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Vitis vinifera L. germplasm diversity: a genetic and ampelometric study in ancient vineyards in the South of Basilicata region (Italy)

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Summary

The evaluation of the existing grapevine biodiversity in several areas still unexplored in Basilicata region has been carried out. A four year's survey in ancient vineyards of Potenza was performed to investigate grapevine biodiversity. 85 collected accessions were subjected to genetic characterization through nine microsatellite markers. A total of 42 genotypes were obtained. The comparison with national and international databases allowed the identification of 26 accessions corresponding to new autochthonous genotypes and minor/local cultivars, in addition 16 international and national cultivars commonly cultivated in several Italian regions were found (data not shown in this work). Results indicated that minor/local cultivars were mainly cultivated in the near regions. The genetic profile of 9 new autochthonous grapevines was described here for the first time. Comparison of the genotypes, allelic frequencies, allelic sizes and ampelometric traits on mature leaves are highlighted. Conservation of new autochthonous and minor/local cultivars in germplasm collections has been carried out by including them in the germplasm collection of CREA-VE in Arezzo in order to save grapevine biodiversity and allowing further agronomical and enological evaluation.

K e y w o r d s : biodiversity; autochthonous *Vitis vinifera*; minor/local cultivars; microsatellites; ampelometry.

Introduction

The territory of Basilicata region is part of the Third Centre of Domestication of *Vitis vinifera* (Del Lungo 2015). The territory of the ancient Enotria, known as the land of the vine cultivated with the support of a pole, spreading from Campania to Calabria, with Lucania (or Basilicata) as the core (Del Lungo *et al.* 2016). Lucanian agricultural landscapes having a "historical character" are concentrated in the hills, where ever since the time of Magna Graecia the crops that epitomize "Mediterraneity" appeared, notably the olive tree and the sub-Mediterranean grapevine (Russo 2012). In this context, the vegetative propagation of *Vitis vinifera* during the centuries has allowed the diffusion of many cultivars through the historic migrations of people. In "Statistics of the Naples Kingdom" published by G. Murat in 1811, more than 150 grape varieties were collected in Lucanian area. In addition, in Ampelographic Bulletins of the Ministry of Agriculture of 1881 approximately 63 wine varieties and other 29 with dual use (wine and table grapes) (Del Lungo *et al.* 2016) were reported. This precious heritage was mostly lost due to migration processes that contributed to increasing of abandonet of vineyards. Nowadays, in Basilicata region, only 38 varieties (registered from 1970 to 1978) are allowed for cultivation in the official regional list and other 19 varieties are under observation (Tab. 1). One Controlled and Guaranteed Denomination of Origin (D.O.C.G) allows the cultivation of 'Aglianico del Vulture' which represents the main variety widespread in Basilicata (Tab. 1). Besides, the Designation of Origin (D.O.C) using four varieties mainly related to the territory of Potenza in the South of Basilicata. The main representative varieties allowed for D.O.C wines are national or international cultivars such as 'Sangiovese', 'Cabernet Sauvignon' and 'Merlot'. Moreover, the autochthonous 'Malvasia (N. and B.) di Basilicata' variety is also admitted. The official technical specifications in D.O.C. approves only the use for winemaking of 10 % to at least 20 % of grape varieties from the official regional list (see Tab. 1 for more details) and, therefore, the utilization of minor/local varieties is not widespread. Unfortunately, the presence of few national and international cultivars allowed for cultivation by the different Designation of Origin (D.O.), as reported previously, has led to a deep reduction of grapevine diversity. Therefore, many autochthonous grapevines have to be protected from extinction. With the purpose of saving grapevine biodiversity, several studies on identification, characterization and conservation of germplasm have being carried out in many countries from national to regional and local level (Gonzales-Andres *et al.* 2007, De Andres *et al.* 2012, Brunori *et al.* 2015, Maletić *et al.* 2015). DNA genotyping by microsatellite markers allows identifying varieties despite of plant phenotype and changes in morphology due to virus infection or lack of vigour (Bergamini *et al.* 2016). Ampelography represents the first step of grapevine identification, however sometimes it is not able to differentiate grapevine varieties. Moreover, the SuperAmpelos software represents a useful tool to help *Vitis* germplasm cataloguing when only ma-

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Table 1

Main Red and White official varieties allowed in the D.O.C. G. and D.O.C. of Basilicata region, old grape varieties grown years ago and varieties currently under observation (Source: www.regione.basilicata.it)

Denomination of Origin (D. O.)	Main Red varieties	Main White varieties	% Official minor or local varieties allowed	Official List of varieties allowed in Basilicata Region	Main old varieties grown years ago
D.O.C.G "Aglianico del Vulture Superiore" D.O.C "Aglianico del Vulture"	Aglianico del Vulture	Malvasia B. di Basilicata	Not allowed	Aglianico N. Aglianicone N. Aleatico N. Asprinio Bianco B. Barbera N. Bombino Bianco B. Bombino Nero N. Cabernet Franc N. Cabernet Sauvignon N. Ciliegiolo N. Cortese B. Falanghina B. Fiano B. Freisa N. Garganega B. Greco Bianco B. Malvasia Bianca di Basilicata B. Malvasia Nera di Basilicata N. Merlot N. Montepulciano N. Moscato Bianco B. Müller-Thurgau B. Nebbiolo N. Pinot Bianco B. Pinot Grigio G. Pinot Nero N. Primitivo N. Refosco dal Peduncolo Rosso N. Sangiovese N. Sauvignon B. Syrah N. Terolengo N. Traminer Aromatico Rs. Trebbiano Toscano B. Verdeca B. Aglianico del Vulture N. Chardonnay B. Manzoni Bianco B.	Aglianico B. Cassano N. Colatamurro N. Giosana B. Rosette N. Jusana B. Malvasia B. Passolara B. Pergola N. Piscialetto B. San Nicola N. Trebbiano Antico B. Uva BiancaAntica B. Uva Bianca e Nera R. Varieties currently under observation Ansonica B. Bellone B. Calabrese N. Castiglione N. Greco Nero N. Grillo B. Malbech N. Malvasia di Lipari B. Moscato Giallo B. Moscato Rosa Rs. Negro Amaro N. Negro Buono N. Pecorello B. Pecorino B. Uva di Troia N. Petit Verdot N. Viogner N. Petit Manseng B. Minutolo B.
D.O.C "Grottino di Roccanova"	Sangiovese Cabernet Sauvignon Malvasia N. di Basilicata Montepulciano		10 %		
D.O.C "Matera"	Sangiovese Cabernet Sauvignon Merlot Primitivo	Malvasia B. di Basilicata Greco Bianco	10-15 %		
D.O.C "Terre dell'Alta Val d'Agri"	Merlot Cabernet Sauvignon Malvasia di Basilicata		10-20 %		

ture leaves are available for description especially in some cases (e.g. ancient and/or abandoned vineyards) (Kullaj *et al.* 2015). The main goals of this research are: (a) to identify and characterize new cultivars; (b) to determine minor/local grapevines currently cultivated and/or being widespread in the south of Basilicata region.

