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RESEARCH ARTICLE

# Venetian olive (*Olea europaea*) germplasm: disclosing the genetic identity of locally grown cultivars suited for typical extra virgin oil productions

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**Abstract** Olive (*Olea europaea* L.) is one of the most important tree crops of the Mediterranean regions. In spite of the increasing appreciation of typical extra virgin olive oils at world level, based on the use of local traditional varieties, very few studies have focused on the genetic characterisation of olive cultivars of regional interest, such as those grown in Veneto, a North-Eastern Italy region. A deep knowledge of the varieties cultivated in this territory is a key step to address the product quality, to increase market demand and to certify the origin of local olive oils. Here we have analyzed olive cultivars and cultivar groups within the olive cultivation area in Veneto, from the Garda Lake to the Euganean and Trevisan hills, by using discriminant SSR markers, in order to obtain a systematic genetic survey of the Veneto regional olive germplasm patrimony. A total of 203 previously uncharacterized olive samples were collected from ancient trees still grown by local farmers. The analyzed samples included also 36 olive reference cultivars from Veneto and neighbour Regions. We found 57 unique molecular profiles out of this set of olive accessions that were split into 15 cultivar groups corresponding to genetically distinct STRUCTURE clusters. Based on a common SSR database, our 239 Venetian accessions were compared with 280 olive reference genotypes representative of the Mediterranean cultivation area. From the genetic structure analysis, it has been observed that 80% of Venetian cultivars clustered in the central Mediterranean group, about 9% and 2% with the eastern and western varieties, respectively, and all the others resulted intermixed among two or three populations. We found that regionally the most common variety was "Casaliva", corresponding to the widely diffused cultivar "Frantoio", while others showed identity with known varieties grown in close regions, such as "Leccino", "Miniol", "Capolga" and "Bianchera". Besides these genotypes, others were not matching any known reference and therefore they could be classified as true local varieties of indigenous origin, possibly deriving from the hybridization and selection made by

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farmers and from their adaptation to the local soil and climate conditions.

**Keywords** *Olea europaea* · Local varieties · SSR genotyping · Population genetic structure · Homonymy · Synonymy · Mislabeling

## Introduction

Olive (*Olea europaea* subsp. *europaea*, $2n = 2x = 46$) is one of the most economically important widely tree fruit crops of the Mediterranean area. The olive tree products represent a significant share of the agricultural economy of the southern European countries. The EU, in fact, is the global leader of olive production, accounting for almost 70% of the total world output and the main net exporter towards non-producing areas such as North America. In terms of overall production, Spain is the biggest producer in the world within the period 2010–2014, followed by Italy and Greece (FAO STAT 2016).

The Mediterranean olive growing countries possess a rich diversity of olive cultivars, including Spain (133 cultivars), France (88 cultivars), Greece (52 cultivars), and Turkey (45 cultivars). Among them, Italy counts on about 600 cultivars, which represents half of the olive germplasm (Bartolini et al. 1998).

Olive grows through the typical Mediterranean climate and its spread to northern latitudes is limited by winter temperatures lower than − 8.3 °C (Ponti et al. 2014). In the coming decades, the olive tree should face the greatest climatic change that has been recorded since its spread into the Mediterranean Basin, and its cultivated area is expected to be reshaped to the predicted future climate (Moriondo et al. 2013). For this reason, regions actually at the northern limit of olive cultivation as Veneto (highest latitude at 45°58′48″N), represent important cradles of variability because there have settled and adapted genotypes able to withstand extreme weather conditions.

The cultivated olive stands out from all the other fruit species for the great varietal heritage still preserved in culture (Lazović et al. 2016). More than 1,200 cultivars have been described (Bartolini et al. 1998) and many others are still waiting to be recognized and characterized (Mousavi et al. 2014). Identification of olive cultivars was based in the past exclusively on morphological and agronomical traits, tree growth habit, resistance to pathogens and pests, fruit and oil composition and phenological parameters (e.g., flowering time, fruit set, etc.) (Grasso et al. 2016; León et al. 2016). However, morphological and phenological descriptors have shown major drawbacks because they provide a small number of polymorphisms and are under environment influence (Gomes et al. 2012; Ayed et al. 2015). Therefore, identification of olive varieties based on phenotypic traits has lead in the past to several confusions (Corrado et al. 2009; Rotondi et al. 2011). On the contrary, molecular markers have been successfully exploited for the characterisation of the olive germplasm and represent very accurate tools to screen, characterize and assess genetic identity of cultivars and to determine genetic diversity and relationships among cultivars (Hannachi et al. 2008; El Bakkali et al. 2013; Díez et al. 2015), with applications in breeding programs and germplasm collection management (Belaj et al. 2012; De la Rosa et al. 2013; Saumitou-Laprade et al. 2017). Microsatellite (simple sequence repeat, SSR) markers represent one of the most popular marker systems for olive DNA genotyping, since they are highly informative, reproducible within and transferable among laboratories, and are thus ideal for developing fingerprinting studies (Baldoni et al. 2009; Gomes et al. 2009; Muzzalupo et al. 2009; Haouane et al. 2011). A great deal of research has been devoted to apply SSR markers for the characterization of olive trees accessions due to the high degree of polymorphism, co-dominant inheritance and simplicity of detection of a high number of marker alleles per locus (Ayed et al. 2016; Díez et al. 2016). In the past, some authors have tested a number of SSR markers on a number of cultivars grown in different regions in order to assess the distribution of variability, establish relationships among varietal populations, infer the origin of cultivars (Mackay et al. 2008; Barazani et al. 2014; Sakar et al. 2016), allocating olive cultivars to their geographic population of origin (Sarri et al. 2006; Díez et al. 2015).

The systematic and unequivocal identification of locally grown varieties represents a key step towards the evaluation, characterization and exploitation of autochthonous locally distributed varieties. In the present study, we have collected and molecularly characterized a large set of olive trees grown in the Veneto region by using a selection of highly

polymorphic SSR markers. The final aim of the study was to establish the consistency of the local germplasm patrimony, to verify the presence of autochthonous varieties in one of the northernmost areas of olive cultivation and to ascertain their possible origin and development.

## Materials and methods

### Plant material and DNA extraction

The area of olive cultivation of the Veneto region (north-eastern Italy), from the Garda lake to the hilly areas of Verona, Vicenza, Padova and Treviso (Fig. 1), was deeply prospected during 2014 and 2015 seasons by experienced staff in order to collect plant material from trees whose estimated age was over 60 years, with the aim of gathering only local accessions and avoid any recent introduction (Table 1). To the 203 previously uncharacterized olive genotypes, other 36 accessions were added, as reference cultivars from Veneto (15) and neighbouring regions/countries and few allochthonous varieties currently cultivated in Veneto (Table 1). Reference samples were derived from the olive germplasm collections of the Experimental Station of Horticulture, Verona (VISF-VR), and the Institute of Biosciences and Bioresources, National Research Council (CNR-IBBR), Italy.

