	265	316
"Palermina"	265	318
"Papalona"	263	316
"Poponcina"	263	316
"Ravenna precoce"	265	316
"Ravenna tardiva"	267	316
"Roana"	265	316
"Roma"	263	316
"San Giorgio"	263	316
"Sant'Anna"	265	316
"Sardinia 5"	267	318
"Sardinia 8"	263	316
"Sotto l'acquavite"	265	318
"Tempio Bonannaro"	267	318
"Turca"	263	316
"Usignano"	263	316
"Vittona"	265	316
"Zambana"	265	316

*An extra peak of 249 bp was amplified

[a] Reported in Marchese *et al*. (2010)

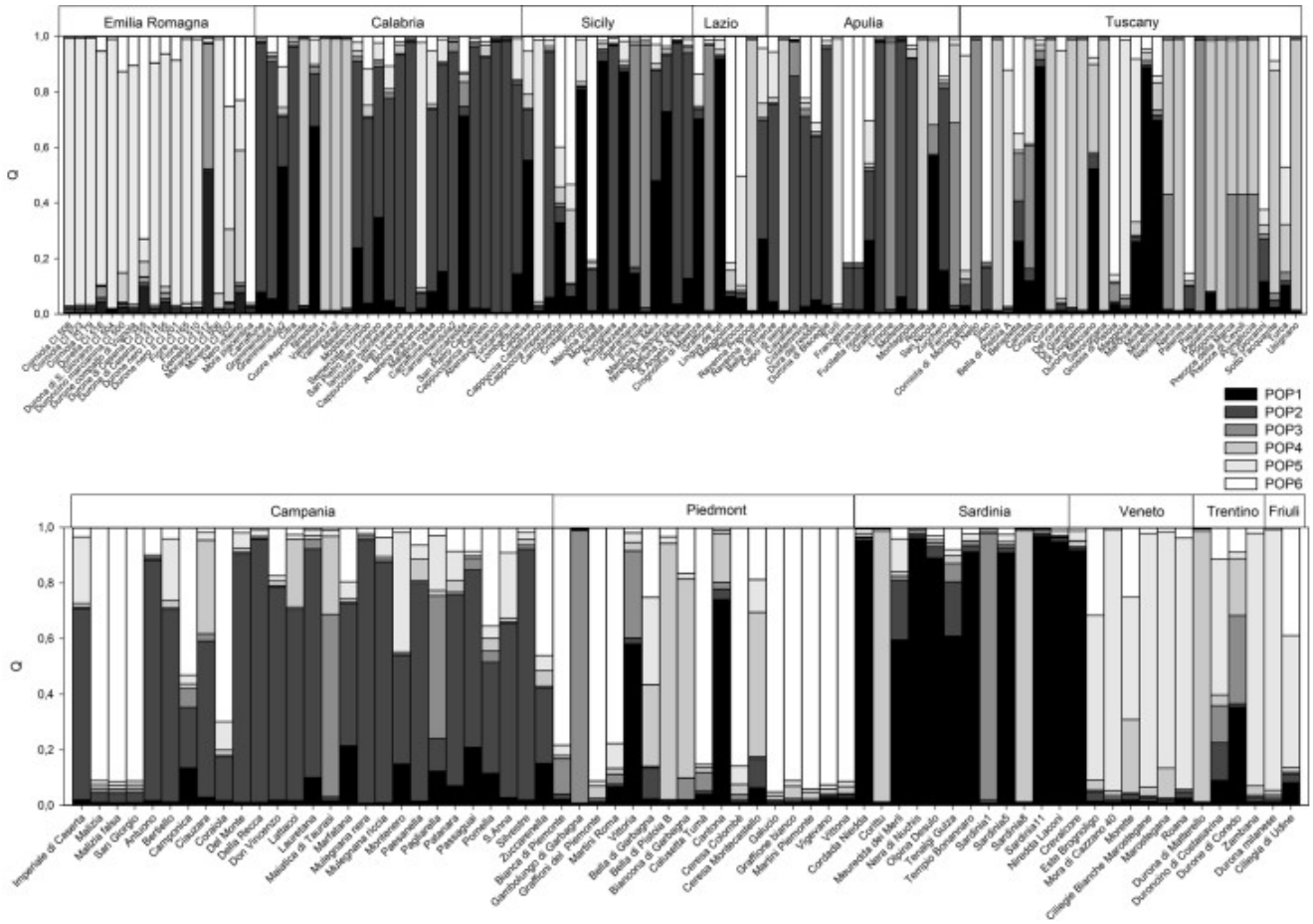

Fig. 1 Genetic structure of 186 Italian sweet cherry landraces, considering $K = 6$. Colours (*black, dark grey, grey, light grey, grey white* and *white*) indicate each of the six groups, defined by the K value. Sweet cherry accessions showing more than one colour may have an intermixed genetic makeup, originated from crossing. The vertical axis designates the membership value 1