Scientia Horticulturae 312 (2023) 111857

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Characterization of red-fleshed pear accessions from Emilia-Romagna region

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ARTICLE INFO

Edited by Wenwu Guo

Keywords:
S-allele
Germplasm
Fingerprinting
SSR
Anthocyanin
Pyrus communis
Genetic diversity

ABSTRACT

Germplasm collections represent a reservoir of traits and genes that might be used in breeding programs to cope with the evolving market demand. Some old pear accessions still cultivated in the Apennine Mountains in Italy possess a red flesh fruit. This paper reports the molecular analysis of 33 red-fleshed pear accessions, collected in different areas of the Emilia-Romagna region and genotyped with 18 simple sequence repeat (SSR) markers with the aim of improving germplasm conservation strategies for old red-fleshed pears and for supporting ongoing breeding programs. The molecular profiles revealed both cases of synonymy and homonymy and only 6 unique genotypes were identified. S-genotypes were also established in order to highlight the genetic relationships among these landraces. Four of the unique genotypes have been clustered based on pomological data.

1. Introduction

Pear cultivation has had a great importance in Italy since Roman times, as evidenced by the description of more than 40 varieties of pears by Pliny the Elder (Hendrick, 1921). In particular, in Emilia-Romagna (ER) pears have been cultivated for a very long time and the environmental variability of this region promoted the development of a rich local germplasm. The ancient landraces must be preserved not only because of their cultural value but also for their high genetic variability, mostly not yet exploited by breeders. Although often lacking in quality in respect to the modern varieties, ancient pear landraces could be used for introgressing valuable traits such as longer shelf-life, precocity of ripening, resilience to environmental or biotic stresses and to introduce peculiar fruit traits, including the red flesh (Sansavini and Ancarani, 2020). The most known variety among the red-fleshed pears in Emilia-Romagna is 'Pera Cocomerina', whose cultivation area is located close to a small village named Verghereto (Forlì-Cesena, Italy). In Italy, 'Pera Cocomerina' is recognised as 'Slow Food' presidium (https://www.slowfood.com/) (https://www.fondazioneslowfood.com/it/presidi-slow-food/pera-cocomerina/) and every year the 'Pera Cocomerina Fair' takes place in the Verghereto village where this pear variety and its by-products (i.e. jam, liquors,..) are promoted (https://www.peracocomerina.it/beta/le-sagre/).

The origin of these accessions is uncertain: red-fleshed pears were first mentioned at the end of XVII century in a manuscript of the Tuscan Academic Pier Antonio Micheli which cited the 'Pera Sanguignola' (literally "bloody pear"). In the following centuries, red-fleshed pears were also reported in France, Belgium and Germany (Leroy, 1867; Downing, 1869; Mas, 1872; Hedrick, 1921). Those heirloom cultivars exhibited many pomological differences in tree habit, ripening time and/or fruit shape, possibly suggesting multiple genotypes. Red flesh is an interesting trait for pear breeding due to the well-known nutraceutical value of anthocyanin in the diet. Their beneficial effects on human health are now widely reported and range from reducing the risk of cardiovascular diseases and preventing the onset of cancer (Seeram et al., 2004; Stevenson and Hurst, 2007; Butelli et al., 2008; Manach et al., 2009; Espley et al., 2014; Antognoni et al., 2020). In particular, the beneficial property of 'Pera Cocomerina' have been already reported in literature by Bucchini et al. (2016), who described the high level of antioxidant compounds contained in these fruits.

There is an increase in the number of varieties with red skin colour on the market for several fruit crops including apricot (Bassi and Foschi, 2019), peaches (Chavez et al., 2019), pears (Brewer and Volz, 2019; Caracciolo et al., 2021) and apples (Chen et al., 2021). The red-flesh trait

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https://doi.org/10.1016/j.scienta.2023.111857
Received 10 November 2022; Received in revised form 23 December 2022; Accepted 14 January 2023
Available online 25 January 2023

was introgressed into many apple cultivars such as 'Red Moon ®', 'Red Love®' and 'Kissabel Rouge®' (Guerra, 2018). The anthocyanin content in the fruit flesh could result in an increased intake of antioxidants in people's diet (Allan et al., 2019).

