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Vitis vinifera subsp. *sylvestris* and *sativa*; so far, so close: a 20 SSR based comparison of the two taxa

S. Imazio[1)], G. De Lorenzis[2)], B. Biagini[2)], D. Maghradze[3)], A. Scienza[2)] and O. Failla[2)]

[1)] Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia, Reggio Emilia, Italy
[2)] Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano, Milano, Italy
[3)] Institute of Horticulture, Viticulture and Oenology, Agricultural University of Georgia, Tbilisi, Georgia

Summary

In *Vitis vinifera* L., the hypothesis of secondary domestication center, located along the wild progenitor distribution areas, is suggestive and credible even if up to now close parentage relationships between domestic (*Vitis vinifera* L. subsp. *sativa* (DC.) Hegi) and wild (*Vitis vinifera* L. subsp. *sylvestris* (Gmel.) Hegi) grapevines have not been detected, possibly due to century long separation of the two subspecies. The aim of this work was to verify the possibility of tracing a flow between the two compartments basing on molecular data and thanks to the availability of a huge dataset comprising 645 wild and more than 1400 cultivated samples. Twenty SSR loci were used to describe and genotype both *sylvestris* and *sativa* compartments. The *sylvestris* samples were all collected in the frame of a three year census in Italy and are representative of the Italian distribution range from north to south. The cultivated *sativa* accessions mainly (1231 samples) belong to the Vassal (INRA-Montpellier) collection, while the remaining (200) were selected in the frame of the Italian grapevine germplasm. Results highlighted a high level of genetic diversity for both wild and cultivated groups. STRUCTURE analysis clearly evidenced the separation of the two compartments and no first or second degree relationships were evidenced between the two subspecies.

K e y w o r d s : domestication; feral forms; gene flows; introgression; parentage analysis.

Introduction

The first centers of domestication of wild grapevines (*Vitis vinifera* L. subsp. *sylvestris* (Gmel.) Hegi), dating back 8,000 years ago, were probably located in the South Caucasus and in the Near East regions, comprising Oriental Anatolia, Syria and the area around Northern Mesopotamia. The distribution area for *V. vinifera sylvestris* is large (Heywood and Zohary 1991) and progenitor populations of the domestic compartment are supposed to be still present in the spontaneous flora of Mediterranean regions, including the Italian Peninsula that seems to be one of the richest under this point of view. A recent census (Biagini *et al.* 2014) in the entire country has verified the condition of Italian populations and allowed to collect a statistically appropriate number of samples from all the populations. At the same time in these last ten years, numerous European and national projects devoted to a better knowledge of European grapevines genetic resources have stimulated a reorganization effort, which included the genotyping of the most important germplasm grapevines collections, giving also the guidelines to identify the accessions "true to typeness". This allows the construction of huge datasets to perform genetic analysis and comparisons.

Till now numerous studies (Imazio *et al.* 2006, Arroyo-Garcia *et al.* 2006, Myles *et al.* 2011, Snoussi *et al.* 2004, Ghaffari *et al.* 2014) have tried to identify genetic links and possible parentages among the wild and *sativa* subspecies. This work represents a first tentative based on a huge dataset, with the highest number of *sylvestris* samples ever based on the 20 SSR allelic profiles.

Material and Methods

The entire dataset was obtained from previous investigations. Wild samples derived from a three years census (Biagini *et al*. 2014) performed on the Italian territory, the cultivated varieties were obtained from the literature (Lacombe *et al*. 2013, De Lorenzis *et al*. 2013, 2014) and private databases as well (data not shown), including a great percentage of Italian varieties. All the samples included were previously genotyped at 20 SSR loci (Laucou *et al*. 2011, Biagini *et al*. 2014, De Lorenzis *et al*. 2013, De Lorenzis *et al*. 2014), and all cultivated accessions were selected basing on the results of the genotyping and structure analysis of collections, to be sure to refer to well described samples with sure provenience, avoiding mistakes.

The 20 SSR allelic profiles obtained from different works were normalized using four reference varieties: 'Cabernet Sauvignon', 'Chardonnay', 'Pinot noir' and 'Sangiovese'. Normalized data were initially analyzed with Identity 1.0 (Wagner and Sefc 1999) and GenAlEx 6.5 (Peakall and Smouse 2006, 2012) software to find perfect matches (synonyms) among the two compartments, or the existence of relationships. As a second step, a comparison with the Excel macro developed in the frame of the GrapeGen06 EU project was performed to identify allele sharing, and GenAlEx 6.5 software was used, once again, to assess the relationship among the wild and the cultivated groups,

Correspondence to: Dr. S. Imazio, Dipartimento di Scienze della Vita, Università di Modena e Reggio Emilia, via Amendola, 2, Padiglione Besta, 42122 Reggio Emilia, Italy. Email: serenaanna.imazio@unimore.it

computing pair-wise Euclidean distance for every pair of accessions and to perform Principal Coordinate Analysis (PCoA) conducted on individual multilocus genotypes with standardized covariance. Bayesian clustering using STRUCTURE 2.34 (Pritchard *et al.* 2000) with admixture model was employed to evaluate the number of inferred population clusters (K) and to assign individuals to their likely population of origin using no prior information. Ten independent runs were assigned per each K value. A burn-in of 20,000 steps was initially adopted and followed by 100,000 Markov chain Monte Carlo steps (Falush *et al.* 2007). The most suitable K value was evaluated based on the method reported in Evanno *et al*. (2005).

