

# Exploration and evaluation of grapevine biodiversity using molecular markers

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*The Vitis vinifera L. species encompasses some thousand varieties mainly conserved in germplasm repositories. The extensive use of molecular markers, especially SSRs, gave the possibility to explore the variability of this species at varietal level in a very quick and powerful way, reassessing ambiguities regarding homonyms, synonyms and even pedigree relationships. Some examples are given. The elaboration of molecular data with software such as GENETIX and STRUCTURE shows that V. vinifera genotypes are very close and form a single and compact group when compared with V. sylvestris or rootstock genotypes. Some Sardinian varieties, such as 'Garnacha tinta', are the only Italian grapes that group very closely to Armenian genotypes, and this fact is very intriguing because Sardinia was hypothesized as a second centre of origin for cultivated grapevine. Other minor Italian varieties, mainly from the North-Eastern part of the Italian peninsula, are the most divergent from Transcaucasian varieties, suggesting that they could be derived from a different domestication event.*

**Keywords:** Vitis, SSR marker, microsatellite analysis, genetic relationship, pedigree

*Untersuchung und Bewertung der Biodiversität von Weinrebe mittels Molekularer Marker. Die Art Vitis vinifera L. umfasst einige tausend Sorten, von denen die meisten in Genbanken erhalten werden. Durch die weitreichende Nutzung Molekularer Marker, speziell der SSR-Marker, ergab sich die Möglichkeit, die Variabilität dieser Art auf Sortenebene schnell und aussagekräftig zu erforschen und so Unklarheiten in Hinblick auf Homonyme und Synonyme und sogar Abstammungsverhältnisse von Sorten aufzuklären. Einige Beispiele werden angeführt. Die Aufbereitung molekularer Daten mittels Software (z. B. GENETIX und STRUCTURE) zeigt, dass V. vinifera-Genotypen sehr nahe verwandt sind und eine einheitliche und kompakte Gruppe bilden, wenn sie mit V. sylvestris- oder Unterlagsgenotypen verglichen werden. Einige sardinische Sorten, wie 'Garnacha tinta', sind die einzigen italienischen Sorten, die armenischen Genotypen sehr nahe stehen. Diese Tatsache ist sehr erstaunlich, da Sardinien hypothetisch als ein zweites Ursprungszentrum für Kulturreben betrachtet wird. Andere, weniger verbreitete italienische Sorten, hauptsächlich aus dem nordöstlichen Teil der Halbinsel, unterscheiden sich am deutlichsten von den transkaukasischen Sorten, sie könnten einem anderen Ereignis der Inkulturnahme entstammen.*

**Schlagwörter:** Vitis, SSR-Marker, Microsatelliten-Analyse, genetische Verwandtschaft, Abstammung

*Vitis vinifera L. with the two subspecies sativa and sylvestris is the only species of Vitis genus indigenous to Eurasia. In an attempt to quantify the number of varieties present in germplasm collections, evaluations performed at the beginning of the 1990ies estimated about 10.000 varieties around the world, but THIS et al. (2006) halved this number to 5.000 based on extensive DNA profiling results.*

*The source of V. vinifera biodiversity and spreading world-wide is due to sexual multiplication, vegetative propagation (cuttings) and somatic mutations. The*

*knowledge of grapevine biodiversity origin is important to understand its evolution, biology and also to find new tools for genetic improvement, based on molecular markers. Moreover, discovery of both parentage and progeny of traditional grape varieties is scientifically valuable and of considerable economic interest.*

*The introduction and massive use of SSR markers since the early 1990ies has gained a lot of new information on synonym and homonym ambiguities, both previously hypothesized by ampelographers or unknown. Using the same markers, researches have been increased in*



Fig. 1: Places where 'Malvasia delle Lipari' and its synonyms have been found

the last years on grapevine varieties pedigree determination (SEFC et al., 2001), contributing to clarify the evolution of the current ampelographic assortment. One of the most interesting examples is the discovery of the origin of dozens of French varieties from a single pair of parents, 'Pinot' and 'Gouais' (BOWERS et al., 1999). Finally, the implementation of new software to perform genetic analyses using these markers allowed describing more precisely the genetic relationship between different genotypes.