Genetic diversity in pear could be efficiently estimated by SSR analysis as demonstrated by the huge number of published papers describing the characterisation of local germplasm in Asia (Ahmed et al., 2015; Erfani et al., 2012; Akçay et al., 2014; Song et al., 2014; Liu et al., 2015; Rana et al., 2015; Suprun et al., 2016), in Europe (Fernàndez--Fernàndez, 2009; Martinelli et al., 2008; Bassil et al., 2009; Sisko et al., 2009; Urbanovich et al., 2011; Miranda et al., 2010; Deliquegiovanni et al., 2012; Gasi et al., 2013; Queiroz et al., 2015; Puskaś et al., 2015; Ferradini et al., 2017; Reim et al., 2017; Bennici et al., 2018; Baccichet et al., 2020; Queiroz et al., 2019; Sau et al., 2020; Bielsa et al., 2021; Velázquez-Barrera et al., 2022) and in Africa (Brini et al., 2008).

The identification of S-allele genotypes and phenotypic characterization can complement SSR analysis and confirm the results obtained through molecular characterization (Martinelli et al., 2008; Bennici et al., 2018 and 2020). S-allele diversity has been widely studied through S-genotyping in Japanese (Gu et al., 2009; Yamamoto et al., 2011) and European pear (Zuccherelli et al., 2002; Sanzol, 2009; Nikzad et al., 2014; Bennici et al., 2020) in order to improve the knowledge about self-incompatibility and giving tools for boosting breeding programs.

In the current study, red-fleshed pear landraces of Emilia-Romagna were examined for their genetic diversity to provide insight on the genetic basis of the trait and promote breeding initiatives for its introduction into new pear varieties.

2. Materials and methods

2.1. Plant material

A total of 33 red-fleshed accessions have been collected and analysed in this study, including 20 samples from private orchards and gardens in Verghereto (FC, Italy), 6 samples from CREA's pear germplasm collection (Forlì, FC Italy), 6 samples from UNIBO's pear germplasm collection (Cadriano, BO, Italy) and one from private nursery (Parma, PR, Italy). Three white-fleshed commercial cultivars, 'Abate Fétel' ('Abbe Fétel'; AF), 'Decana del Comizio' ('Doyenne du Comice'; DC) and 'William Bon Chretien' (W; also known as 'Bartlett'), obtained from the UNIBO collection were also included in this study as reference (Table S1).

2.2. DNA extraction

Young leaves were collected in springtime and stored at −80 °C. DNA was extracted by using a CTAB protocol (Mercado et al., 1999). Genomic DNA was quantified by NanodropTM ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and diluted to 50 ng/μL as a working solution.

2.3. SSR genotyping, cluster analysis and S-allele genotyping

A panel of 18 SSR markers was chosen among the most used in literature: 14 of them are included in a former list recommended by the ECPGR (European Cooperative Programme for Plant Genetic Resources) *Malus/Pyrus* working group (Evans et al., 2009) and four SSR markers were selected to be located in other chromosomes not covered.

Forward primers were labelled with four different fluorescent dyes (6-FAM, PET, HEX or NED) in order to combine PCR products in a single electrophoretic run. The list of primer and their characteristics are reported in supplementary material Table S2.

The PCR reactions were performed with the Thermal Cycler 2700 GeneAmp PCR System (ABI Prism) in 1 μL of DNA solution and 9 μL of master mix prepared according to Sau et al. (2020) but by using the AmpliTaq Gold (Thermo Scientific, Wilmington, USA) as DNA polymerase. The reaction cycling conditions were as follows: initial denaturation step of 10 min at 95 °C, followed by 6 cycles using a touchdown amplification program with an annealing temperature reduced by 1 °C per cycle from 60 °C to 55 °C; then 32 cycles, each consisting of 30 s denaturation at 95 °C, 90 s annealing at 55 °C and 60 s elongation at 72 °C and the last cycle ends with a final 10 min extension at 72 °C.

Nine pooling groups of 2 SSRs labelled with different fluorescent dyes (Table S2) and characterised by different fragment lengths were designed for SSR genotyping by ABI PRISM 3730 DNA analyser. PCR products were pooled in a ratio of 1:1. One μl of each PCR product was added to 8 μl of formamide containing 0.2 μl of GeneScan 500 LIZ size standard (Applied Biosystem). Fragments were analysed and visually scored using Peak Scanner v.1.0 (Applied Biosystem).