Young leaves were harvested from this overall set of 239 single trees. Total DNA was extracted from 100 mg of fresh leaves using CTAB method (Doyle and Doyle 1987), with slight modifications (Angiolillo et al. 1999). Plant tissues were manually ground using mortar and pestle, liquid nitrogen and 600 µL of preheated CTAB buffer were added to each sample with 1.2 µL β-mercaptoethanol. Samples were incubated in water bath at 60 °C for 60 min, inverting the tubes vigorously every 15 min. After incubation, 600 µL of chloroform/isoamyl alcohol (24:1) were added and mixed by inverting tubes. Next, the tubes were centrifuged at 10,000 rpm for 15 min and the upper aqueous phase was transferred into 1.5 mL tubes. Two µL RNase (20 mg/mL stock) were added and incubated at 37 °C for 15 min. After that 400 µL of cold isopropanol were added and mixed by inverting tubes, followed by centrifugation at maximum speed for 20 min. The supernatant was discarded and the pellet was washed twice using 400 µL of 70% cold ethanol then centrifuged at maximum speed for 10 min. The supernatant was decanted and pellet was air dried. Genomic DNA was finally resuspended in 50 µL of sterile water. The

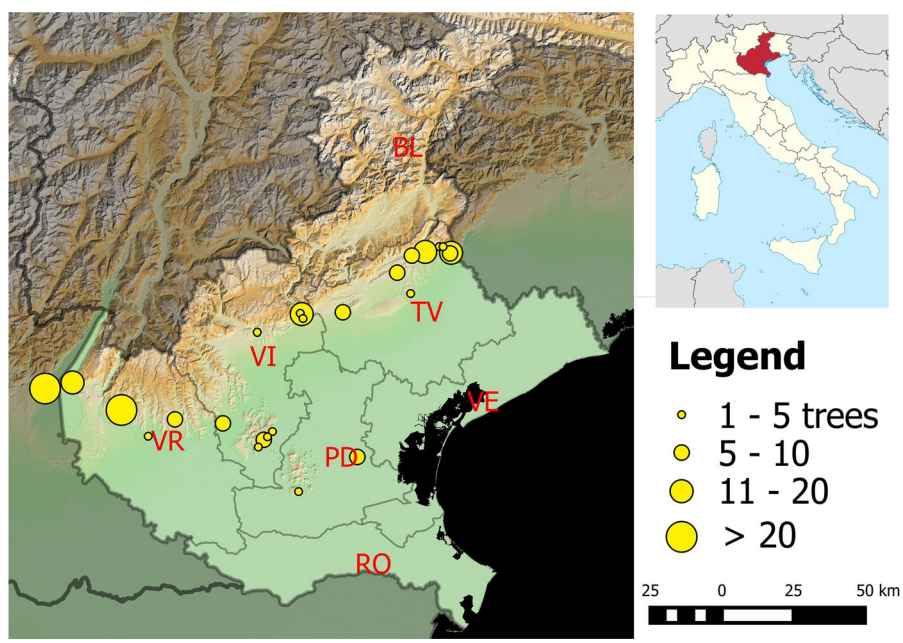



**Fig. 1** Map of the sampling sites: the area of olive cultivation of the Veneto region (north-eastern Italy), from the Garda lake to the hilly areas of Verona, Vicenza, Padova and Treviso provinces, where leaf samples were collected from more than 60 years old olive trees

**Table 1** Comprehensive list of the 239 olive accessions analyzed in this work and corresponding collection sites in Veneto (north-eastern Italy)

| Sample ID | Collection site | Sample ID | Collection site | Sample ID | Collection site |
|---|---|---|---|---|---|
| ASO1 | Asolo (TV) | PDG8 | Pove del Grappa (VI) | TDB14 | Torri del Benaco (VR) |
| ASO2 | Asolo (TV) | PDG9 | Pove del Grappa (VI) | TDB15 | Torri del Benaco (VR) |
| ASO7 | Asolo (TV) | PDG10 | Pove del Grappa (VI) | TDB16 | Torri del Benaco (VR) |
| ASO8 | Asolo (TV) | PDG11 | Marsan (VI) | TDB17 | Torri del Benaco (VR) |
| ASO9 | Asolo (TV) | PDG12 | Marsan (VI) | TDB18 | Torri del Benaco (VR) |
| ASO12 | Asolo (TV) | SAR1 | Sarmede (TV) | TDB19 | Torri del Benaco (VR) |
| ASO13 | Asolo (TV) | SAR2 | Sarmede (TV) | TDV1 | Toara di Villaga (VI) |
| ASO14 | Asolo (TV) | SAR3 | Sarmede (TV) | TDV2 | Toara di Villaga (VI) |
| ASO15 | Asolo (TV) | SAR4 | Sarmede (TV) | TDV4 | Toara di Villaga (VI) |
| ASO16 | Asolo (TV) | SAR5 | Sarmede (TV) | VGO1 | Verona (VR) |
| BAR1 | Barbarano (VI) | SAR6 | Sarmede (TV) | VGO2 | Verona (VR) |
| BAR2 | Barbarano (VI) | SDM1 | Selva di Montebello (VI) | VIV1 | Vittorio Veneto (TV) |
| BAR3 | Barbarano (VI) | SDM2 | Selva di Montebello (VI) | VIV2 | Vittorio Veneto (TV) |
| BAR4 | Barbarano (VI) | SDM3 | Selva di Montebello (VI) | VIV3 | Vittorio Veneto (TV) |
| BAR5 | Barbarano (VI) | SDM4 | Selva di Montebello (VI) | VIV4 | Vittorio Veneto (TV) |
| BAR6 | Barbarano (VI) | SDM5 | Selva di Montebello (VI) | VIV5 | Vittorio Veneto (TV) |
| BAS1 | Bassano (VI) | SDM6 | Selva di Montebello (VI) | VIV6 | Vittorio Veneto (TV) |
| BAS2 | Marostica (VI) | SDM7 | Selva di Montebello (VI) | VIV7 | Vittorio Veneto (TV) |
| BAS3 | Marostica (VI) | SFB1 | San Felice del Benaco (BS) | VIV8 | Vittorio Veneto (TV) |
| COR1 | Cordignano (TV) | SFB2 | San Felice del Benaco (BS) | VIV9 | Vittorio Veneto (TV) |
| COR2 | Cordignano (TV) | SFB3 | San Felice del Benaco (BS) | VIV10 | Vittorio Veneto (TV) |
| COR3 | Cordignano (TV) | SFB4 | San Felice del Benaco (BS) | VIV11 | Vittorio Veneto (TV) |
| COR4 | Cordignano (TV) | SFB5 | San Felice del Benaco (BS) | VIV12 | Vittorio Veneto (TV) |
| COR5 | Cordignano (TV) | SFB6 | San Felice del Benaco (BS) | VIV13 | Vittorio Veneto (TV) |
| COR6 | Cordignano (TV) | SFB7 | San Felice del Benaco (BS) | VIV15 | Vittorio Veneto (TV) |