Material and Methods

Plant material: A survey in several growing areas in the south of Basilicata region was carried out between 2011 and 2015 searching grapevine accessions in ancient and/or abandoned vineyards. A total of 85 plant accessions, supposed to display particular traits, were collected. Samples of young fresh leaves were collected in the field and stored at -80 °C for the following molecular characterization.

Microsatellite analysis and grapevine microsatellite databases consulted: The nine microsatellites recommended by the European project GRAPEGEN06 were utilized for identification of grapevines (Maul *et al.* 2012). The following nine STMS loci were utilized: VVS2 (Thomas and Scott 1993); VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32 (Bowers *et al.* 1996, 1999); ssr VrZAG62 and ssrVrZAG79 (Sefc *et al.* 1999). PCR reaction and amplified product analyses were carried out according to Bergamini *et al.* (2013). Genetic profile of accessions were compared to several national (www.vitisdb.it, D'Onofrio and Scalabrelli 2014; www.catalogoviti.politicheagricole.it, Zavaglia *et al*. 2014; the database of CREA-VE of Turi, Antonacci *et al*. 2012) and international databases (www.vivc.de and www.eu-vitis.de, Maul *et al.* 2012) in order to match each genotype to the correspondend variety when possible. Genotypes with no association found in the databases were identified by a code ("BAS" followed by a progressive number).

Ampelography: Mature leaves were collected in ancient and/or abandoned vineyards. Moreover, few main ampelographic characters (mature leaf and wooden shoot) were performed on each collected mature leaf on the

base of a list of descriptors developed by the Organization Internationale de la Vigne et du Vin (OIV 2009). In addition, budding, flowering, veraison and ripening time were added based on winegrowers personal communication.

Ampelometry: Twenty mature leaves for each genotype were collected and processed by SuperAmpelos software (Ver. 2.0. Comunità Monastica S.S. Pietro e Paolo. Germagno, Verbania, Italy). The medium profile of "mature leaf" was performed for each genotype. Ampelometric data were standardized in order to transform all characters in a comparable scale for the following statistical analysis.

Statistical analysis: The number of alleles, allele frequencies, expected (He) and observed (Ho) heterozygosity and probability of identity (PI) were calculated using GenAlEx version 6.5 software (Peakall and Smouse 2012). Dendrograms based on genotype data were constructed using PAST software (Paleontological statistics software package for education and data analysis. Paleontologia Electronica 4, 1:9). Principal Component Analysis (PCA) of standardized ampelometric data was obtained using the package "FactoMineR" Ver. 1.35 in R software, version 3.3.2 (20-02-2017).

Results and Discussion

The investigations of grapevine diversity in the south of Basilicata region was carried out to identify and catalogue the genetic heritage of this territory. Despite the large utilization of national and international cultivars also in this territory, this work focuses the attention of unknown grapevines and minor/local cultivars in order to preserve them from extinction. From 2011 to 2015, a total of 85 plant accessions were collected.

Microsatellite analysis: All nine analyzed loci were polymorphic. A total of 69 alleles were produced among the 26 individuals. Number of different alleles (Na), effective number of alleles (Ne), Shannon's information index (I), observation heterozygosity (Ho), expected heterozygosity (He), Fixation index (F) and probability of identity (PI) were calculated (Tab. 2). Locus VVS2 had the lowest number of alleles (6), while locus VVMD28 was the most polymorphic with 10 alleles. The average of alleles per locus was 7.66. The effective number of alleles (Ne) was lower for each locus investigated and it ranged from 3.704 to 6.693 with an average of 5.002. The highest Shannon's information index (I) value was observed in the VVMD28 locus (2.049), while the VrZAG79 locus had the lowest value with an average of 1.536 among SSR loci (Tab. 2). Shannon's information index is an important index for reflecting the level of polymorphism. The lowest Ho was 0.731 (VVMD25) while the highest value (0.923) was for VVMD32, with an average of 0.838. The lowest and the highest He values were respectively 0.851 and 0.730 for VVMD28 and VVMD25, with an average of 0.793. In all cases the observed Heterozygosity (Ho) showed values higher than expected one (He), except for VVMD27. All microsatellites amplified homozygous loci in at least three genotypes (VVS2, VVMD28 and VVMD32). VVMD2 and VVMD25 amplified homozygous loci in six out of twenty-six genotypes. 'Guarnaccia B.', BAS02 and 'Marchione B.' revealed three homozygous loci among all nine microsatellites investigated. The fixation index F ranged from -0.013 and -0.158 with an average of -0.056. The probability of identity (PI) revealed a very low probability that two unrelated individuals will have an identical genotype for each single SSR marker. In fact, the PI calculated for individual loci ranged from 0.039 for the most discriminating locus VVMD28 to 0.115 for the least discriminating VVMD25. The probability that two individuals drawn randomly in a given population share identical genotypes at the nine loci analyzed is about four chances in a billion (3.631×10^{-11}) considering that this probability becomes very small if many highly polymorphic loci are considered. Allele size and frequencies of the alleles for each of the microsatellites are shown in Tab. 3. The total number of alleles was 25. For each of the loci, there was at least one allele with a frequency higher than 0.20, two alleles for loci VVMD5, VVMD7, VrZAG62, VrZAG79. Only in VVMD25 there were two alleles with a frequency higher than 0.3. In addition, the loci showing three alleles with a frequency higher than 20 % were VVS2 and VVMD32. In VrZAG79, the allele 14 represented around 40 % of the total number of allele frequency. Conversely, 14 alleles highlighted frequency below 5 %. According-