## Materials and Methods

### Plant material

Grapevine accessions coming from CRA-VIT collections, commercial nurseries and private vineyards

were analyzed.

### DNA profiling

Genotyping for variety identification was performed with 11 SSR loci (CRESPAN et al., 2006). For a profound pedigree relationship hypothesis, up to 36 SSR markers were analysed in specific cases (CRESPAN et al., 2008; CIPRIANI et al., 2010).

### Statistics on molecular data and genotype correlations evaluation

Different software (e. g. Identity, Cervus, Kingroup, NTSYS, GENETIX and STRUCTURE) was used to elaborate SSR data in order to get statistics on loci polymorphism, probability of identity, pedigree likelihood ratios and clustering genotypes.

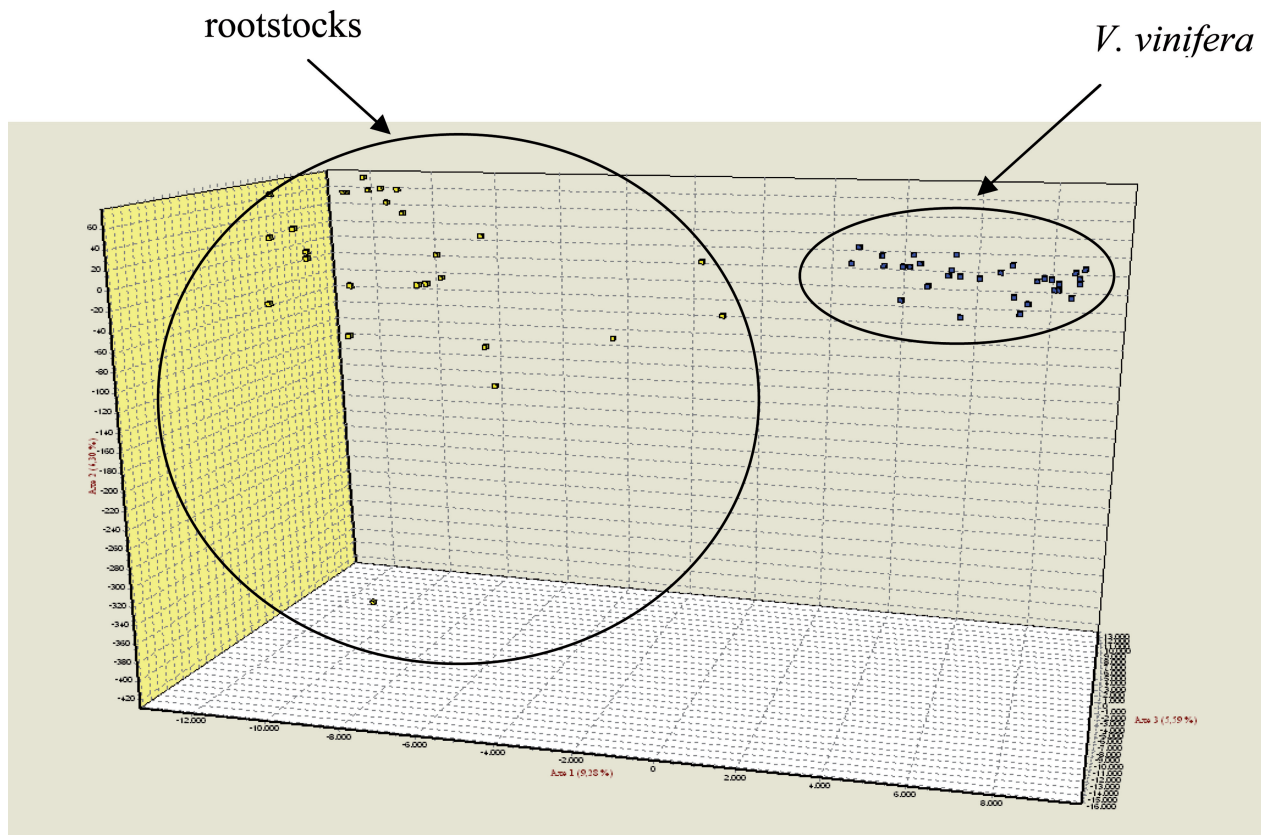


Fig. 2: Analysis of functional correspondence of 27 rootstock genotypes and a selection of 34 highly different *Vitis vinifera* genotypes