The SSR data were organised as a square matrix to be analysed by NTsys 2.0 (Rohlf, 1988). The cluster analysis was carried out by using the DICE coefficient (Dice, 1945) and the relative dendrogram was calculated by using the Unweighted Pair-Group Method (UPGMA). The results were used to identify synonyms and homonyms and unique genotypes. The number of alleles per locus (k), the expected and observed heterozygosity (HExp and HObs) and the polymorphism information content (PIC) of unique genotypes were estimated using CERVUS Software v3.0.3 (Kalinowski et al., 2007). The frequency of null alleles, was calculated by using the maximum likelihood (ML) estimator of Kalinowski (2007)

S-allele combinations on the unique genotypes has been determined as reported by Nikzad et al. (2014). The PCR products obtained with the S-allele consensus primers (PycomC1F1 and PycomC5R1) were separated by 1% agarose gels. Based on the amplicon lengths, allele-specific primers were used to confirm the S-genotypes (Table S3; Nikzad et al., 2014).

2.4. Fruit quality analysis

At the ripening time, fruit length (FL), fruit diameter (FD), fruit weight (FW), flesh firmness (FF), soluble solid content (SSC), juice pH and titratable acidity (TA) have been determined both on the four unique genotypes and on the three commercial cultivars (AF, W, DC) used as a reference. FF was determined using a digital penetrometer (Güss Fruit Texture Analyzer equipped with a 8-mm tip, Strand, South Africa) taking two measurements per fruit. SSC was measured in single fruits using a digital refractometer (Atago Pocket Refractometer PAL-1, Tokyo, Japan). TA was measured by titration of the juice obtained from a pool of 10 fruits with a NaOH 0.5 M solution (Crison Titromatic 1S, Barcelona, Spain). The flesh red colour intensity was visually evaluated by using a 0 to 5 scale (0 corresponding to the absence of colour and 5 to its highest intensity). This evaluation was performed at four different positions within the fruit sections: seed locule (SL), fruit core (FC), fruit flesh (FF) and under-skin region (US). All analysis has been performed at ripening time on samples of 10 fruits from three plants per accession from the UNIBO's pear germplasm collection (Cadriano, BO, Italy). 'Cocomerina Selvatica LaCasa' and 'Incrocio Sant'Alessio' were excluded from this analysis because these accessions were single trees grown in other locations and the different pedoclimatic conditions could impact the amount of anthocyanin in the fruit flesh.

A cluster analysis was carried out by analysing the phenotypic data with the dissimilarity index of Canberra available on the software package NTSYSpc 2.0 (Rohlf, 1988).

3. Results

3.1. Genetic and cluster analysis

The 18 selected SSR markers amplified 133 alleles with an average of 7.389 alleles per locus. The number of alleles ranged from 4 ofCH04e03 to 11 ofCH03g07. The frequencies of the allele in each locus were

reported in supplementary material (Table S4). The expected heterozygosity (HExp) ranged from 0.471 (CH04e03) to 0.915 (CH01d09 and CH04c07) with an average value of 0.795. The observed heterozygosity (HObs) ranged from 0.333 (CH01a02 and GD147) to 1.000 (CH01f07 and CH01d08) with an average of 0.729. The Polymorphism Information Content (PIC) value indicated that the most informative loci were CH04c07 and CH01d09, both with the value of 0.850; the lowest value was observed in CH04e03, with 0.409 (Table 1). The frequency of null alleles, as calculated by Cervus using the maximum likelihood (ML) estimator of Kalinowski (2007), is negligible (data not shown). This observation is supported by the fact that almost all the analysed samples were in heterozygosis. for most of the loci while just a few were in putative homozygosis.

Cluster analysis elucidated genetic relationships among varieties and four groups of synonyms were identified (Fig. 1).

Group 1 includes 6 accessions with 6 different names: 'Pera Sanguigna', 'Pera Cocomera', 'Pera Vinata' from the CREA germplasm collection, 'Salama' and 'Pera Polpa Rossa' from UNIBO germplasm collection and 'Ingurien' from a private nursery in Parma. 'Pera Sanguigna' (PS) was selected as the reference for this group.