Table 2

Number of total genotypes (N), number of different alleles (Na), number of effective alleles (Ne), Shannon's information index (I), observed (Ho) and expected (He) heterozigosity, Fixation index (F) and probability of identity (PI) of the nine SSR markers for the grape cultivars analyzed in this study

Table locus	N	Na	Ne	I	Ho	He	F	PI
VVS2	26	6	4.811	1.633	0.846	0.792	-0.068	0.075
VVMD5	26	7	5.240	1.779	0.846	0.808	-0.046	0.062
VVMD7	26	8	5.200	1.814	0.846	0.808	-0.048	0.062
VVMD27	26	7	4.537	1.680	0.769	0.780	-0.013	0.077
VrZAG62	26	9	6.145	1.926	0.885	0.837	-0.057	0.047
VrZAG79	26	7	3.756	1.536	0.808	0.734	-0.101	0.109
VVMD25	26	8	3.704	1.539	0.731	0.730	-0.001	0.115
VVMD28	26	10	6.693	2.049	0.885	0.851	-0.040	0.039
VVMD32	26	7	4.934	1.691	0.923	0.797	-0.158	0.071
Mean	26	7.667	5.002	1.739	0.838	0.793	-0.056	-
Cumulative	-	69	45.02	-	-	-	-	3.631×10^{-11}

Table 3

Allele Size (AS) and allele frequency (AF) for the nine SSR markers in the grape cultivars analyzed in this work

SSR loci																	
VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79		VVMD25		VVMD28		VVMD32	
AS	AF	AS	AF	AS	AF	AS	AF	AS	AF	AS	AF	AS	AF	AS	AF	AS	AF
10	0.269	4	0.250	8	0.288	4	0.365	12	0.019	6	0.135	4	0.154	12	0.154	15	0.135
12	0.154	6	0.077	12	0.077	6	0.154	14	0.212	8	0.019	6	0.365	18	0.096	17	0.269
20	0.212	10	0.269	16	0.154	8	0.038	16	0.019	10	0.077	8	0.038	20	0.115	20	0.019
22	0.231	12	0.058	18	0.250	10	0.173	18	0.019	12	0.019	14	0.019	28	0.154	21	0.212
24	0.019	14	0.115	20	0.077	14	0.135	20	0.173	14	0.404	16	0.019	29	0.019	23	0.115
28	0.115	18	0.173	22	0.096	16	0.019	22	0.135	20	0.077	20	0.327	30	0.038	27	0.019
-	-	24	0.058	24	0.019	19	0.155	26	0.212	22	0.269	28	0.058	32	0.250	37	0.231
-	-	-	-	32	0.038	-	-	28	0.077	-	-	32	0.019	28	0.019	-	-
-	-	-	-	-	-	-	-	30	0.135	-	-	-	-	42	0.115	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	44	0.038	-	-

ly, the most informative marker for the present study was VVMD28 with a PI of 0.041 as reported in another study (Moreno-Sanz *et al.* 2011). In addition, the least informative marker was VVMD25.

Cultivar identification: From 85 accessions presumed to be different according to winegrowers personal communication, 42 allelic profiles were obtained. Identity was assigned by comparison with the consulted grapevine SSR databases and bibliography. Among accessions, the identity of 16 genotypes corresponded to widespread national and international cultivars (not shown). Moreover, 17 genotypes were identified as minor/local cultivars. Likely, some genotypes, such as 'Bellone B.', 'Castiglione N.', 'Grillo' and 'Uva di Troia', are currently under observation (see Tab. 1). The grapevine genetic profiles identified by the code "BAS" followed by a number from 1 to 9 are described here for the first time (Tab. 4). BAS01, BAS02 and BAS03 have a white berry colour and they were found in two growing areas such as *Chiaromonte* and *Lagonegro*; other 5 new accessions are red cultivars and they were discovered in four different growing areas of Basilicata region (*Maratea*, *Rivello*, *Sant'Arcangelo* and *Roccanova*). The BAS05 is the only cultivar with a pink berry colour found in *Maratea*.

Berry colour, growing area and allelic sizes of genotypes obtained for the nine SSR microsatellite loci analyzed are shown in Tab. 4. The majority of known minor or local varieties are currently cultivated in several regions in the south of Italy, whereas 'Pavana N.' is mainly cultivated in Trentino Alto Adige. Based on microsatellite profiles, a dendrogram of genetic similarity was generated by computing UPGMA method using Correlation matrix, where the cophenetic correlation was 0.6568 (Fig. 1). Cluster analysis of the data allows grouping minor/local known cultivars together with new ones in order to evidence the genetic relationship among all genotypes described in this study. Three main groups were distinguished, indicating several origins for grapevine germplasm in the south of Basilica-

