



Use of Molecular Markers (SSRs) and Public Databases in *Vitis Vinifera* L. as the Main Case of Efficient Crop Cultivar Identification



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Mini Review

Grape (*Vitis vinifera* L.) is a main crop providing both fresh produce (table grape) and a main transformed beverage (wine) of ancient origin. It is grown in several countries in the world producing relevant business value as 8 million hectares of vineyards are estimated at the global level (<http://www.fao.org/faostat>). Despite the slowdown of the wine market after the financial crisis, the new century brought about a growth of 75% in volume and a doubling in value in 15 years in the international market [1].

Loss of biodiversity has occurred in grape as in many other crops because of the large cultivation of a limited number of highly productive and widely adapted cultivars, therefore the scientific community supported by different stakeholders has undertaken a large effort to preserve minor vines in different types of collections (at public institutions but also in private farms).

The number of existing grape cultivars is generally estimated as several thousands [2], however the Working Group on Vitis referring to the European Cooperative Programme for Plant Genetic Resources (ECPGR) reports 27,000 accessions of grape held only in European collections (first meeting, 2003) and in a second report (2012) the group is still seeking to solve the problem of cultivar synonyms and the presence of duplications in spite of the passport data available for about 35,000 accessions from European countries.

In general, local produce has gained a large appeal with consumers, this including local wines. Consumers perceive local foods as a mean to improve the sustainability of the system by reducing the carbon and water footprint and by providing new sale opportunities for local wineries, especially of small-medium size. Marketing of traditional varieties is also exploiting the perception of consumers to be part of biodiversity conservation connected to historical aspects of the region and it uses adjectives such as “indigenous, rare, neglected, recovered”. Concerning grape, the development of oenotourism has raised awareness in consumers of the so-called terroir and the request of local wines produced using autochthonal grape varieties. In addition, there is a general trend to drink wines with a lower alcohol and phenolics content which is typical of older varieties [3].

In view of the large number of vines reported worldwide a proper identification system is necessary. The aims of plant variety identification are several including breeding programs, cultivar registration and protection and subsequently its seed (or cutting) production and trade. Cultivar identification is usually addressed via morphological descriptors and (more recently) molecular markers. Discrimination between varieties within a species may be difficult. PBR/PVR registration requires varieties to be morphologically distinct, uniform and stable (DUS) [4], however field trials and testing for varietal identification or verification based on morphological criteria may be costly and time-consuming. Environmental factors can influence

the expression of morphological characteristics, thus causing variation between environments and across years.

Morphological descriptors have been used for many years in grape cultivar identification and they have been coded by the Organization Internationale de la Vigne et du Vin (OIV) for more than 600 different traits (2nd Edition of the OIV Descriptor list for grape varieties and *Vitis* species). Ampelography is the term of use to refer to grape morphological analysis as a science to distinguish grapevines from a phenotypical point of view [5]. In more recent times softwares such as Superampelo have been developed to aid the morphological scoring assisted by Image analysis [6]. Interestingly a morphometric analysis of leaf shape via Elliptical Fourier Descriptors (EFD) and generalized Procrustes analysis combined to Genome Wide Association Analysis (GWAS) of 1,200 USDA cultivars allowed the discovery of genomic regions associated to leaf morphological traits [7].

Recently, molecular markers based on DNA, have become an essential tool for genotyping and therefore for the identification of plant (grape) varieties. Molecular markers have the advantage

of being stable and independent from the environmental conditions compared to morphological descriptors. Among the several types of markers (reviewed recently in [8]) the grape scientific community has chosen Simple Sequence Repeats (SSR) for cultivar identification because of their combination of polymorphism, reproducibility, and their codominant nature [9,10]. Two European projects (Genres081/GrapeGen06) allowed the same community to agree on a set of 6 SSR markers (VVS2, VVMD5, VVMD7, VVMD27, VrZag62, VrZag79) added to the OIV register in 2009 which were lately increase of 3 more (VVMD25, VVMD28 e VVMD32) as reported in Table 1. Alleles are conveniently expressed as relative base pair distance to the shortest allele size (n) found within the Genres081. It is considered that two different plants having the same profile for the 9 SSR loci represent the same grape genotype. The mentioned SSR set is not able to distinguish among somatic variants which are common in grape as natural mutations occurring because of the vegetative type of propagation; those showing modifications in major traits can be commercialized as new cultivars and their identification is of economic importance.