**Table 1** continued

| Sample ID | Collection site | Sample ID | Collection site | Sample ID | Collection site |
|---|---|---|---|---|---|
| COR7 | Cordignano (TV) | SFB8 | San Felice del Benaco (BS) | VIV16 | Vittorio Veneto (TV) |
| COR8 | Cordignano (TV) | SFB9 | San Felice del Benaco (BS) | VISF1 | ISF Verona (VR) |
| COR9 | Cordignano (TV) | SFB10 | San Felice del Benaco (BS) | VISF2 | ISF Verona (VR) |
| COR10 | Cordignano (TV) | SFB11 | San Felice del Benaco (BS) | VISF3 | ISF Verona (VR) |
| COR11 | Cordignano (TV) | SFB12 | San Felice del Benaco (BS) | VISF4 | ISF Verona (VR) |
| COR12 | Cordignano (TV) | SFB13 | San Felice del Benaco (BS) | VISF5 | ISF Verona (VR) |
| CRO1 | Sarmede (TV) | SFB14 | San Felice del Benaco (BS) | VISF6 | ISF Verona (VR) |
| CRO2 | Sarmede (TV) | SFB15 | San Felice del Benaco (BS) | VISF7 | ISF Verona (VR) |
| FRE1 | Fregona (TV) | SFB16 | San Felice del Benaco (BS) | VISF8 | ISF Verona (VR) |
| LEG1 | Legnaro (PD) | SFB17 | San Felice del Benaco (BS) | VISF9 | ISF Verona (VR) |
| LEG2 | Legnaro (PD) | SFB18 | San Felice del Benaco (BS) | VISF10 | ISF Verona (VR) |
| LEG3 | Legnaro (PD) | SFB19 | San Felice del Benaco (BS) | VISF11 | ISF Verona (VR) |
| LEG4 | Legnaro (PD) | SFB20 | San Felice del Benaco (BS) | VISF12 | ISF Verona (VR) |
| LEG5 | Legnaro (PD) | SFB21 | San Felice del Benaco (BS) | VISF13 | ISF Verona (VR) |
| LEG6 | Legnaro (PD) | SFB22 | San Felice del Benaco (BS) | VISF14 | ISF Verona (VR) |
| LUG1 | Lugo (VI) | SFB23 | San Felice del Benaco (BS) | VISF15 | ISF Verona (VR) |
| LUG2 | Lugo (VI) | SOL1 | Vittorio Veneto (TV) | VISF16 | ISF Verona (VR) |
| LUG3 | Lugo (VI) | SOL2 | Solagna (VI) | VISF17 | ISF Verona (VR) |
| MEZ1 | Mezzane (VR) | SOL3 | Pieve di Soligo (TV) | VISF18 | ISF Verona (VR) |
| MEZ2 | Mezzane (VR) | SOL4 | Pieve di Soligo (TV) | VISF19 | ISF Verona (VR) |
| MEZ3 | Mezzane (VR) | SOL5 | Pieve di Soligo (TV) | VISF20 | ISF Verona (VR) |
| MEZ4 | Mezzane (VR) | SOL6 | Pieve di Soligo (TV) | VISF21 | ISF Verona (VR) |
| MEZ5 | Mezzane (VR) | SOL7 | Pieve di Soligo (TV) | VISF22 | ISF Verona (VR) |
| MEZ6 | Mezzane (VR) | SOL8 | Pieve di Soligo (TV) | VISF23 | ISF Verona (VR) |
| MEZ7 | Mezzane (VR) | SOL9 | Pieve di Soligo (TV) | VISF24 | ISF Verona (VR) |
| MON1 | Sarmede (TV) | SOL10 | Solagna (VI) | VISF25 | ISF Verona (VR) |
| MON5 | Sarmede (TV) | SUS1 | Susegana (TV) | VISF26 | ISF Verona (VR) |
| MON7 | Sarmede (TV) | SUS2 | Susegana (TV) | VISF27 | ISF Verona (VR) |
| MOS1 | Mossano (VI) | PDG1 | Pove del Grappa (VI) | TAR9 | Tarzo (TV) |
| MOS2 | Mossano (VI) | PDG2 | Marostica (VI) | TDB1 | Torri del Benaco (VR) |

**Table 1** continued

| Sample ID | Collection site | Sample ID | Collection site | Sample ID | Collection site |
|---|---|---|---|---|---|
| MOS3 | Mossano (VI) | PDG3 | Pove del Grappa (VI) | TDB2 | Torri del Benaco (VR) |
| MOS4 | Mossano (VI) | PDG4 | Pove del Grappa (VI) | TDB3 | Torri del Benaco (VR) |
| MOS5 | Mossano (VI) | PDG5 | Pove del Grappa (VI) | TDB4 | Torri del Benaco (VR) |
| NAN1 | Nanto (VI) | PDG6 | Pove del Grappa (VI) | TDB5 | Torri del Benaco (VR) |
| NAN2 | Nanto (VI) | PDG7 | Pove del Grappa (VI) | TDB6 | Torri del Benaco (VR) |
| NAN3 | Nanto (VI) | TAR1 | Tarzo (TV) | TDB7 | Torri del Benaco (VR) |
| PAD1 | Baone (PD) | TAR2 | Tarzo (TV) | TDB8 | Torri del Benaco (VR) |
| PAD2 | Baone (PD) | TAR3 | Tarzo (TV) | TDB9 | Torri del Benaco (VR) |
| PAD3 | Baone (PD) | TAR4 | Tarzo (TV) | TDB10 | Torri del Benaco (VR) |
| PAD4 | Baone (PD) | TAR5 | Tarzo (TV) | TDB11 | Torri del Benaco (VR) |
| PAR1 | Parma (PR) | TAR6 | Tarzo (TV) | TDB12 | Torri del Benaco (VR) |
| PAR2 | Parma (PR) | TAR7 | Tarzo (TV) | TDB13 | Torri del Benaco (VR) |
| PAR3 | Parma (PR) | TAR8 | Tarzo (TV) | | |
| **BELVEDERE**[b] | Veneto | **ROSSANEL**[a] | Veneto | **COLOMBINA**[a] | Emilia Romagna |
| **CASALIVA**[a] | Veneto | **TONDA DI VILLA**[a] | Veneto | **GHIACCIOLO**[a] | Emilia Romagna |
| **COMPOSTARO**[a] | Veneto | **TREPP**[b] | Veneto | **GRAPPUDA**[a] | Emilia Romagna |
| **CORNILAR**[b] | Veneto | **BIANCHERA**[a] | Friuli V.G. | **BAIA**[a] | Lombardy |
| **FAVAROL**[a] | Veneto | **BUGA**[b] | Friuli V.G. | **GRAPPOLO**[a] | Tuscany |
| **FORT**[a] | Veneto | **CARBONA**[b] | Friuli V.G. | **LECCINO**[a] | Tuscany |
| **LESS**[b] | Veneto | **CRNICA**[a] | Friuli V.G. | **MAURINO**[a] | Tuscany |
| **GARGNA**[a] | Veneto | **DROBNICA**[b] | Friuli V.G. | **MORAIOLO**[a] | Tuscany |
| **GRIGNAN**[a] | Veneto | **GORGAZZO**[b] | Friuli V.G. | **OBLICA**[a] | Croatia |
| **MINIOL**[a] | Veneto | **ROCCA BERNARDA**[b] | Friuli V.G. | **PIGNOLA**[b] | Liguria |
| **RAZZA**[b] | Veneto | **CAPOLGA**[a] | Emilia Romagna | **POSOLELLA**[b] | Abruzzo |
| **REGINA**[b] | Veneto | **CARBUNCION**[b] | Emilia Romagna | **OLIVELLO**[b] | Campania |

The province of each site is reported in brackets: *BS* Brescia, *TV* Treviso, *PD* Padova, *VI* Vicenza, *VR* Verona. Samples include a total of 203 local accessions and 36 reference cultivars from Veneto and neighbor Regions collected from the olive germplasm collections of the Experimental Station of Horticulture, Verona, and the Institute of Biosciences and Bioresources, National Research Council (CNR-IBBR), Italy. Reference samples are highlighted in bold

[a]Reference varieties, source material from the olive germplasm collection of VISF-Verona (VR)

[b]Reference varieties, source material from the olive germplasm collection of CNR-IBBR (Italy)

integrity of extracted DNA was estimated by electrophoresis on a 1% agarose/1 × TAE gel containing 1 × Sybr Safe DNA stain (Thermo Scientific). Purity and quantity of DNA extracts were assessed by means of the NanoDrop 2000c UV–Vis Spectrophotometer (Thermo Scientific).