Group 2 clusters together all trees attributable to the landrace 'Cocomerina Tardiva' (CT; known also with the name 'Cocomerina Invernale'). Most of the samples were collected in the Verghereto area. 'Cocomerina Tardiva' samples from UNIBO and CREA were used as reference.

Group 3 includes the samples of 'Cocomerina Precoce' (CP; with references from both UNIBO and CREA germplasm collections). All the samples denominated with this name from the Verghereto area are included in this cluster. An accession of 'Briaca' maintained in the UNIBO collection ('Briaca Ghetti') was unexpectedly included in this group.

The last group is composed of two 'Briaca' (B) accessions present in the UNIBO and CREA collections (group 4). Despite their common name, the three analysed 'Briaca' accessions showed two different molecular profiles.

The other two red-fleshed accessions, 'Cocomerina Selvatica La Casa' (CS) and 'Incrocio S. Alessio' (IA) resulted as unique genotypes and very diverse in respect to all the other genotypes.

3.2. Unique genotype S-allele determination

All the unique genotypes identified with the cluster analysis were analysed at first by using the consensus primer approach and based on the estimated fragment lengths; subsequently, a panel of allele-specific primers were used for confirming all *S*-allele attributions (Table 3).

Results evidenced the presence of 6 different S alleles in the 6 analysed accessions (*S101, S104, S105, S108, S120* and *S125*) with the allele *S104* that is the most frequent one being present in three accessions (CP, CT and IA). The allele *S120* was detected in CT and CS and *S125* in CP and PS. The remaining alleles *S101, S105* and *S108* were present only once in the analysed samples. For the samples PS only one allele was clearly identified (*S125*) and more research will be needed for identifying the second one (Table 2).

Table 1
The number of alleles (k), the observed (HObs) and expected (HExp) heterozygosity, the polymorphic information content (PIC) were reported for each SSR locus tested in the 9 analysed unique genotypes of *P. communis*.

Locus	K	HObs	HExp	PIC
CH01D09	10	0.889	0.915	0.850
CH05C06	7	0.889	0.771	0.696
CH01F07	8	1.000	0.889	0.820
CH02B10	8	0.778	0.869	0.798
CH01Vf	8	0.556	0.876	0.806
CH02C09	7	0.556	0.745	0.679
EMPC11	7	0.778	0.739	0.670
CH03D12	7	0.778	0.824	0.747
EMPC117	7	0.889	0.824	0.753
CH04E03	4	0.556	0.471	0.409
GD147	5	0.333	0.549	0.485
GD96	6	0.444	0.739	0.669
CH01D08	6	1.000	0.797	0.718
CH03G07	11	0.778	0.882	0.819
CH04C07	10	0.778	0.915	0.850
CH01A09	7	0.889	0.791	0.712
CH01H10	6	0.333	0.797	0.718
CH01H02	9	0.889	0.908	0.842
Average	7,39	0,729	0,795	0,725

3.3. Fruit quality analyses

Four of the 6 unique genotypes and the 3 reference cultivars were also analysed for their fruit quality features and for the intensity and distribution of the red colour in the fruits (Table 3). The red fleshed genotypes IA and CS were not analysed because they are single trees located in a not comparable environment.

Based on the variance analysis among cultivars, statistical differences of significant level were observed in most of the considered parameters such as fruit diameter, fruit length, fruit weight, fruit firmness and sugar. In fact, only pH did not show significant differences among samples. As expected, all the fruit size-related traits were higher in reference cultivars than in the red-fleshed genotypes.

CT reached the highest firmness values 8.12 kg/cm^2, when compared to the other cultivar. When it comes to sugar content in the fruit (SSC), AF cultivar reached the lowest value (15.7), while CT cultivar had the highest ones (19.7). About the flesh colour trait, the reddest one was CT within the highest values for each parameter. No significant differences could be identified among the other three accessions.

Regarding different intensity and position of red colour CP and B looks quite similar, with the highest concentration of anthocyanin in the fruit core and some reddish spots all over the flesh. PS has a characteristic strong red colour ring around the fruit core. The flesh has very strong pigmentation in CT, as well as the fruit core. Also, in these fruits the red was not uniformly spread but it appeared in patches (Fig. 2).

A new cluster analysis was carried out by using a dissimilarity index (Canberra) with these analytical data (Fig. 3 B). In both graphs CP, CT and PS were clustered together. Reference varieties are grouped along with each other in both the analyses. Nevertheless, they showed a more marked similarity in the fruit quality related graph.