Results

In order to trace a flow between *sativa* and *sylvestris* compartments and to discover possible first or second degree relationships between them, more than 2,000 wild and cultivated grapevine samples, including the most important Italian cultivars and the rare ones as well, were compared. Identity and GenAlEx softwares were unable to identify synonyms and first or second degree relationships. On the other side, the adoption of the GrapeGen06 Excel macro, helped in the identification of few matching profiles between the two compartments. Six samples from the wild compartment proved to be very similar to 'Trebbiano Toscano' (syn. 'Ugni Blanc') and 'Sangiovese'. The GrapeGen06 Excel macro identified 20 cultivated accessions having 30 % of loci in common with some *sylvestris* ones, but matches were not enough to identify and confirm the existence of first or second degree relationship.

GenAlEx was used to define a genetic distance matrix used in computing principal coordinate analysis (PCoA) on the entire dataset. The two dimensional projections of PCoA analysis per each samples were plotted in a 2-D dimension scattered plot (Fig. 1). The first two principal coordinates accounted for 18.5 % of total variability and divided the two taxa clearly in distinct groups. The few overlapping areas were related to sampling mistakes, identified by GrapeGen06 Excel macro.

STRUCTURE software was used to verify admixtures. The tentative of defining population (K) numbers was performed allowing the software to draw different scenarios with population number increasing from 1 to 10. Optimal K estimated the most likely number of populations at K = 2. Consistent with multivariate analysis, structure clustering highlighted two groups: one for *sativa* accessions and the other for *sylvestris* individuals (Fig. 2).

Discussion

Although cultivated grapes are easily distinguishable from wild individuals due to their differences in some phenotypic traits (*e.g.* flower sex, berry and cluster size and shape), the distinction of feral cultivated forms, escaped from vineyards and survived without any agronomical treatment, and the recognition of *sylvestris* individuals is not simple even for expert eyes.

Few size mismatches among the allelic fingerprint made GenAlEx and Identity unable to identify the pres-

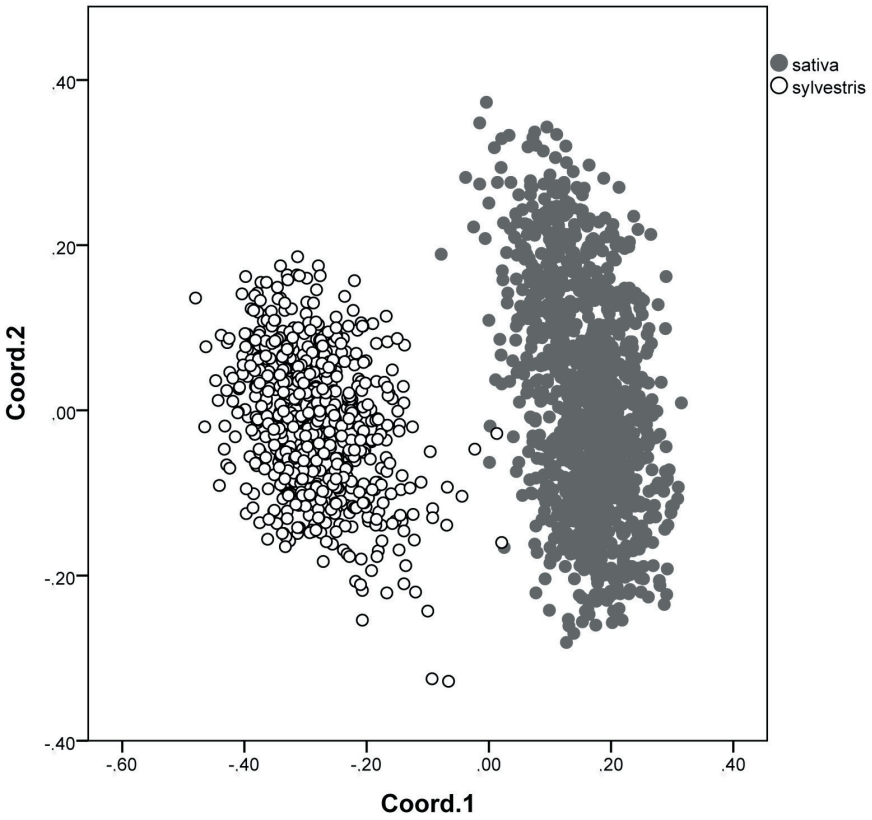

Fig. 1: Principal Coordinate Analysis (PCoA) scatterplot of Italian wild grapevines and European grapevine cultivars based on 20 SSR markers.