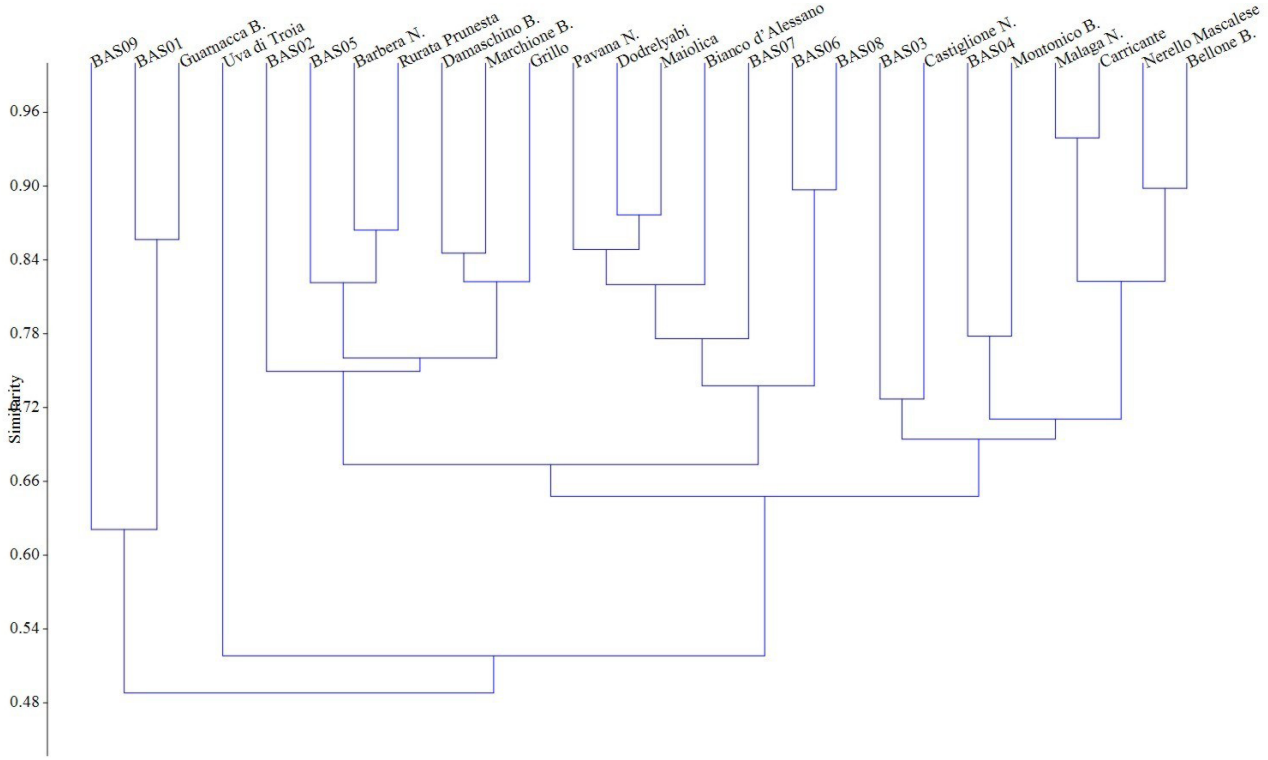

Fig. 1: Genetic dendrogram of the twenty-six grape cultivars investigated in this study. It was generated by computing UPGMA method using Correlation matrix, where the cophenetic correlation was 0.6568

ta. It was not always possible to correlate between clustering and growing area where genotypes were found. Also no relationship was evident between clustering and berry colour with the exception of the sub-cluster a composed by 'Grillo', 'Marchione B.' and 'Damaschino' (level of similarity of 0.825) and the sub-cluster 'Guarnaccia B.' and BAS01. Instead, in Fig. 1, it is possible to distinguish at least three main clusters and four outlier genotypes, reported in the upper part of the figure. The first cluster was mainly characterized by cultivars from the south of Italy with the exception of 'Bellone B.' native of Lazio region (Central Italy). 'Bellone B.' is widespread and cultivated in the Tuscia area as reported in Muganu *et al.* 2009. 'Nerello mascalese' and 'Carricante' are important cultivars of Sicily with an ancient origin (Carimi *et al.* 2010). Their genetic relationship suggests a common origin from Contea of Mascali in Etna city (Sestini 1812). The new accession BAS04 highlighted genetic similarity with 'Montonico B.', which is the second parent of the 'Sicilian Catarratto' (Crespan *et al.* 2017). Alba *et al.* (2015b and 2016) described for the first time the accession 'Malaga N.' that showed a strong similarity (up to 0.925) with the Sicilian 'Carricante'. The accession is currently preserved in the germplasm collection of CREA-VE of Turi. The white BAS03 evidenced a similarity with the autochthonous cultivar of Cosenza (Calabria region) 'Castiglione N.' (Mazzei and Zappala 1965). Among the pairs, the least distance in the first cluster was observed for the 'Malaga N.' and 'Carricante' (0.95 level of similarity). The second cluster was characterized by two main sub-clusters. In this case, the relatedness of the new accessions BAS07, BAS06 and BAS08 was evidenced. In fact, BAS08 and BAS06 shared high genetic similarity; moreover, BAS07 appeared similar to some minor/local cultivar of Puglia ('Bianco D'Alessano'), Abruzzo ('Maiolica'), Veneto ('Pavana N.') and 'Dodrelyabi' accession with Georgian origin (VIVIC: www.vivc.de). Among the pairs, in the second cluster, the least distance was evidenced for BAS06 and BAS08. In the third group, the white BAS02 and the pink BAS05 were related to 'Barbera' and 'Rurata prunesta'. 'Barbera' is one of the most important red-grape variety grown in the north of Italy, being, since the 1970s, the base cultivar for the production of high quality wines. Until now, 33 clones have been selected and registered in Italy. Other white cultivar, such as the Sicilians 'Grillo' and 'Damaschino' and the ancient autochthonous 'Marchione', which originated from Valle d'Itria (Puglia region) are highlighted in the same cluster. The outliers are the pink BAS09 and the white BAS01 that evidenced a high genetic similarity with the 'Guarnaccia B.' of Consenza (Calabria region) with a genetic level of similarity around 0.85. Some of the cultivars reported in this study had already been detected previously (Schneider *et al.* 2014, Mercati *et al.* 2016), but others are investigated here for the first time. Some new accessions

Table 4

Allele size in a relative base pair distance to allele size "n" as coded by OIV of twenty-six genotypes at 9 SSRs, homozygous loci are reported in bold. Berry colour and Growing area are highlighted