## Results and Discussion

### Clarifying homonyms and synonyms: Some examples

**On 'Wildbacher'.** Sixteen accessions of 'Wildbacher' coming from different European collections and from private vineyards in Styria and Italy were compared. Five different molecular profiles were found, showing that the designation 'Wildbacher' comprises a group of homonyms; only two of them are of agronomic interest: 'Wildbacher blau' and 'Wildbacher Spätblau'. These two varieties are morphologically and genetically very similar, the most important difference being linked to berry ripening time (MENEGETTI et al., 2009). 'Wildbacher blau' is grown also in Italy, in Treviso's province, where acidity levels at ripening are lower than in Styria, producing more coloured and better balanced wines.

**On 'Malvasia delle Lipari'.** The history of the variety 'Malvasia delle Lipari' can be considered a good ex-

ample of how SSR markers can contribute to the knowledge of a variety spreading over space and time. 'Malvasia delle Lipari' was considered a unique and very ancient variety grown on the Lipari's archipelagous (Sicily island) until the discovery that 'Malvasia di Sardegna', 'Greco di Gerace' (Italy), 'Malvasia de Sitges' (Spain) and 'Malvasia dubrovačka' (Croatia), previously believed to be distinct varieties and grown in very distant places, showed the same molecular profile. Ampelographic comparison supported this conclusion, too, indicating that they are all the same variety, because they do not differ in any important morphological trait. Therefore, this genotype is scattered all over the Mediterranean area and as far as Balearic islands, Canaries and Madeira along naval routes (Fig. 1). It represents one of the oldest known Malvasias, famous for its very special and aromatic wine. Historical investigation indicates that this genotype could have been imported from Greece, even if, so far, the search in Greek databases has not confirmed this theory (CRESPAN et al., 2006).

### Limits of SSR markers in identifying clones

SSR markers are very effective in distinguishing individuals originated through a sexual reproduction, but are not useful to differentiate clones belonging to the same variety, even if synonymous accessions can differ from each other in traits that could be important for wine production and for plant morphology, like berry colour and shape, cluster dimension, leaf morphology and so on. Actually, some additional SSR alleles can be found in the same plant but only rarely and almost in a chimeric state (FRANKS et al., 2002; CRESPIAN, 2004). A strong correlation can be hypothesized about the level of SSR polymorphisms in clones and variety age, as is the case of 'Pinot noir' vs. 'Chardonnay' (RIAZ et al., 2002) or 'Savagnin' vs. 'Riesling' (PELSY et al., 2010). In both cases, the older variety shows a higher level of SSR polymorphisms after the analysis of a comparable number of clones.

### Genetic variability between *V. vinifera* and rootstocks

A great conservation of microsatellite loci was found within the *Vitis* genus (DI GASPERO et al., 1999). For some SSR loci the distribution of allele frequencies mainly encompasses the entire range of lengths found in both groups, *V. vinifera* and rootstocks. Nevertheless, there are some loci in which the distribution of the alleles in the two groups clearly differs, to the point that for some length intervals, the alleles are specific to just one group. These alleles belong to either the lowest or the highest molecular weight range, or to both extremes, depending on the locus. These traits may be useful for understanding if a variety could be a hybrid, showing a genetic contribution from American species. Using GENETIX software a very clear separation of the two groups has been obtained, with *V. vinifera* va-

rieties very close to each other (Fig. 2) (CRESPIAN et al., 2009).

### Pedigree discovery: The example of Muscat family

Many researches contributed to discover kinships among varieties. Even if the time of origin cannot be determined, these studies outline the order of appearance of a number of varieties. One of these reconstructions regards the Muscat family, whose two most ancient founders, 'Muscat blanc à petits grains' and 'Muscat of Alexandria', showed to be linked by a first degree relationship (CRESPIAN and MILANI, 2001). In a very recent paper by CIPRIANI et al. (2010) it has been discovered that 'Muscat of Alexandria' surprisingly resulted from the cross 'Muscat blanc à petits grains' x 'Axina de tres bias', which was never suspected to be involved in Muscat pedigrees. Therefore 'Muscat blanc à petits grains' remains the oldest ancestor of this family. The second parent, 'Axina de tres bias' is an ancient black-berried variety without any agronomic interest. It is also known as 'Trifera', 'Uva di tre volte' and 'Tre volte l'anno', names which refer to its ability to flower and crop three times a year under favourable climatic conditions (Fig. 3).