SSR and chloroplast DNA marker analysis

Ten highly polymorphic SSR markers were chosen on the basis of their effectiveness of discrimination (Baldoni et al. 2009): DCA3, DCA5, DCA9, DCA16, DCA18 (Sefc et al. 2000), EMO90 (De la Rosa et al. 2002), GAPU71b, GAPU101, GAPU103A (Carriero et al. 2002) and UDO-043 (Cipriani et al. 2002).

PCR amplifications were performed in a reaction volume of 25 μL containing 25 ng of template DNA, 10 × PCR buffer, 2,5 mM of each dNTP, 10 pmol of each primer (forward primer labeled with FAM, NED, PET or VIC fluorescent dyes) and 2 U of Perfect Taq DNA Polymerase (5 PRIME, Eppendorf). Amplifications were performed on the thermal cycler PCR System 9600 (Applied Biosystems, Foster City, CA, USA), using the following cycling conditions: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, annealing temperatures as suggested by the authors for 30 s and 72 °C for 25 s, followed by a final elongation step at 72 °C for 30 min. The resulting PCR products were first visualized by 2% agarose gel electrophoresis and then loaded onto an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Output data were analyzed using GeneMapper 3.7 (Applied Biosystems).

The 36 reference varieties were also analyzed by means of chloroplast (cpDNA) markers. To detect maternal inheritance of the Venetian samples, cpDNA genotyping was performed by using 29 chloroplast SSRs and INDELs along with 20 single nucleotide polymorphisms (SNPs), the latter through SNaPshot technique (Mariotti et al. 2010; Hosseini-Mazinani et al. 2014, Mousavi et al. 2017). Data obtained from this survey were compared with those of Mediterranean cultivars previously published (Mariotti et al. 2010; Besnard et al. 2013).

Marker data analysis

The overall molecular data were used for computation of genetic similarity and diversity statistics. Frequency analyses were performed on a total of 239 olive genotypes including the new accessions and the reference cultivars (Table 1). Intra-population genetic statistics, such as observed number of alleles (Na), effective number of alleles (Ne), observed (Ho) and expected (He) heterozygosity estimates and heterozygous deficiency (F), and the presence of private alleles for the Venetian varieties were calculated for each single marker locus using GenAlEx 6.5 (Peakall and Smouse 2006, 2012).

The Wright's inbreeding coefficients Fis, Fit and Fst, and gene flow (Nm) estimates were calculated across all marker loci by PopGene 1.21 (Yeh et al. 1997). The Wright's inbreeding coefficients express a measure of heterozygote deficiency (−) or excess (+) of single individuals compared to accession groups and to total population, respectively, while Fst is the degree of genetic differentiation.

The overall molecular data were used for computation of genetic similarity estimates in all possible pairwise combinations using the simple matching coefficient for the construction of neighbour joining (NJ) dendrograms and the determination of centroids of single accessions, including the reference cultivars. Molecular phylogenetic analyses were performed by MEGA7 (Kumar et al. 2016). Bootstrap-based data analysis was performed using the Darwin 6.0 software (Perrier and Jacquemoud-Collet 2006), and branches of the tree were tested with 1000 replicates. The unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm from Sneath and Sokal (1973) was used to generate circular dendrograms based on Nei's (1973) genetic distances among the subgroups of accessions as well as to group and plot centroids according to the Principal Coordinates Analysis (PCoA), as reported by Rohlf (1998).

To establish the Bayesian relationships between Venetian genotypes and a large collection of olive cultivars representative of the Mediterranean cultivated olives, previously published SSR-based data (Sarri et al. 2006; Baldoni et al. 2009; Trujillo et al. 2014; Mousavi et al. 2017) were also included in the STRUCTURE 2.3.4 analysis (Pritchard et al. 2009), running 100 replicates MCMCs with a burn-in period of 100,000 followed by a sampling period of 100,000 for 500,000 iterations, applied for each number of clusters (K). The number of possible clusters ranged from 1 to 10, considering independent alleles and admixture of individuals. Bayesian analysis divided sampled individuals into a number of clusters (K) and the most likely value of K was estimated using delta K (Evanno et al. 2005), performed by STRUCTURE Harvester (Earl 2012).

To test whether among the local varieties have recently occurred cases of inter-crossing and local selection of progenies and also to establish parent–offspring relationships among all Veneto genotypes, we performed a parentage analysis using CERVUS 3.0.3 (Kalinowski et al. 2007). All combinations were analyzed and only those offspring sets generated by direct crossing were accounted.

## Results

### Relationships among Veneto varieties and genetic diversity statistics

After calculating the genetic similarity matrix and constructing a phylogenetic tree (Fig. 2) of all Veneto olive samples on the basis of overall SSR data, we found that numerous local samples resulted genetically identical to some reference cultivars and could be unequivocally assigned to their genotypes (Supplementary Figure 1S). Among these, the highest number of samples (59) was assigned to the cv. Casaliva, which showed also an identical profile with other reference local cultivars, such as Belvedere, Razza and Gorgazzo. A total of 18 genotypes were identical to cv. Tonda di Villa and two other cultivars, Buga and Carbona, from the close Friuli region, 16 to cv. Leccino (recently introduced in Veneto), 15 to the local cv. Grignan, 14 to cv. Capolga (neighboring Emilia Romagna region), cv. Trepp (Veneto region) and cv. Drobnica (Friuli region), eight to cv. Gargnà, seven to cv. Miniol and cv. Rossanel, six to cv. Less, four to cv. Baia (Lombardia region), four to cv. Compostaro, three to cv. Fort, only one to each of the cv. Grappolo, Crnica, Favarol, Regina and Moraiolo. Reference cultivars Bianchera and Rocca Bernarda resulted identical. None of the other local samples has fully matched with any of the other reference cultivars. Other olive trees revealed not identity but a high genetic similarity and resulted closely related to the cultivars Casaliva, Grappolo, Compostaro, Maurino, Colombina, Leccino, Favarol, Miniol, Capolga, Grappuda, Oblica, Tonda di Villa and Grignan, with bootstrap values greater than 85%. Some local samples resulted clonally identical among each other, such as SDM3-6-7 close to cv. Compostaro, SAR3-4-5 close to cv. Oblica and VISF5-6-SOL4 close to cv. Grignan, whereas other clonal groups resulted interspersed along the NJ dendrogram, such as SFB8-22, TDB16-