N°	Cultivar	Berry colour	Growing area	VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79		VVMD25		VVMD28		VVMD32	
1	Guarnacca Bianca	White	Carbone	20	28	4	18	**16**	**16**	**4**	**4**	20	30	14	22	**20**	**20**	12	32	17	21
2	Nerello Mascalese	Black	Carbone	10	20	4	14	8	18	**4**	**4**	22	28	14	22	**6**	**6**	12	28	17	21
3	**BAS01**	White	Chiaromonte	20	28	6	18	16	18	4	6	28	30	**22**	**22**	**20**	**20**	12	20	15	17
4	**BAS02**	White	Lagonegro	**22**	**22**	**18**	**18**	**18**	**18**	14	19	14	20	6	20	6	20	18	32	15	37
5	**BAS03**	White	Lagonegro	10	28	4	24	**20**	**20**	6	14	16	20	8	22	8	16	**32**	**32**	**21**	**21**
6	Pavana Nera	Black	Lagonegro	10	20	10	14	8	18	6	19	20	30	14	22	6	20	12	28	27	37
7	Bellone Bianco	White	Lagonegro	12	22	6	10	8	16	**4**	**4**	14	30	14	22	4	6	18	44	17	23
8	Dodrelyabi	Pink	Lauria	10	22	4	14	16	24	4	10	**30**	**30**	14	14	4	20	12	28	17	37
9	**BAS04**	Black	Maratea	**12**	**12**	4	10	8	16	4	10	20	22	6	20	**20**	**20**	**42**	**42**	17	21
10	**BAS05**	Pink	Maratea	12	22	10	18	8	16	**6**	**6**	14	22	6	20	6	20	20	42	17	23
11	**BAS06**	Black	Maratea	12	22	4	10	8	22	**4**	**4**	20	26	6	10	4	20	12	32	21	21
12	Bianco d'Alessano	White	Maratea	20	22	**10**	**10**	8	22	6	10	14	26	6	22	6	20	20	30	23	37
13	Montonico Bianco	White	Maratea	20	22	**10**	**10**	8	18	4	10	14	26	**14**	**14**	6	20	30	32	15	17
14	**BAS07**	Black	Rivello	10	22	4	10	8	32	**4**	**4**	20	28	14	22	6	6	12	18	21	37
15	Damaschino Bianco	White	Rivello	20	22	6	18	8	12	4	19	12	14	14	20	6	20	32	42	15	37
16	Grillo	White	Rivello	**20**	**20**	12	18	8	18	6	19	14	30	**14**	**14**	4	8	20	42	21	37
17	**BAS08**	Black	Sant'Arcangelo	22	24	10	18	18	22	4	14	**26**	**26**	6	14	4	20	12	38	21	23
18	Uva di Troia	Black	Sant'Arcangelo	20	28	4	10	12	22	14	19	14	26	**14**	**14**	28	32	28	32	15	37
19	**BAS09**	Pink	San Severino	10	28	6	24	18	20	10	14	**26**	**26**	14	22	6	28	18	20	17	23
20	Castiglione Nero	Black	San Severino	10	20	10	14	16	32	14	19	20	22	12	14	6	14	28	32	17	23
21	Malaga N.	Black	San Severino	10	12	4	12	12	18	6	8	14	26	10	22	**6**	**6**	28	32	15	17
22	Maiolica	Black	San Severino	10	28	4	14	8	18	4	10	22	28	14	22	6	28	20	29	17	37
23	Marchione Bianco	White	San Severino	10	22	10	24	**8**	**8**	4	16	14	22	10	14	**6**	**6**	**32**	**32**	17	37
24	Carricante	White	San Severino	**10**	**10**	4	14	8	18	4	10	20	22	14	22	4	6	28	32	21	20
25	Barbera Nera	Black	Roccanova	10	12	**4**	**4**	18	22	10	14	18	26	6	22	4	20	18	44	17	37
26	Rurata Prunesta	Black	Trecchina	10	12	12	18	12	20	8	10	14	26	10	14	4	20	28	42	15	37

are probably autochthonous and relatives of the main cultivated varieties in the south of Italy. Further investigations are necessary to confirm this hypothesis starting from the molecular analysis of more loci.

Ampelography and ampelometric traits: Main ampelographic characters are highlighted in Tab. 5. The characters were, however, not sufficient to distinguish among all genotypes, consequently ampelometric study was performed. In recent times, ampelometry represents an important tool in grapevine morphological description for the metric calculations of the main traits of a "mature leaf". The normality test in "FactoMineR" package of R software was firstly applied in order to assess the normality distribution of ampelometric data and, subsequently subjected to Principal Component Analysis (Tab. 6 and Fig. 2). Eigenvalues, percentage of variation and eigenvectors were calculated (Tab. 6).

In addition, values with higher weights on the determination of PCA axes were marked in bold. The first three axes explained a total of 76 % variability. The first two components explained respectively 55.38 % and 11.59 % of total variability. Along the first axis, different behaviour of genotypes was shown by the mean of lateral veins traits. In fact, ON2, ON3, O3N4 and O4N5 represented the highest eigenvectors (weights upper than 0.952). In addition, the distance between petiolar point and the ned of lateral vein N4 (ON4) gave also the highest positive weights (0.965). In the second axis, which accounted for 11.59 % of variability, only the angle between N1 and N2 (α and β) at first bifurcation determined differences in genotypes showing eigenvectors weight of 0.832 and 0.601 respectively. In the third axis (9.03 % of variation), the ratio between lateral veins versus central vein (N1) discriminated genotypes. In particular, 0.498, 0.828 and 0.664 eigenvectors weight were observed in R2, R3 and R4 respectively. Results suggest how few ampelometric traits are sufficient to discriminate grapevine as reported by Alba *et al.* (2015a) and Preiner *et al.* (2014). The results evidenced and confirmed the efficiency of ampelometric traits like main veins, their ratio and angles between main veins allow differentiating and discriminating grape genotypes on the base of "mature leaf" score. In addition, ampelometric data are more accurate and objective than ampelographic descriptions that are mainly related to the experience of the technician that collects the sample. The similarity matrix of genetic data was compared to the ampelometric traits recorded for each genotype using Mantel test in Past software (Ver. 3.11). No correlation was found between the two matrices ($r = 0$). Accordingly, molecular and ampelometric traits could not be combined in a unique analysis.