### Evaluation of genetic variability within *V. vinifera* and searching for genetic relationships among varieties of different geographic origin

Transcaucasia is considered to be the first domestication area of cultivated grapevine. Very recently we have had the possibility to compare varieties from Armenia and Georgia (24 genotypes) with 271 varieties, mainly European, 174 of which from Italy, searching for possible

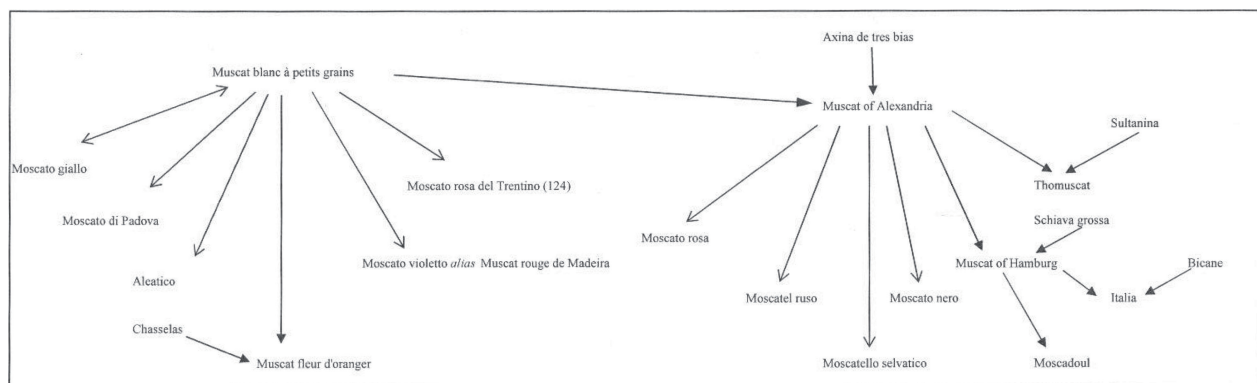


Fig. 3: The Muscat family pedigree (double arrows indicate a highly uncertain descend direction, single arrows indicate a probable direction of crosses, bold arrows indicate a safe direction)