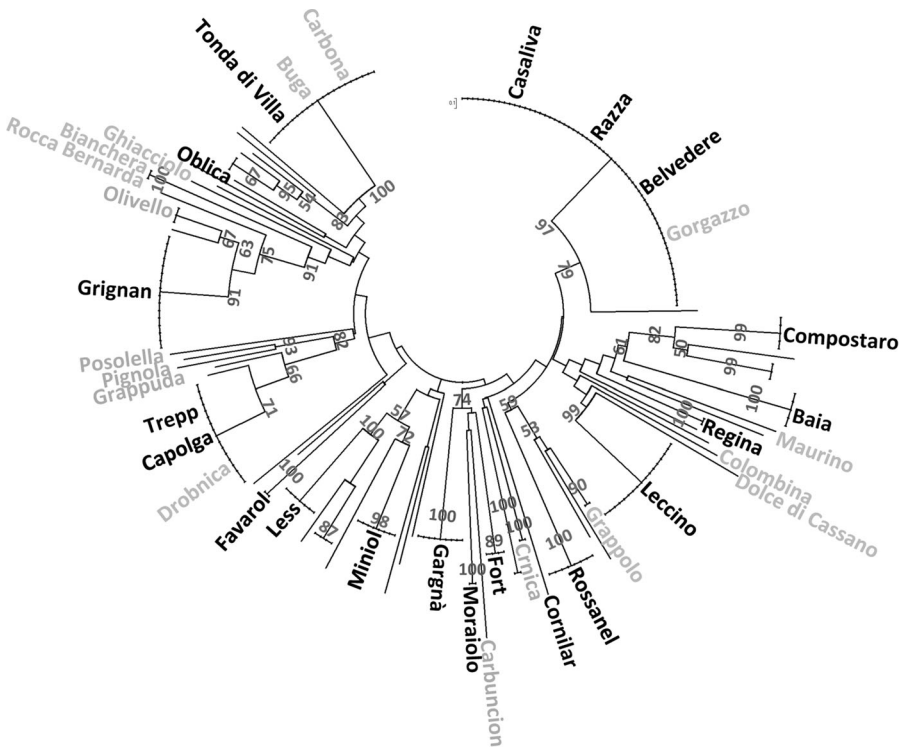



**Fig. 2** Phylogenetic tree depicting the genetic relationships among all 203 olive accessions sampled in the Veneto region: this tree was constructed using the genetic similarity matrix calculated for all pair-wise comparisons on the basis of overall SSR marker data (the names of the reference cultivars, including putative synonymies, that were found genetically identical to local accessions for which could be unequivocally assigned their identification genotypes are written in black, whereas for the reference cultivars without matches among local accessions the names are written in grey)

17-19, BAR6, SFB3-16 and VISF21-22-25. Finally, 28 unique profiles were found and did not match with any of the reference cultivars considered in this study, including the set used for Bayesian analysis.

The main descriptive statistics of the 57 unique profiles resulted from the analysis of 239 samples, including local samples and reference cultivars from Veneto and neighbour Regions, are reported (Table 2). A total of 108 alleles was detected across the assayed SSR loci, with an average number of Na = 11.1 and with EMO90 and DCA9 loci showing the minimum (6) and maximum (17) values, respectively. The average number of effective alleles was Ne = 4.3. Values of null alleles resulted very low at all loci, with a mean value of −0.067. The average estimate of the information index of diversity was equal to I = 1.62, ranging between 1.023 and 1.962, whereas the calculated PIC values for the individual marker loci resulted high at all loci and ranged from 0.551 (DCA05) to 0.853 (DCA09), with an average value equal to 0.749. The estimates of observed heterozygosity were higher than expected ones at all marker loci, with an overall value of Ho = 91.4% (SE 0.019) and He = 74.3% (SE 0.028), respectively. Moreover, the Nei's unbiased genetic diversity was as equal to uHe = 74.4%. The fixation index (F), equivalent to the inbreeding coefficient, was negative at all loci, averaging −0.243 (Table 2).

All Venetian samples and genotypes were then divided into 15 subgroups, according to the mean genetic similarity estimates and principal coordinates (Fig. 3), and the main inbreeding coefficients and gene flow values were calculated for each marker locus and over all accessions (Table 3). The results showed that all Wright's inbreeding coefficients were negative and on average equal to Fis = −0.86 and Fit = −0.16, indicating a significant excess of heterozygosity for the olive accessions of the Venetian germplasm. Moreover, the degree of genetic differentiation (Fst = 0.38), revealed that only 38% of the genetic variation was found among cultivar groups and that as much as 62% of the total polymorphism was distributed within cultivar groups. The narrow genetic differentiation among subgroups over the investigated SSR loci was confirmed by the gene flow estimates that was as low as Nm = 0.41 (Table 3). The analysis of chloroplast DNA polymorphisms on the 15 reference Venetian genotypes, corresponding to each subgroup, revealed no differences among these samples, by both cpSSRs and cpSNPs. Comparing their molecular profiles with what previously published, it was observed that all of them hold the E1.1 chlorotype, shown by 90% of the Mediterranean cultivated varieties. These data were not used for other analyses because no differences were detected.

**Table 2** Descriptive statistics of the 57 unique profiles resulted from the analysis of Venetian olive genotypes and reference cultivars for each SSR marker locus

| Locus ID | $N_a$ | $N_e$ | NA | I | PIC | $H_o$ | $H_e$ | F |
|---|---|---|---|---|---|---|---|---|
| DCA3 | 11.000 | 5.897 | − 0.0449 | 1.962 | 0.835 | 0.983 | 0.830 | − 0.184 |
| DCA5 | 8.000 | 2.270 | − 0.1309 | 1.023 | 0.551 | 0.780 | 0.559 | − 0.394 |
| DCA9 | 17.000 | 5.140 | 0.0072 | 1.896 | 0.853 | 0.892 | 0.805 | − 0.108 |
| DCA16 | 11.000 | 4.342 | − 0.0151 | 1.727 | 0.770 | 0.870 | 0.770 | − 0.131 |
| DCA18 | 8.000 | 2.891 | − 0.1274 | 1.253 | 0.674 | 0.892 | 0.654 | − 0.364 |
| EMO90 | 6.000 | 3.352 | − 0.1393 | 1.315 | 0.654 | 0.954 | 0.702 | − 0.360 |
| GAPU71B | 10.000 | 3.158 | − 0.0912 | 1.348 | 0.703 | 0.946 | 0.683 | − 0.385 |
| GAPU101 | 12.000 | 4.923 | − 0.0439 | 1.861 | 0.793 | 0.921 | 0.797 | − 0.156 |
| GAPU103A | 13.000 | 5.048 | − 0.0362 | 1.908 | 0.812 | 0.933 | 0.802 | − 0.164 |
| UDO-043 | 14.000 | 5.638 | − 0.0484 | 1.906 | 0.843 | 0.971 | 0.823 | − 0.180 |
| Mean | 11.100 | 4.266 | − 0.0670 | 1.620 | 0.749 | 0.914 | 0.743 | − 0.243 |
| SE | 1.059 | 0.399 | n.a. | 0.110 | 0.032 | 0.019 | 0.028 | 0.037 |

Average number of observed alleles (Na), effective number of alleles (Ne) per marker locus, null alleles (NA), estimates of Shannon's information index of diversity (I), polymorphism information content (PIC), values of observed (Ho) and expected heterozygosity (He), inbreeding coefficient (F). The mean values and standard errors over all loci are also reported for the population as a whole