Conclusion

Results obtained in this work contribute to the study of the wide genetic diversity of grapevines in several growing areas in the south of Basilicata region. Starting from 85 accessions collected in ancient vineyards, 26 profiles were characterized as new autochthonous and minor/local grapevines. The growing areas investigated are rather small, otherwise they resulted rich in terms of biodiversity. High grapevine diversity was found in *San Severino, Maratea* and *Lagonegro* with 6, 5 and 4 cultivars found respectively. *Maratea* was the richest in terms of variability with three new autochthonous genotypes discovered. Among 26 accessions, 17 genotypes matched with minor/local grapevines currently cultivated mainly in the south of Italy, whereas 9 new genotypes, considered endangered, are

Table 5

Main ampelographic mean values of OIV codes for mature leaf, wooden shoot and budding, flowering, veraison and ripening time of grape cultivars analyzed in this study

N°	Cultivar	Berry colour	65	67	68	69	75	80	83-1	306	101	103	301	302	303	304
1	Guarnacca bianca	White	5	3	2	5	3	3	3	1	1	3	5	5	7	7
2	Nerello Mascalese	Black	7	3	2	5	1	3	3	1	1	2	3	5	5	6
3	**BAS01**	White	6	3	3	5	3	1	3	1	1	2	5	5	5	5
4	**BAS02**	White	3	2	3	5	5	2	2	1	1	2	3	3	3	3
5	**BAS03**	White	3	2	3	5	1	1	3	1	2	3	3	5	6	3
6	Pavana nera	Black	5	3	3	3	5	1	1	2	3	3	3	5	5	5
7	Bellone Bianco	White	3	3	3	7	5	2	1	1	2	3	3	5	5	5
8	Dodrelyabi	Pink	7	3	4	7	7	3	3	2	1	3	5	5	7	7
9	**BAS04**	Black	7	4	3	5	5	3	1	2	1	3	5	5	5	5
10	**BAS05**	Pink	5	4	3	4	3	2	3	2	2	3	3	3	3	3
11	**BAS06**	Black	5	3	3	5	5	3	3	3	2	3	3	3	3	3
12	Bianco d'Alessano	White	5	4	3	7	1	3	3	1	1	2	3	3	3	3
13	Montonico Bianco	White	5	3	3	7	1	1	1	1	2	2	5	5	5	5
14	**BAS07**	Black	5	4	3	7	3	1	1	2	2	3	5	5	5	5
15	Damaschino Bianco	White	7	4	3	5	1	3	3	1	1	2	5	3	5	5
16	Grillo	White	5	4	3	5	1	3	3	1	1	2	3	5	5	6
17	**BAS08**	Black	6	3	3	5	1	1	1	2	1	2	5	5	5	5
18	Uva di Troia	Black	5	4	3	5	1	3	1	2	1	3	5	5	6	5
19	**BAS09**	Pink	5	3	3	5	3	1	1	1	1	3	7	6	5	7
20	Castiglione Nero	Black	5	3	3	3	1	1	3	1	1	2	5	5	6	6
21	Malaga N.	Black	6	4	3	3	5	3	3	2	1	3	5	5	6	6
22	Maiolica	Black	5	5	3	3	3	2	3	2	2	3	3	5	5	6
23	Marchione Bianco	White	5	2	4	7	5	3	1	2	2	3	5	6	5	5
24	Carricante	White	7	3	3	3	1	1	1	1	1	3	5	6	6	6
25	Barbera Nera	Black	5	3	3	7	3	3	3	4	2	2	3	5	5	6
26	Rurata Prunesta	Black	5	4	2	7	1	1	3	2	3	2	3	5	6	5

Table 6

Principal component analysis: eigenvalues, eigenvectors and percent of variation accounted for the first three principal components on ampelometric data of twenty-six grape cultivars

Trait description			Dim. 1	Dim. 2	Dim. 3
		Eigenvalue	12.18	2.55	1.98
		Variance (%)	55.38	11.59	9.03
		Cumulative (%)		66.97	76
	Abbreviation	Units	Eigenvectors		
Length of leaf	Le	mm	0.949	-0.054	-0.199
Width of leaf	Wi	mm	0.947	0.101	-0.049
Length of leaf + length of petiole	Lepet	mm	0.917	-0.022	-0.184
Length of petiole	OP	mm	0.819	0.129	-0.056
Length of central vein N1	ON1	mm	0.927	-0.175	-0.250
Length of lateral vein N2	ON2	mm	**0.959**	-0.107	0.004
Length of lateral vein N3	ON3	mm	**0.952**	-0.075	-0.032
Distance between petiolar point and the ned of lateral vein N4	ON4	mm	**0.965**	-0.044	-0.083
Length of lateral vein N4	O3N4	mm	**0.962**	0.013	0.110
Length of lateral vein N5	O4N5	mm	**0.956**	0.055	0.014
Lateral vein N3, distance between petiolar sinus and the end of lateral vein N4	OO3	mm	0.870	-0.279	-0.154
Distance between petiolar sinus and the lateral upper sinus	OS	mm	0.885	0.002	-0.119
Distance between petiolar sinus and the lateral lower sinus	OI	mm	0.918	0.036	-0.076
Angle between N1 and N2 at the first bifurcation	α	°	0.191	**0.832**	-0.208
Angle between N2 and N3 at the first bifurcation	β	°	0.253	**0.601**	-0.338
Angle between N3 and N4	γ	°	-0.066	0.423	-0.072
Width of angle of petiolar sinus	π	°	-0.074	-0.918	0.055
Ratio between OP and N1	RP	ratio	-0.329	0.404	0.317
Ratio between N2 and N1	R2	ratio	0.504	-0.134	**0.498**
Ratio between N3 and N1	R3	ratio	0.408	0.052	**0.828**
Ratio between N4 and N1	R4	ratio	0.467	0.275	**0.664**
Ratio between N5 and N1	R5	ratio	0.405	0.405	0.370