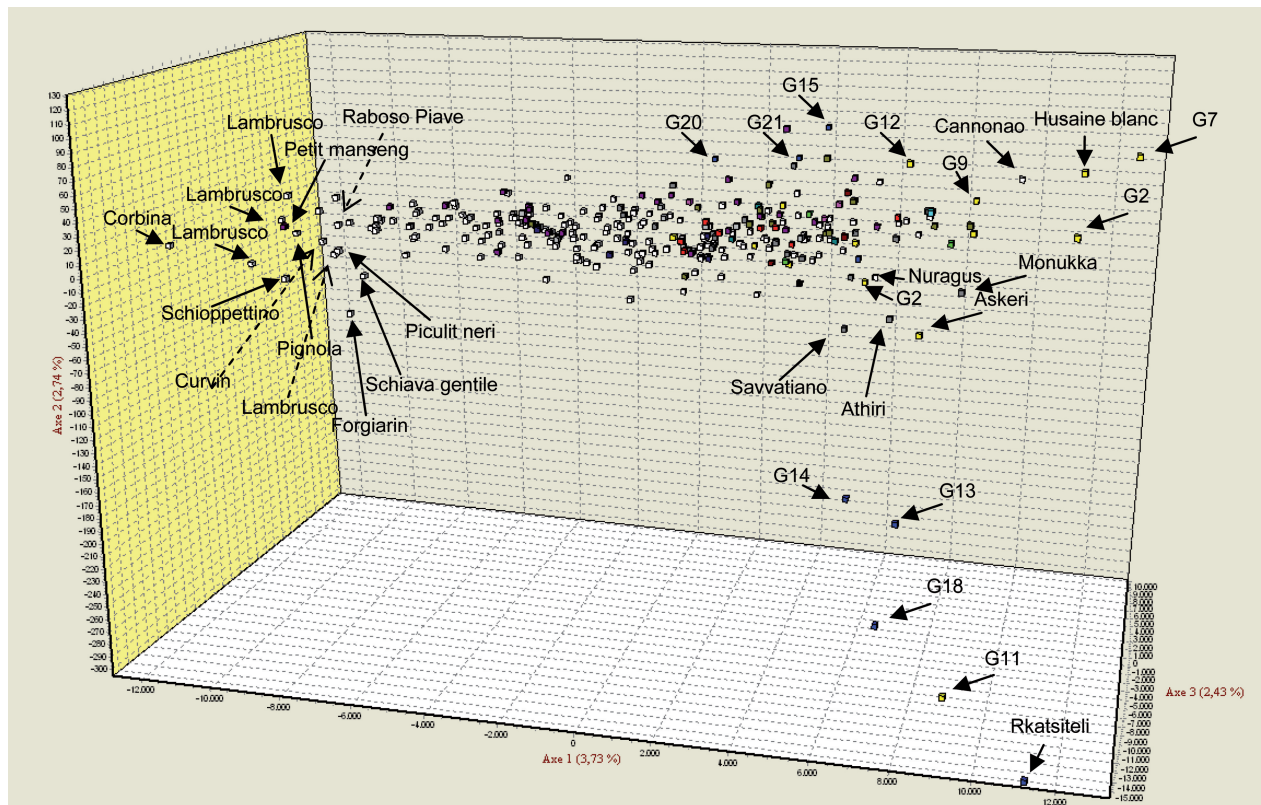


Fig. 4: AFC graph: Sardinian varieties are close to Armenian varieties; at the opposite side Lambruscos and some other minor varieties from North East Italy ('G' plus number: unknown genotype from Armenia or Georgia)

genetic similarities with GENETIX software (FRARE et al., 2010). A lot of interesting information was gained from the AFC graphic (Fig. 4). The first general observation is that all genotypes are very close, the axis 1 showing the greatest discrimination capacity with a very low value: 3.73 %. Secondly, even if the number of Armenian and Georgian samples is poor, they decidedly occupy the right part of the graph and are highly spread all over the three-dimensional space, much more than all other varieties. This is not surprising, given that Armenia and Georgia were the first centres of origin of *Vitis vinifera* and therefore the greatest genetic variability is expected here. The Italian varieties are spread along the horizontal axis and some of them remain alone on the left side of the graph: they are ancient minor varieties of North East Italy such as 'Corbina', 'Raboso Piave', 'Schioppettino', 'Ciavalgian', 'Berzaminio' and also the most known Refoscos and Lambruscos. The Austrian varieties such as 'Portugieser blau', 'Veltliner', 'Franconia', 'Heunisch weiß' and the two most important 'Wildbacher' are largely spaced in the middle of the AFC graph.

It is very interesting to underline that the Lambruscos are considered to be very close to *sylvestris* grapes, and it will be an intriguing topic to scrutinize this preliminary indication, because North East Italy could be another centre of origin of *sativa* grapes. Another surprising observation was to find 'Garnacha tinta' (*alias* 'Grenache', 'Cannonao', 'Tocai rosso') on the right side of the graph, together with many Sardinian varieties, showing to be more close to Armenian and Georgian varieties than all other Italian genotypes. This fact is very intriguing because Sardinia was hypothesized as a second centre of origin for cultivated grapes (GRASSI et al., 2003). The Georgian 'Rkatsiteli' is the most isolated variety, localized at the right side in the bottom. The analysis performed on the same genotypes with STRUCTURE software to infer K value, i.e. the number of genetic clusters or gene pools in this set of genotypes, failed to reveal any maximum value of  $\ln P(D)$  over the range of K values from 1 to 20. Therefore all these varieties could not be divided into sub-populations (data not shown). The same has happened in a more recent study performed on 745 unique genotypes

of *V. vinifera* from CRA-VIT collections: the analysis with 34 new SSR loci, characterized by repeats of three, four and five nucleotides in tandem, could not divide them in any sub-population (CIPRIANI et al., 2010). On the contrary, ZECCA et al. (2009) very clearly separated *V. vinifera sativa* from *sylvestris* genotypes using the same program, showing that the different genetic structure of these two groups can be recognized by SSR markers.

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