In contrast to the genetic data-related dendrogram, 'Briaca' was included in the red fleshed cluster within the phenotype analysis.

4. Discussion

In this study the molecular characterization of a panel of 33 accessions collected in a very narrow area of the Emilia-Romagna Apennines and from two germplasm collections resulted in the identification of six unique genotypes. Four of these genotypes were also characterised for their fruit quality features and for the red colour distribution and intensity in order to estimate how much the genetic diversity determined by SSRs reflects the phenotypic variability. The importance of characterising pear germplasm collections with molecular markers, particularly by SSRs, was widely reported in literature as a tool for assessing pear genetic diversity (Fernàndez-Fernàndez, 2009; Evans et al., 2009; Sehic et al., 2012; Dequigiovanni et al., 2012; Urrestarazu et al., 2015).

The panel of 18 specific SSR markers allowed to identify four clusters of unique genotypes. Regarding the effectiveness of each marker, as already reported in literature, CH01D09 and CH01F07a were found to have a high discrimination power. Moreover, CH04E03 had shown low levels of PIC as previously reported (Gasi et al., 2013; Queiroz et al., 2015; Baccichet et al., 2020; Queiroz et al., 2019; Sau et al., 2020; Bielsa et al., 2021).

CP and CT genotypes were confirmed as well-known varieties and only a misnomer was found. At the opposite, PS had shown several