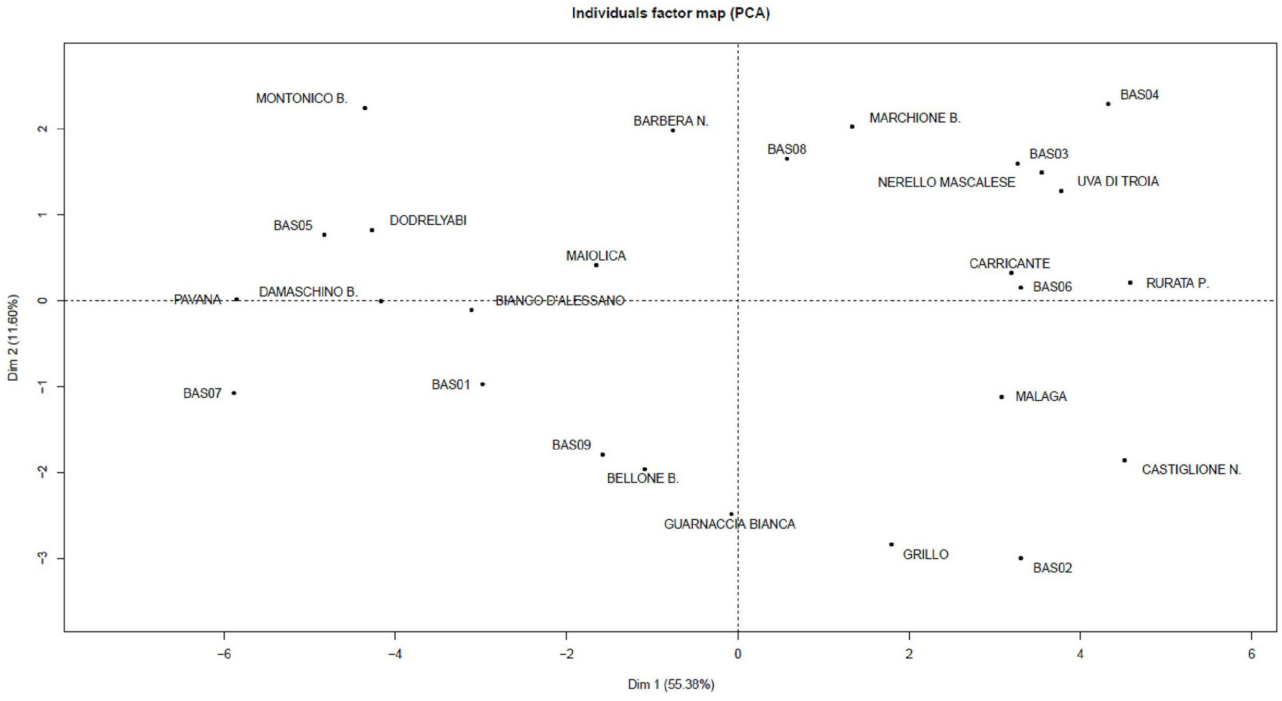

Fig. 2: Graphic representation of twenty-six grape genotypes according to axes 1 and 2 on the base of principal component analysis performed on twenty-two ampelometric traits.

presented here for the first time. Ampelometric traits confirmed the importance of lateral main veins, their ratio and angles in characterizing *Vitis vinifera* L. germplasm. The conservation of these unique plant material was secured by introduction into the existing collection of a certified wine farm for germplasm preservation in *Roccanova* and in the CREA-VE collection in Arezzo. The maintenance of endangered cultivars highlighted in this work allow future agronomical and enological evaluation to enhance and improve the typical traits of Basilicata southern territory.

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References

Alba, V.; Bergamini, C.; Gasparro, C.; Mazzone, F.; Roccotelli, S.; Antonacci, D.; Caputo, A. R.; Del Lungo, S.; Dal Monte, G.; Cirigliano, P.; Labagnara, T.; Pisani, F.; Cerbino, D.; 2016: Basivin-Sud la

Ricerca del Germplasma Viticolo in Basilicata. Mario Adda Editore, Bari, Italy.

Alba, V.; Bergamini, C.; Gasparro, C.; Mazzone, F.; Roccotelli, S.; Antonacci, D.; Caputo, A. R.; Del Lungo, S.; Dal Monte, G.; Cirigliano, P.; Labagnara, T.; Pisani, F.; Cerbino, D.; 2015b: Il Progetto Basivin_SUD Recupero e Valorizzazione delle Principali Varietà Locali e dei Vitigni Autoctoni Minori in Basilicata. Mario Adda Editore, Bari, Italy.

Alba, V.; Bergamini, C.; Genghi, R.; Gasparro, M.; Perniola, R.; Antonacci, D.; 2015a: Ampelometric leaf trait and SSR loci selection for a multivariate statistica approach in *Vitis Vinifera* L. Biodiversity management. Mol. Biotechnol. **57**, 709-719.

Antonacci, D.; Caputo, A. R.; Gasparro, M.; Bergamini, C.; 2012: Il database di *Vitis vinifera* del CREA-UTV di Turi. Proc. IXth Convegno Nazionale Biodiversità, CIHEAM - IAM, September 5-7, 2012, Valenzano, Italy.

Bergamini, C.; Caputo, A. R.; Gasparro, M.; Perniola, R.; Cardone, M. F.; Antonacci, D.; 2013: Evidences for an alternative genealogy of 'Sangiovese'. Mol. Biotechnol. **53**, 278-288.

Bergamini, C.; Perniola, R.; Cardone, M. F.; Gasarlo, M.; Pepe, R.; Caputo, A. R.; Antonacci, D.; 2016: The molecular characterization by SSRs reveals a new South Italian kinship and the origin of the cultivar Uva di troia. SpringerPlus **5**, Art. 1562.

Bowers, J. E.; Dangl, G. S.; Vignani, R.; Meredith, C. P.; 1996: Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome **39**, 628-633.

Bowers, J. E.; Dangl, G. S.; Vignani, R.; Meredith, C. P. 1999: Development and characterization of additional microsatellite DNA markers for grape. Am. J. Enol. Vitic. **50**, 243-246.

Brunori, E.; Cirigliano, P.; Biasi, R.; 2015: Sustainable use of genetic resources: the Characterization of Italian local grapevine variety ("Grechetto rosso") and its own landscape. Vitis **54**, 261-264.

Carimi, F.; Mercati, F.; Abbate, L.; Sunseri, F. 2010: Microsatellite analyses for evaluation of genetic diversity among Sicilian grapevine cultivars. Genet. Res. Crop Evol. **57**, 703.

Crespan, M.; Storchi, P.; Migliaro, D.; 2017: Mantonico bianco grapevine cultivar is the second parent of the Sicilian Catarratto. Am. J. Enol. Vitic. **68**, 258-262.