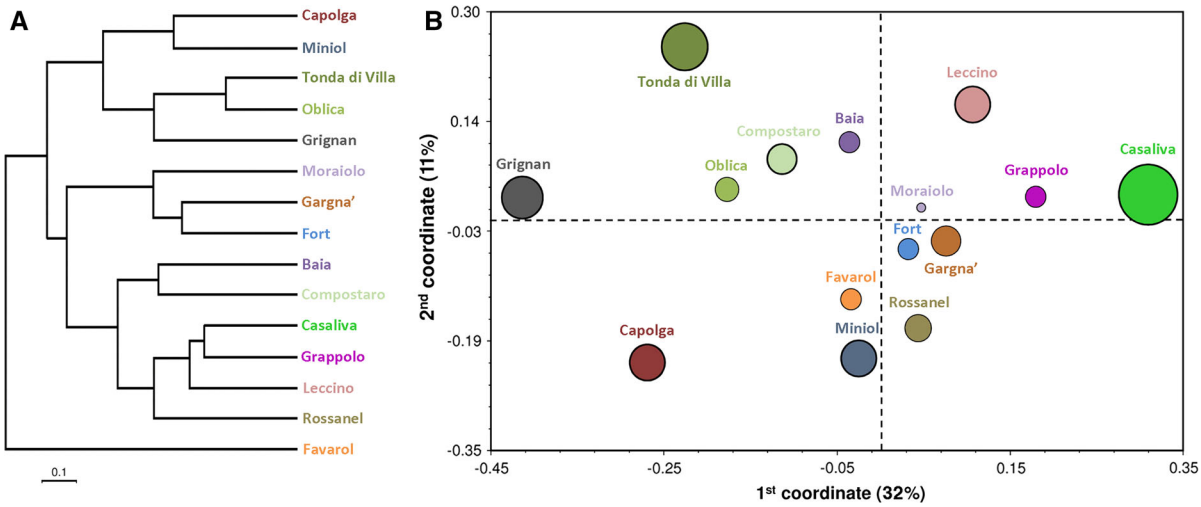



**Fig. 3** Genetic similarities and relationships among all olive accessions of the Veneto region: the UPGMA dendrogram deriving from mean genetic similarity estimates supports the existence of 15 distinct varietal genotypes (**a**), whereas the genetic relationships among these subgroups are depicted by 2D centroids plotted according to principal coordinate analysis (**b**). Based on the whole set of SSR marker alleles, the first two principal axes explain about 43% of the total genetic variation among groups

**Table 3** Summary of F-statistics and gene flow (Nm) for individual marker loci over 15 groups derived from the level of similarity within groups

| Locus ID | F-statistics | | | Nm |
|---|---|---|---|---|
| | $F_{is}$ | $F_{it}$ | $F_{st}$ | |
| DCA03 | − 0.84 | − 0.15 | 0.38 | 0.41 |
| DCA05 | − 0.93 | − 0.26 | 0.35 | 0.47 |
| DCA09 | − 0.77 | − 0.10 | 0.38 | 0.41 |
| DCA016 | − 0.94 | − 0.13 | 0.41 | 0.35 |
| DCA018 | − 0.89 | − 0.29 | 0.32 | 0.53 |
| EMO90 | − 0.93 | − 0.34 | 0.30 | 0.57 |
| GAPU71B | − 0.84 | − 0.23 | 0.33 | 0.51 |
| GAPU101 | − 0.81 | − 0.06 | 0.41 | 0.36 |
| GAPU103A | − 0.83 | 0.02 | 0.46 | 0.29 |
| UDO-043 | − 0.88 | − 0.14 | 0.39 | 0.39 |
| Mean | − 0.86 | − 0.16 | 0.38 | 0.41 |
| SD | 0.06 | 0.11 | 0.05 | 0.09 |

Mean values and standard deviations are also reported

## Structure analysis and phylogenetic relationships of Venetian accessions with the Mediterranean varieties

The whole data set, including 57 unique profiles from the Venetian subgroup including reference cultivars and 280 Mediterranean cultivars, was submitted to genetic structure analysis, based on all molecular marker data, in order to evaluate the membership of the Venetian genotypes into Bayesian groups. When Veneto trees were analyzed alone, the existence of 15 distinct genotypes was hypothesized for the olive germplasm cultivated in this Region (Supplementary Figure 2S). The existence of 15 distinct varietal genotypes was also supported by the dendrogram deriving from mean genetic similarity estimates (Fig. 3, panel A). Genetic relationships among these 15 subgroups were further investigated by defining varietal genotype centroids according to principal coordinate analysis (Fig. 3, panel B). Based on the whole set of SSR marker alleles, the first two principal axes explained about 43% of the total genetic variation among groups, of which 32% attributed to the first coordinate and 11% to the second one. The main descriptive statistics of genetic diversity were also computed for the single cultivar groups that correspond to genotypically distinct STRUCTURE clusters (Supplementary Table 1S). The unbiased measures of genetic identity and genetic diversity among the 15 olive cultivar groups in all pair-wise comparisons were also calculated (Supplementary Table 2S). We found that the cultivar group "Tonda di Villa" was closely related to the cultivar group "Oblica", with a coefficient of genetic identity equal to 76%, followed by "Casaliva" and "Grappolo", showing a 72% of

genetic identity. The lowest value of genetic identity was scored between the cultivar groups "Casaliva" and "Grignan" (20%). It is worth mentioning that private marker alleles were found for five Venetian genotypes and the cv. Grignan (Supplementary Table 3S).

The Structure analysis related to 57 unique genotypes derived from the Venetian set together with the Mediterranean cultivars (280), for a total of 337 genetically distinct accessions, the uppermost hierarchal level of population structure suggested a clear peak of the log probability of the data at K = 3. Only one cultivar, Bianchera, was mainly related to the western Mediterranean group of varieties with a high admixture output. Most of the Venetian cultivars (80%) clustered in the central Mediterranean group with a very robust level of membership. Five genotypes (8.8%) were clearly assigned to the eastern group, while all the other samples (9%) were intermixed among two or three populations (Fig. 4).

The CERVUS parentage analysis has shown many cases of direct kinship, with some accessions likely representing hybrid progenies resulting from the cross of other local varieties or the cross between local and reference varieties. In particular, samples BAR1, VISF5 and VISF12, with 0 mismatches, were found to derive from crosses Capolga × VISF5, Miniol × SFB13 and BAR1 × MEZ4, respectively. A remarkable result refers to the finding that the cv. Casaliva likely originated, with 0 mismatches, from crosses between "Leccino" and "Crnica" genotypes. In all other cases, one or two mismatches were observed and therefore they were not taken into account (data not shown).

## Discussion

### The introduction of the olive cultivation in the area

The olive tree can not withstand temperatures below −12 °C and may not be cultivated in inland zones. The foothill areas of Veneto (north-east Italy), well exposed, within a short distance from the sea or under the relieving effect of Garda Lake, represent the most northern latitude of olive diffusion. In these territories, an extensive traditional olive cultivation is established, representing an important source of agriculture incomes, a characteristic element of the sub-alpine landscape and a reservoir of genetic variation never explored to date.

The presence of *Olea* pollen in the area (Salcedo, Vicenza) has been detected dating back to the Late Glacial (approximately 10,000 years BCE) (Follieri 1984), together with other Mediterranean elements, such as *Quercus* and *Phillyrea*. *Olea* pollen records have been found in Polcenigo (Pordenone), in a Late Neolithic site (5960–5260 cal. yr BP), likely deriving from surrounding areas (Pini 2004). Pollen and charcoal records found at Lago della Costa (Colli Euganei, Padova) document the cultivation of *Castanea*, *Juglans*, *Olea* and Cerealia during the Bronze Age (4150–2750 cal. BP) (Kaltenrieder et al. 2010).