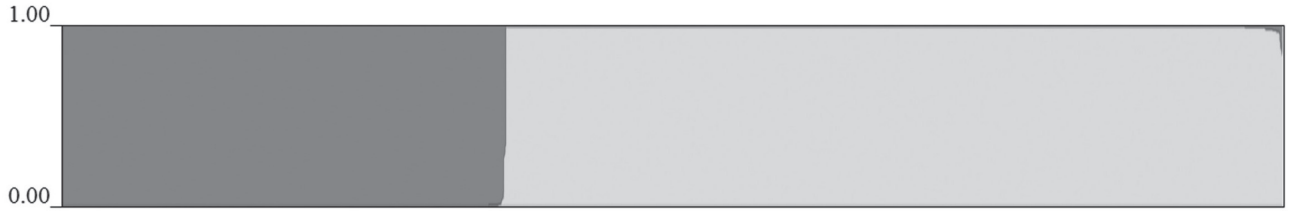

Fig. 2: Two-cluster bar plots resulted from STRUCTURE analysis. Y-axis: estimated membership proportion. Each sample is shown as vertical line divided into differently colored segments representing the estimated membership proportions in the two ancestral genetic clusters. Dark grey = sylvestris compartment; Light grey = sativa compartment.

ence of 'Trebbiano Toscano' and 'Sangiovese' among the *sylvestris* accessions. Nevertheless, the identification, by GrapeGen06 Excel macro, of these few identities among the wild and cultivated compartments was expected due to their not clear *sylvestris* habitus. The identification of 'Sangiovese' SSR profile was not surprising, indeed it is one of the most cultivated grapevine varieties in Italy and it is spread from North to South of the Italian territory (Bergamini *et al*. 2012, De Lorenzis *et al*. 2014).

The fact that only 6 out of 645 accessions were sampling mistakes, resulting feral forms of cultivated individuals, is very comforting and encouraging, suggesting the goodness of sampling strategy and the great value of the *sylvestris* collection used in this work (Biagini *et al.* 2014). Moreover, previous evidences about the goodness of this *sylvestris* dataset were obtained when a restricted set of samples (about 150 samples) was used to test the diagnostic potential of VVIB23 sex flower marker discriminating among female, male and hermaphrodite individuals (Battilana *et al.* 2013). The absence of first or second degree relationships between the two compartments confirmed what already stated and discussed in previous literature. For example, even though De Andrés *et al*. (2012) proposed a genetic contribution of wild accessions from Spain to current Western cultivars, based on the analyses of genetic relationships between wild and cultivated grapevine compartments, only 10 putative cases of spontaneous hybrids involving a set of 418 wild and cultivated plants collected from the Iberian Peninsula were identified. Other works addressing the lack of close relationships between wild individuals and cultivars have been published: Myles *et al*. (2011); Garfì *et al*. (2013); Imazio *et al*. (2013). Moreover, genetic evidences showed that a moderate to high gene-flow is achievable between wild and cultivated grapes (Di Vecchi-Staraz *et al*. 2009, Arroyo-Garcia *et al*. 2006).

The clear separation of two subspecies in distinct groups, highlighted by multivariate analysis and corroborated by STRUCTURE analysis, is a common result when wild European accessions and grape cultivars are compared. A similar distinction between the two compartments was highlighted by De Andrés *et al*. (2012), when the wild and cultivated individuals collected in Spain were analysed by 20 SSRs, as already stated by Ghaffari *et al*. (2014), analysing the Tunisian germplasm by 261 SNPs and Zdunić *et al*. (2014), analysing wild accessions maintained in the National Clonal Germplasm Repository (US Department of Agriculture, Davis, CA), in the collection of the Institute for Adriatic Crops and Karst Reclamation, Split, Croatia and collected from different sites in Albania.

Conclusions

The main conclusion arising from this study, also in comparison with similar ones, was that the putative western European secondary domestication center (dating back approximately from 4,000 to 2,000 years ago) may be probably detected only by searching for introgression remains from the wild into the domestic gene pools, as a consequence of several and successive spontaneous crossings and selection processes. Therefore, it seems to be quite impossible to trace a connection between the two taxa based on the identification of close parentage relationships. Moreover, the wild accessions, nowadays linked to the cultivated forms by first or second degree relationships, could be related to recent and transient events of spontaneous gene flow from *sativa* towards *sylvestris* individuals. These events are documented to be mediated by wind- and insect-dispersed pollen (Brantifs 1978), rather than by seed dissemination (Grassi *et al*. 2006).

Have there been one or several centres of domestication of the grapevine? The question is not settled (Bouquet 2008), at least for the Italian peninsula. Nevertheless, the search for possible "founding population" in the Italian Flora has to be continued and increased. Furthermore, the wild germplasm can represent an authentic new source of genes for grapevine breeding (resistant genes to biotic and abiotic stresses) as well as an important component of the Mediterranean autochthonous Flora to be saved from genetic erosion, due to human action, including intensive riverbank and forest management and disappearance of their most appropriate habitats.

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