De Andrés, M. T.; Benito, A.; Pérez-Rivera, G.; Ocete, R.; López, M. A.; Gaforio, L.; Muñoz, G.; Cabello, F.; Martínez-Zapater, J. M.; Arroyo-García, R.; 2012: Genetic diversity of wild grapevine populations in Spain and their genetic relationship with cultivated grapevines. Mol. Ecol. **21**, 800-816.

Del Lungo, S.; 2015: Centro Terziario di Domesticazione: la Topografia Antica e la Genetica in Enotria, dalla Siriche alla Multivarietà Viticola della Lucania. Il Progetto Basivin-SUD, chapter 3.

Del Lungo, S.; Caputo, A. R.; Gasparro, M.; Alba, V.; Bergamini, C.; Roccotelli, S.; Mazzone, F.; Pisani, F.; 2016: Lucania as the heart of III vine domestication center: The rediscovery of autochthonous vines. 39th World Congress of Vine and Wine. BIO Web Conf. **7**, 01021.

D'Onofrio, C.; Scalabrelli, G.; 2014: The software for a universal grapevine database. Acta Hort. **1046**, 665-672

Gonzáles-Andrés, F.; Martín, J. P.; Yuste, J.; Rubio, J. A.; Arranz, C.; Ortiz, J. M.; 2007: Identification and molecular biodiversity of autochthonous grapevine cultivars in the "Comarca del Bierzo", Leon, Spain. Vitis **46**, 71-76.

Kullaj, E.; Bacu, A.; Thomaj, F.; Ficu, H.; Argyriou, A.; 2015: Albanian grapevine cultivars: Preliminary results of molecular, phenolic and ampelometric profiles and relatedness. Vitis **54**, 111-113.

Maletić, E.; Pejić, I.; Karoglan Kontić, J.; Zdunić, G.; Preiner, D.; Šimon, S.; Andabaka, Ž.; Žulj Mihaljević, M.; Bubola, M.; Marković, Z.; Stubić, D.; Mucalo, A.; 2015: Ampelographic and genetic characterization of Croatian grapevine varieties. Vitis **54**, 93-98.

Maul, E.; Sudharma, K. N.; Kecke, S.; Marx, G.; Müller, C.; Audeguin, L.; Boselli, M.; Boursiquot, J.; Bucchetti, B.; Cabello, F.; Carraro, R.; Crespan, M.; De Andrés, M. T.; Eiras Dias, J.; Ekhvaia, J.; Gaforio, L.; Gardiman, M.; Grando, S.; Gyropoulos, D.; Jandurova, O.; Kiss, E.; Karoglan Kontić, J.; Kozmá, P.; Lacombe, T; Laucou, V.; Legrand, D.; Maghradze.; Marioni, D.; Maletić, E.; Moreira, F.; Muñoz-Organero, G; Nakhuttrishvili, G.; Pejić, I.; Peterlunger, E.; Pitsoli, D.; Pospíšilová, D.; Preiner, D.; Raieno, C.; Šimon, S.; Staraz, M.; Zulini, L.; Bacilieri, R.; This, P.; 2012: The European *Vitis* Database (www.eu.-vitis.de) – a technical innovation through an online uploading and interaction modification system. Vitis **51**, 79-85.

Mazzei, A.; Zappala', A.; 1965: Principali Vitigni da Vino Coltivati in Italia - Volume IV, Ministero dell'Agricoltura e delle Foreste

Mercati, F.; De Lorenzis, G.; Brancdoro, L.; Lupini, A.; Abenavoli, M. R.; Barbagallo, M. G.; Di Lorenzo, R.; Scienza, A.; Sunseri, F.; 2016: High-throughput 18K SNP array to assess genetic variability of the main grapevine cultivars from Sicily Tree Genet. Genomes **12**, 59.

Ministero d'Agricoltura, Industria e Commercio, Direzione Dell'Agricoltura; 1881: Bollettino Ampelografico, FASCICOLO XV.

Moreno-Sanz, P.; Loureiro, M. D.; Suarez, B.; 2011: Microsatellite characterization of grapevine (*Vitis vinifera* L.) genetic diversity in Asturia (Northen Spain) Sci. Hortic. **129**, 433-440

Muganu, M.; Dangl, G.; Aradhya, M.; Frediani, M.; Scossa, A.; Stover, E.; 2009: Ampelographic and DNA Characterization of local grapevine accessions of the Tuscia area (Latium, Italy). Am. J. Enol. Vitic**.** **60**, 110-115.

OIV 2009. 2nd edition of the OIV descriptor list for grape varieties and *Vitis* species. Organisation International de la Vigne et du Vin, Paris, France-International Plant Genetic Resources Institute, Rome, Italy (www.oiv.org).

Peakall, R.; Smouse, P. E.; 2012: GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research an update. Bioinformatics **28**, 2537-2539 http://bioinformatics.oxfordjournals.org/content/28/19/2537

Preiner, D.; Safner, T.; Karoglan Kontić, J.; Marković, Z.; Simon, S.; Maletić, E. 2014: Analysis of phillometric parameters efficiency in discrimination of Croatian native *V. vinifera* cultivars. Vitis, **44**, 67-72

Russo, S.; 2012: Basilicata. In: Environmental History Springer, Italian Historical Rural Landscapes. Chapter **1**, 453-465.

Sefc, K. M.; Regner, F.; Turetschek, E.; Glossl, J.; Steinkellner, H.; 1999: Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genom. **42**, 367-373.

Sestini, D.; 1812: Dei vini di Mascali della Sicilia. Accademia dei Georgofili, Firenze, Manuscript 12 C.

Schneider, A.; Raimondi, S.; Pirolo, C. S.; Marionidi, D. T.; Ruffa, P.; Venerito, P; La Notte, P.; 2014: Genetic characterization of grape cultivars from Apulia (southern Italy) and synonymies in other Mediterranean regions. Am. J. Enol. Vitic. **65**, 244-249

Thomas, M. R.; Scott, N. S.; 1993: Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor. Appl. Genet. **86**, 985-990.

Zavaglia, C.; Pecile, M.; Bavaresco, L.; Gardiman, M.; Pezza, L.; Mancusi, D.; d'Aristotile, M. 2014: Il Registro Nazionale delle Varietà e cloni di Vite, In: Acta Italus Hortus, CONAVI 2014, July 01-03, 2014 Foggia.

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