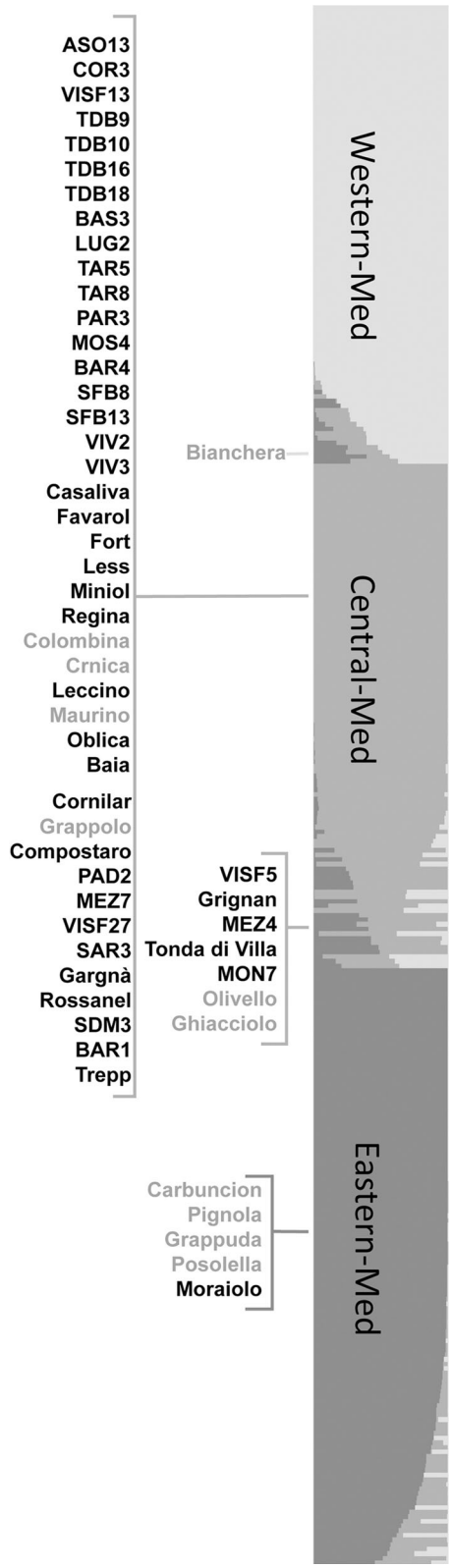
During the Roman Empire, the production of olive oil, albeit limited, assumed a certain importance in Veneto from the first half of the second century BC (Buonopane 2009). According to Latin literature, during the 1st century BC, the best olive oils were produced exclusively from some regions, among others, Istria, very close to Veneto and belonging at that time at the same Augustan Decima Regio (Gaius Plinius Secundus 1984).

In the early Middle Ages some Veneto manors were specialized in olive production, particularly near Verona (Varanini et al. 1994; Curtis and Campopiano 2014). In the tenth century, the great Venetian monasteries owned land cultivated with olives and obtained olive oil, used not only for food but also for worship services and for lighting (Cipriani and Mazzocchin 2004).

It is still completely unknown when first cultivated olives were introduced in the Veneto region and where this genetic material came from, but the analysis of molecular data of the genotypes currently established in the area should allow inferring some hypotheses on these important issues.

### First evidences on the origin and spreading of Veneto olives

This work represents the first wide survey of the olives cultivated in Veneto region, aimed at establishing the consistency of the local germplasm patrimony, verifying the presence of autochthonous varieties and ascertaining their possible origin and development. This information will help to notice an important gene pool well adapted to difficult climatic growing conditions, to better exploit this germplasm and to warrant




◀**Fig. 4** Population genetic structure analysis of the whole set of olive samples, including 203 Venetian local trees (57 different genotypes) and 280 Mediterranean reference cultivars, for a total of 337 olive accessions. The uppermost hierarchal level of population structure suggested a clear peak at K = 3: most of the Venetian genotypes (80%) clustered in the central Mediterranean group, five (8.8%) within the eastern and one (1.7%) within the western varieties, while all the others resulted intermixed among two or three populations (the names of the local genotypes and reference cultivars belonging to the Veneto region are written in black)

the genetic authenticity of local olive cultivars and olive oils.

The olive trees collected from different places of Veneto region (Italy) were selected based on historical searches, interviews to growers and field technical assistants. Only over 60 years old plants were selected for analysis, in order to avoid collecting plant material recently introduced in the area from abroad. To this set of trees, a further group of territorial varieties previously collected was added, being part of a collection of regional olive germplasm at the Horticulture Experimental Station of Verona, which includes also a few varieties from neighbouring regions (Emilia Romagna, Friuli, Lombardy and Tuscany).

First evidences demonstrated that "Casaliva" represents the most widely distributed variety along the Veneto region, as it is present in numerous valleys from the Garda Lake to the foothills of the Venetian Prealps. Moreover, this variety was found genetically undistinguishable from cv. Frantoio, as already reported by Muzzalupo et al. (2014). Then, cv. Casaliva is followed, in terms of number of identical genotypes, by two cultivars such as Tonda di Villa and Grignan. Other varieties, including cv. Gargnà, cv. Rossanel, cv. Compostaro, cv. Miniol and cv. Fort, are also largely spread, meanwhile varieties known in other regions, as cv. Capolga (Emilia Romagna), cv. Baia (Lombardy), cv. Crnica (Friuli), cv. Leccino, cv. Moraiolo and cv. Grappolo (Tuscany), have found in Veneto another great area of cultivation.

Interestingly, some varieties are also widely spread, although to them it has not been assigned any name and their identity remained unknown. Lastly, in Veneto are also present single-tree genotypes, they may represent either the survivors of paleo-varieties diffused in the past and then brought to extinction due to the change of varietal assortment for agronomical,

commercial or climatic reasons (Díez et al. 2011, 2012; Lazovic et al. 2016; Hosseini-Mazinani et al. 2014), or the products of recent hybridizations, waiting to be vegetatively propagated and diffused in cultivation.

The values of Fixation Index, ranging from 0 to − 0.326, indicated an excess of heterozygosity, probably due to selection for heterozygous trees. This observation was confirmed by the negative Fis and Fit values, revealing a significant excess of heterozygosity for the olive accessions of the Veneto germplasm. Some private alleles were detected in such a small population and geographically restricted area.

Most of the Veneto varieties clustered within the Central Mediterranean varietal population and, substantially, any of the Venetian samples was fully assigned neither to the Eastern Mediterranean population nor to the Western one. Their membership to the E1.1 chlorotype, the most widespread among the Mediterranean varieties, represents a further evidence of the introduction of varieties coming from the East of the Mediterranean, where this chlorotype is highly widespread (Besnard et al. 2013; Mousavi et al. 2017). Indeed, in Veneto the presence of oleasters has never been reported, while Venice since the Middle Ages was the Mediterranean harbor with the most extensive trades with the Middle and Far East.

Local varieties may have developed since ancient times, they may have undergone a strong isolation and lack of gene flow for long periods, up to a more recent introduction of some varieties that have hybridized with local ones to give rise to those rare genotypes or those represented by a few trees. This could also justify the excess of heterozygosity. Presumably, some of them represent varieties established long time ago within the region, whilst others might represent the cross products among the most ancient varieties.

The variety "Casaliva" deserves further attention because the presence of the same genotype from Veneto to the South of the Italy peninsula raises the question about its origin and the entry site in Italy. Based on the considerations made on the agro-climatic conditions of Veneto, it is difficult to assume that it has entered through this region, while it seems more plausible that it may have spread since Roman times starting directly from Rome, then conquering gradually the different regions adjacent, to the extreme north and the extreme south. The topic deserves a specific further study. A remarkable finding about cv. Casaliva (corresponding to cv. Frantoio) is that, based on SSR data, this variety seems to be originated from, or to be closely related with, both cv. Leccino and cv. Crnica. However, any hypothesis on the origin of these varieties is possible and genealogical relationships could only be reconstructed using additional markers, including SNPs for nuclear sequences.