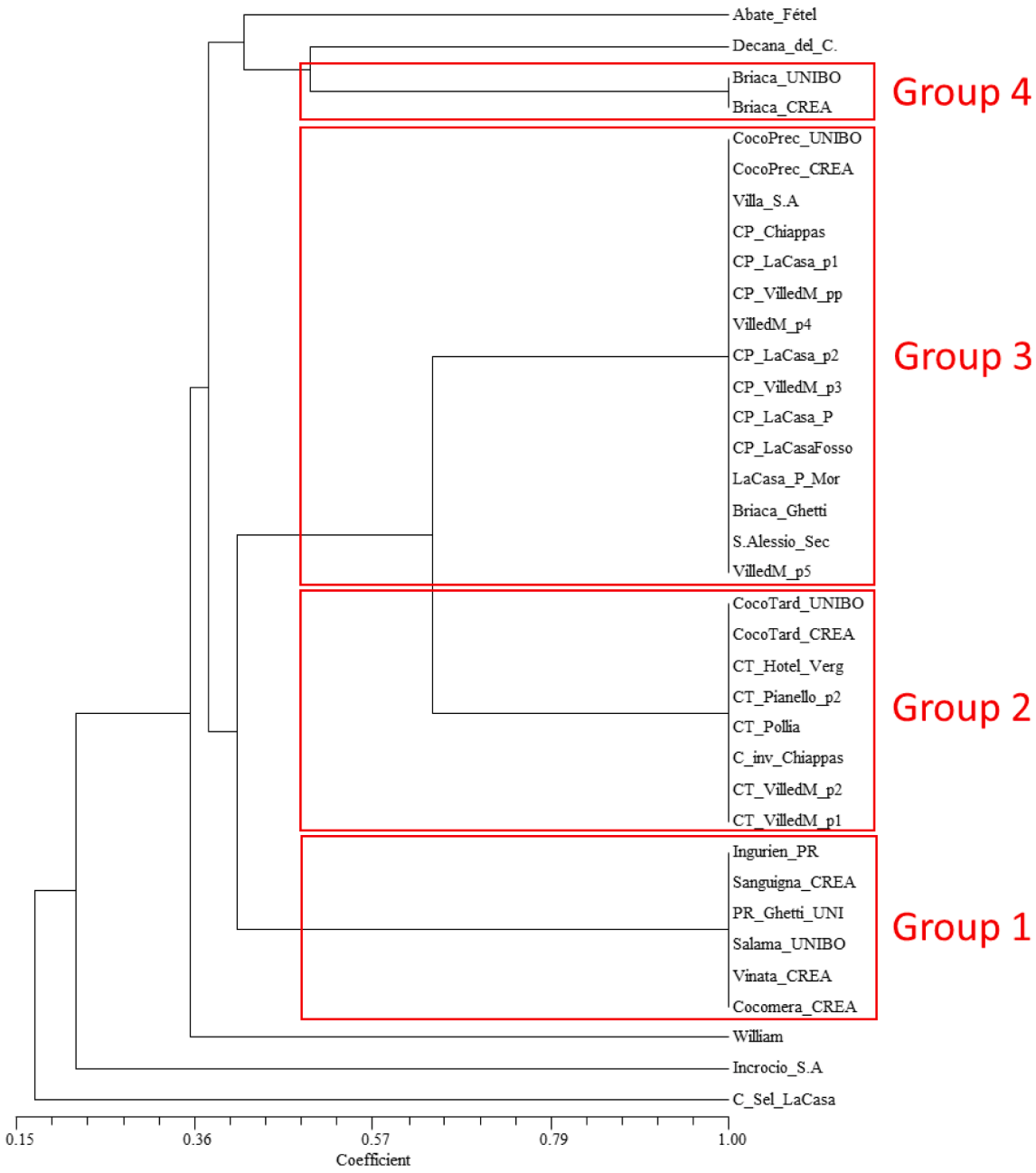

Fig. 1. Dendrogram carried out by NTSYS using the Dice similarity index among the 33 pear landraces analysed. Three commercial cultivars were included as reference.

Table 2
S-Allele combinations of each unique genotype determined by using consensus and allele-specific primers. The fragment size indicates the band size identified by using consensus primers.

Genotype	fragment size (bp)	S-alleles
Cocomerina Tardiva	750/800	*S104/S120*
Cocomerina Precoce	750/1700	*S104/S125*
Briaca	650/1300	*S105/S101*
Pera Sanguigna	1700	*S125*
Cocomerina Sel LaCasa	800/1300	*S120/S101*
Incrocio S. Alessio	680/750	*S108/S104*

different accession names all related to the same genotype.

The large genetic distance observed for CS and IA might have originated by hybridisation with other *Pyrus* species, such as *Pyrus pyraster*, that are widespread in the upper Apennine Mountains as already reported by Bennici et al. (2018). The genetic diversity analysis of *Pyrus* collection performed by Montanari et al. (2019) provides further hint that red fleshed may be connected to the wild species, particularly with *Pyrus pyraster* since three red-fleshed genotypes ('Sanguignole', 'Rottkottig' and 'Summer Blood Birne') were included in the admixture group between *Pyrus communis* and *Pyrus pyraster*. Further investigations should be conducted to determine whether the red-fleshed trait is present in the local wild pear populations and to figure out in which direction the gene flow occurred.

The identification of synonyms and homonyms highlighted the importance of determining the true-to-types plants to be used as reference for the correct conservation of these genotypes and for preserving them against a possible genetic erosion. The availability of well-genotyped plants, as references, is also important for supporting the correct nursery propagation of 'Cocomerina Precoce' and 'Cocomerina

Table 3
Fruit quality data determined at the ripening time: fruit weight (FW), flesh firmness (FFi), Soluble Solid Content (SSC), seed locules red intensity (SL), fruit core red intensity (FC), fruit flesh red intensity (FF), under fruit skin red intensity (US), titratable acidity (TA), pH of the juice, fruit length (FL), fruit diameter (FD), fruit length and fruit diameter ratio (FL/FD).