Table 1: SSR markers adopted by the international scientific community as diagnostic for the identity of two grape plants.

OIV Code	SSR Marker	Size Range	Largest known Allele
OIV 801	SSR-marker VVS2	123/124-161/162	n+38
OIV 802	SSR-marker VVMD5	222-268	n+46
OIV 803	SSR-marker VVMD7	231-265	n+34
OIV 804	SSR-marker VVMD27	171-219	n+44
OIV 805	SSR-marker VrZAG62	174-220	n+46
OIV 806	SSR-marker VrZAG79	235/236-261/262	n+26
OIV 807	SSR-marker VVMD25	235-271	n+36
OIV 808	SSR-marker VVMD28	216-280	n+64
OIV 809	SSR-marker VVMD32	235-292	n+57

The important step undertaken by the grape community was to establish public databases that store the OIV descriptors with a particular focus on the SSR data. Every laboratory can undertake cultivar analysis by using a reference cultivar from the database itself to adjust the length of the 9 SSRs, such length in fact may vary of few bases due to experimental error depending on laboratory conditions. Databases accept novel SSR data after

proper standardization. There are a few databases available as reported in Table 2. Many thousand genotypes are stored and by standardization of the analysis it is possible to compare the SSR profile of an unknown vine to have the chance to verify its identity; it is worth mentioning that SSR profiles are updated constantly.

Table 2: List of *Vitis* sp. databases storing information's on several characters (ampelographic, molecular markers etc.).

Database	Features	Link
Vitis International Variety Catalogue (VIVC)	SSR profiles at 9 loci for 3675 genotypes available	http://www.vivc.de
European Vitis Database	SSR profiles at 9 loci for 4315 genotypes available; n+x OIV coding is used; registering for database access is required	http://www.eu-vitis.de
Registro Nazionale delle Varietà di Vite	SSR profiles at 12 loci for 554 genotypes available; n+x OIV coding is used	http://catalogoviti.politicheagricole.it/catalogo.php
Italian Vitis Database		http://www.vitisdb.it
Swiss Vitis Microsatellite Database	SSR profile for the vines grown in Switzerland, approx. 170 genotypes	http://www1.unine.ch/svmd
Pl@nt grape - Le catalogue des vignescultivées en France	SSR profiles at 9 loci	http://plantgrape.plantnet-project.org/it/cepages

RéseauFrançais de Conservatoires de Vigne	registering for database access is required	https://bioweb.supagro.inra.fr/collections_vigne/Home.php
Nat'l Clonal Germplasm Rep - Tree Fruit & Nut Crops & Grapes: Davis, CA- USA	SSR profiles at 8 loci for 224 genotypes	https://www.ars.usda.gov/pacific-west-area/davis-ca/natl-clonal-germplasm-rep-tree-fruit-nut-crops-grapes/docs/grape-ssr-fingerprinting/main/

In Italy the CREA-VE Center at Conegliano Veneto (TV) offers a service of grape identification (http://sito.entecra.it/portale/cra_avviso.php?id=13755&tipo=&lingua=IT) based on an updated core set of 11 SSRs and analysis of its own database of genotypes [11]. The new SSR toolkit can distinguish somatic variants with particular reference to berry colour [12].

Given the large effort of the international scientific grape community to collect, standardize and share several types of cultivar descriptors, we envisage grape as the main crop whose cultivar identification is best coded and approachable by small-medium size laboratories at the molecular level.

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