## The impact of these findings on the production of Veneto olive oil

The increasing demand of regional extra virgin olive oils (EVOO) reflects the attention paid by consumers to high quality EVOO products, due to their distinct sensory and nutraceutical properties, and their cultural, landscape and ecological value. Olive oil composition, in fact, highly builds upon the genotype (varieties) and on their interaction with the environment where olive trees are grown. Superior olive oils usually derive from areas at high latitudes or high altitudes, where temperatures along fruit ripening remain lower compared to hot areas (Rondanini et al. 2014) and where fruits are harvested and immediately processed for oil extraction (Beltran et al. 2016). These conditions are all represented in the northern areas of olive tree cultivation and, among them, Veneto represents one of the iconic areas for the production of the most outstanding EVOO products.

Previous studies showed that EVOO from the cultivar "Grignan", a variety endemically cultivated in the area surrounding the Garda Lake and Verona, is characterized by aroma profiles which distinguish this olive oil from most Italian and European olive oils and have highly appreciated sensory characters (Recchia et al. 2012; Vezzaro et al. 2011).

In order to further explore the potential biodiversity of olive genotypes present within the Veneto region for the subsequent valorisation of monovarietal olive oils with unique distinguished characters, we have undertaken an in-depth and as systematic as possible genetic analysis of the Veneto olive germplasm.

Besides being an essential step towards the unequivocal identification of locally grown varieties, to uncover cases of synonymy and homonymy, this very detailed survey of the Veneto olive genotypes also represents the way to the identification of genotypes with potentially interesting characters for the production of extra virgin olive oils with unique compositional features. A complete genetic

description of the Veneto germplasm will also set a corner stone for traceability of monovarietal olive oils.

Interestingly, the SSR method used in this study was able to uniquely recognize and unambiguously differentiate each of the 239 cultivated olive accessions under study (unless for the cases of clonal origin) and confirmed the high efficiency of the SSR markers in that aspect.

The biggest cluster was found for "Casaliva", which included 59 accessions that could not be genetically distinguished from the reference cultivar "Frantoio", the most widespread cultivar in Italy. Similarly, 16 accessions were assigned to the group of the cultivar "Leccino". The remaining 128 accessions, excluding the 36 reference cultivars, could be assigned to other fourteen subgroups of genotypes, each of them unequivocally identified on the basis of known locally cultivated cultivars used as references. This evidence shows that many locally grown cultivars, namely "Tonda di Villa", "Compostaro", "Fort", "Rossanel", "Favarol" and "Grignan", have been spread widely over different Veneto provinces by clonal propagation (Albertini et al. 2011) through cuttings or grafting methods. In detail, the cultivar "Grignan", endemic in the area surrounding Verona and the Garda Lake, was found to be present in several accessions in Treviso and Verona provinces, and it appeared highly similar to the cultivar "Olivello". Differently, "Tonda di Villa" was found almost exclusively located in eastern Veneto (Treviso) and within a cluster distinct from all cultivars present in the database. Similarly, several plants widespread throughout the Veneto area resulted to belong to clusters of varieties exclusively grown in the region, such as "Gargnà", "Miniol", "Fort", "Compostaro", "Rossanel" and "Favarol". Besides, the presence in the eastern part of Veneto of some accessions with high similarity with the cultivar "Oblica" and of one accession with the cultivar "Crnica" supports a past introduction of Slovenian cultivars through its close border. Interestingly, nine accessions revealed genetic similarity and to be closely related, but not identical, to two reference cultivars "Tonda di Villa" and "Oblica", and having bootstrap values of genetic similarity greater than 85%. Our results show that the unique genetic distance pattern of six accessions collected from six different collection sites in Veneto region, designated ASO13, COR10, LUG2, TAR5, TAR8, and VISF13, did not match with any of the reference cultivars of SSR olive database. Also in terms of their genotypic structure, these accessions displayed to have an admixed genetic background and showed a low membership value to the associated cultivar group. These accessions may probably represent new putative hybrid progenies or could be local ancient cultivars with somatic mutations raising intracultivar variation, as already reported for other olive genotypes (Banilas et al. 2003; Baali-Cherif and Besnard 2005; Baldoni et al. 2006; Belaj et al. 2007, 2010; Besnard et al. 2013; Marra et al. 2013; Díez et al. 2016). Furthermore, not only these accessions revealed high levels of admixed haplotypes but also there were four accessions, designated as MON7, PAR3, TDB10 and VISF27, which represent novel and peculiar olive genotypes probably maintained through asexual propagation methods. Consequently, these admixture accessions may represent a valuable resource of genetic variation for future breeding programs of local olive cultivars and for better defining the Veneto olive germplasm.

The PIC value of the SSR marker loci used in this study was very high and this finding confirmed that all chosen loci are highly variable, informative and suitable for systemic individual cultivar identification and cultivar group discrimination in olive. Statistically, a total of 110 marker alleles over ten SSR genomic loci utilized in this study showed an average number of observed alleles per locus Na = 9. This number is comparable with the 135 marker alleles with an average of 9.6 per locus published by Lopes et al. (2004), and 67 marker alleles with an average 8.4 found among Sicilian accessions reported by Las Casas et al. (2014). Similarly, 75 marker alleles with an average of 6.8 were reported for olive cultivars in Southern Italy by Muzzalupo et al. (2009), and higher than 104 marker alleles with average 3.6 reported by Cipriani et al. (2002). The higher number of markers alleles, equal to 159 with an average of 13.2, noticed by Sarri et al. (2006), may be explained by the higher number of accessions considered, coming from fourteen Mediterranean countries, and different multilocus SSR markers.

The observed heterozygosity values of each cultivar group were significant compared with the expected values and Nei's genetic diversity, equivalent to the expected heterozygosity, indicating that the significant excess of heterozygous loci in the olive genome and the Veneto olive populations may exhibit a high level

of outbreeding. This is also confirmed by the negative values of Wright's inbreeding coefficients. The total value of observed heterozygosity for Veneto olive cultivar groups appeared very high in comparison to the previously reported ones by other Italian accessions, e.g. 65% by Sarri et al. (2006), 62% by Marti et al. (2015), 60% by Muzzalupo et al. (2014), suggesting a remarkable large genetic variation amongst the Veneto germplasm sources.

Concerning the genetic variation and differentiation levels, the estimated fixation index suggested that the vast majority (62%) of the total genetic variation was found within cultivar groups and the relatively low remaining part (38%) was widespread among cultivar groups. The narrow genetic differentiation among subgroups was confirmed by the gene flow whose estimate was very low ($< 1$).

## Conclusions

Our work represents a first comprehensive and systematic molecular study of the Veneto olive germplasm, from the western side of Garda Lake to the Euganean and eastern Trevisan hills. This contributes to the correct assignment of cultivars grown in Veneto within the general framework of the Italian olive cultivated germplasm. We also provided genetic information about the autochthonous Veneto cultivars, which will represent not only a significant resource for future breeding programs, but also an irreplaceable stock of locally adapted material for future economic valorization and genetic authentication (i.e., traceability) of extra virgin top quality local olive oil.

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**Compliance with ethical standards**

**Competing interest** The authors declare that they have no conflict of interests.

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