Genotype	FW		FFi		SSC		SL		FC		FF		US		TA		pH		FL (mm)		FD (mm)		FL/FD			
Briaca	43.5		cd		4.76		bc		17.9		b		2.3		b		1.0		b		1.0		b		0.0		b	
Pera Sanguigna	44.7		c		5.46		b		18.9		a		2.7		ab		1.3		b		1.0		b		0.0		b	
Cocomerina Precoce	45.9		c		4.67		Bc		18.4		a		2.2		b		1.0		b		1.0		b		0.0		b	
Cocomerina Tardiva	37.1		d		8.12		a		19.7		a		3.2		a		2.8		a		2.6		a		3.0		a	
Abate Fétel	151.3		a		4.25		c		15.7		c		0.0		c		0.0		c		0.0		c		0.0		c	
Decana del Comizio	154.2		a		5.01		bc		18.2		ab		0.0		c		0.0		c		0.0		c		0.0		c	
William	139.8		b		4.49		bc		16.4		bc		0.0		c		0.0		c		0.0		c		0.0		c	
Briaca	43.5	cd	4.76	bc	17.9	b	2.3	b	1.0	b	1.0	b	0.0	b	5.1	ab	4.2	a	38.9	de	41.2	cd	0.94	d		
Pera Sanguigna	44.7	c	5.46	b	18.9	a	2.7	ab	1.3	b	1.0	b	0.0	b	4.5	b	4.3	a	44.5	d	43.3	c	1.02	d		
Cocomerina Precoce	45.9	c	4.67	Bc	18.4	a	2.2	b	1.0	b	1.0	b	0.0	b	5.2	a	4.4	a	43.3	d	45.5	c	0.95	d		
Cocomerina Tardiva	37.1	d	8.12	a	19.7	a	3.2	a	2.8	a	2.6	a	3.0	a	4.6	ab	4.2	a	35.2	e	35.3	d	0.99	d		
Abate Fétel	151.3	a	4.25	c	15.7	c	0.0	c	0.0	c	0.0	c	0.0	b	4.8	ab	4.2	a	133.9	a	62.5	b	2.14	a		
Decana del Comizio	154.2	a	5.01	bc	18.2	ab	0.0	c	0.0	c	0.0	c	0.0	b	5.1	ab	4.1	a	100.6	c	68.4	a	1.47	c		
William	139.8	b	4.49	bc	16.4	bc	0.0	c	0.0	c	0.0	c	0.0	b	4.9	ab	4.2	a	120.1	b	63.3	b	1.89	b		

* Data followed by different letters are significantly different (ANOVA followed by Tukey test. $p < 0.05$).

Tardiva' for which there is an interest among pear growers of the Verghereto district. It should be remembered that all the names given to red-fleshed landraces referred to their peculiar trait, which could be the reason why there were so many cases of homonyms and synonyms. For example, 'Briaca' is the most used term in Tuscany for red-fleshed pears and, probably, in the past, with the names 'Briaca' or 'Briaco' were also used for trees that were clearly ascribable to the accessions 'Cocomerina Tardiva' and 'Cocomerina Precoce' as reported in previous works (Camangi et al., 2006; Martinelli et al., 2008; Ferradini et al., 2017; Pastore et al., 2020).

The use of the consensus (Sanzol and Robbins, 2008) and allele-specific primers (Sanzol, 2009; Nikzad et al., 2014) demonstrated to be a very efficient method for the determination of the *S* allele combinations of pear modern varieties and old landraces (Sanzol, 2009; Nikzad Gharehaghaji et al., 2015; Shi et al., 2018; Bagheri and Ershadi, 2020; Bennici et al., 2020; Gasi et al., 2020). Nevertheless, a 'Pera Sanguigna' S-allele has not been identified. The S-genotypes obtained from the analysis allowed to establish that all the accessions are inter-fertile. The presence of an allele in common in most of the red-fleshed varieties present in a very narrow environment also support the possible presence of genetic relationships among these genotypes.

For a better estimation of this aspect the results of the genetic diversity determined by SSRs have been compared with those obtained by using a dissimilarity index for analysing the variability present in analytical data.

The two dendrograms obtained using these two approaches evidenced a clear separation between the red-fleshed landraces and the references 'Abate Fétel', 'William' and 'Decana'. Considering the red-fleshed landraces, both approaches possibly indicated a relationship between 'Cocomerina Tardiva' and 'Cocomerina Precoce', as expected considering that they originated in the same area. The evidence that these two varieties share at least one common allele for each examined locus, including *S*-locus *S104* allele, suggest that they may be very closely related: indeed, this segregation pattern could be compatible with a direct kinship such as mother-daughter. On the other hand, their kinship with 'Pera Sanguigna' seems to be less strong, but still consistent due to the sharing of several alleles all over the characterised loci, among which the most significant was the partaken S-locus S125 allele within CP. Interestingly, this allele resulted to be infrequent within the varieties of the Italian germplasm (Bennici et al., 2020).

5. Conclusions

The identification of unique references for the main four red-fleshed accessions will pave the way to their propagation since the emerging interest in these old landraces. In particular, a 'Cocomerina' pear consortium was established to promote the cultivation of these accessions in the upper Savio valley (FC, Italy). The organisation harvests the fruit each year and either sells the pears unprocessed or transformed into jams and liquors. The 'Slow Food Presidium' has counted all the surviving trees, assisted farmers with harvesting, and it has the purpose to establish an educational and experimental orchard where the plants may be propagated.

The knowledge of the S-allele combinations in the six unique red-fleshed genotypes is very important to properly design the new orchards since the increasing interest and demand of this type of fruit. The right choice of cultivar combination, with compatible *S*-genotype, could significantly improve the fruit set and therefore the field productivity.

Concerning this fascinating trait in pear, it should be advisable to analyse more samples collected in different areas, for example, testing other European red-fleshed landraces could further shed light about the origin of the trait. Nevertheless, finding more genotypes that possess this trait may be the key to develop a molecular marker that might be very helpful for the ongoing pear breeding programs.

Fig. 2. Different intensity and position of red colour in fruits of red-fleshed accessions fruits. 'Cocomerina Precoce' (top left); 'Cocomerina Tardiva' (top right); 'Briaca' (bottom left) and 'Pera Sanguigna' (bottom right). Fruit equatorial section (left) and lateral shape (right) are represented. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

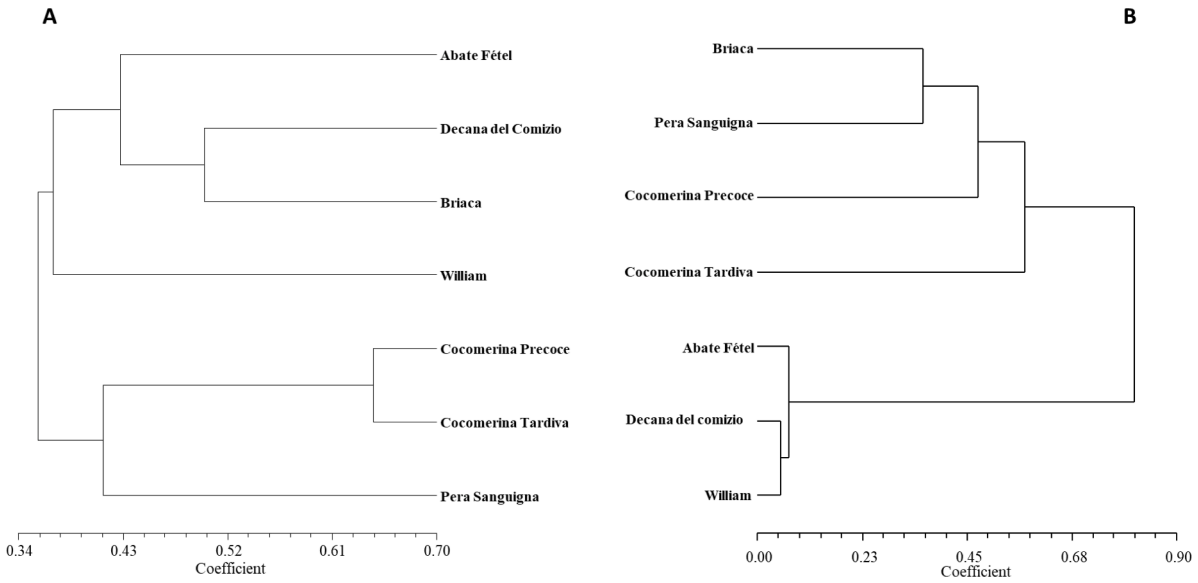

Fig. 3. Dendrograms calculated based on genotypic (A; SSR with the DICE similarity index) and phenotypic data (B; with Canberra dissimilarity index) among 4 red-fleshed pear landraces and the 3 commercial cultivars used as a reference. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

CRediT authorship contribution statement

Lorenzo Bergonzoni: Conceptualization, Investigation, Formal analysis, Methodology, Writing – review & editing. **Sara Alessandri:** Investigation, Formal analysis, Methodology, Writing – review & editing. **Cecilia Domenichini:** Investigation, Formal analysis, Methodology, Writing – review & editing. **Luca Dondini:** Conceptualization, Methodology, Writing – review & editing. **Giuseppina Caracciolo:** Investigation, Formal analysis, Methodology, Writing – review & editing. **Marco Pietrella:** Investigation, Formal analysis, Methodology, Writing – review & editing. **Gianluca Baruzzi:** Conceptualization, Methodology, Writing – review & editing. **Stefano Tartarini:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.111857.

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