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State of the art in grapevine variety and clone identification through polymorphism in DNA molecular markers

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Rapid and certain identification of grapevine clones is of topical interest in modern viticulture, especially with regard to the genetic control of propagation material.

In the beginning of the twentieth century, a specific branch of research called "ampelography" was established to identify grapevine varieties, biotypes and clones. The term derives from the Greek *ampelos*, for vine, and *graphon*, for description, as ampelography is based on visual observation of morphological and phenological characters (Viala and Vermorel, 1909). Currently ampelography is employed by international organizations such as the *Office International de la Vigne et du Vin* (OIV), the *International Board for Plant Genetic Resources* (IBPGR) and the *International Union for the Protection of New Varieties of Plants* (UPOV).

This type of genotypic identification of grapevines presents, however, some limitations. First and foremost, as the method is based on visual observations it is dependent on the subjectivity of experienced operators (ampelographers). Ampelographic description - based primarily on observation of the morphology of sprouts, adult leaves and clusters - is limited to adult plants during vegetative periods, and for this reason varietal and clonal impurities can only be detected some years after the planting of a vineyard. Furthermore, phenotypical changes induced by the cultivation environment, nutritional shortages or possible viral infections often make varietal identification difficult, and sometimes impossible. In fact, for correct varietal identification accessions must grow in the same vineyard and be virus free. However, given the great number of vine varieties (5,000-15,000), even when plants are grouped in a single vineyard, it is extremely difficult to differentiate them all simply on the basis of their morphological and phenological characteristics.

Thus, the availability of a rapid, practical, objective system to identify vine varieties is desirable to satisfy the need for quality control in nursery production, for legal protection of new selections, and as a fundamental tool to recover autochthonous vines with the aim of preserving biodiversity. In this context, as a support for the classic methods of varietal identification through morphological characters, DNA molecular markers are of fundamental importance. Among these, microsatellites are the most frequently used due to their high repeatability and reliability, high level of polymorphism and thus the degree of information they can provide, and their co-dominant nature that permits analysis of parentage. In addition, microsatellites have made it possible to develop numerous vine-related databases, including the "Database Viticolo Italiano" (www.vitisdb.it) which is managed by the author of the present abstract.

Although microsatellites allow rapid and certain identification of vines, they generally do not present sufficient polymorphism to distinguish biotypes and clones, even if some researchers have stated that analyzing an elevated number of microsatellite loci they were able to distinguish clones of Pinot and Carmenère.

Amplified Fragment Length Polymorphisms (AFLPs), a type of molecular marker that uses digestion of DNA with restriction enzymes, are much more polymorphic than microsatellites. Numerous publications have evidenced the ability of these markers to distinguish clones of various species, however they have not found application for legal purposes in the genotyping of clones due to problems of instability related to the use of restriction enzymes.

DNA molecular markers seem more stable, making it possible to combine AFLP with other markers such as SAMPL (Selective Amplification of Microsatellite Polymorfic Loci), M-AFLP (microsatellites amplified fragment length polymorphism) and S-SAP (Sequenze-Specific Amplification Polymorfism). The first two combine AFLP with microsatellites, while the latter combines AFLP technique with molecular markers based on transposons insertion polymorphism. In particular, S-SAP seems to be more stable than AFLP, and recently it was demonstrated that they are efficient in discriminating clones.

Another recently introduced DNA molecular marker for grapevine is Single Nucleotide Polymorphisms (SNPs). This type of marker has developed rapidly following sequencing of the entire grapevine genome. The resequencing of a large number of vines within the context of a specific European project has made it possible to plan an array which allows rapid, simultaneous analysis of more than 18,000 SNPs at a reasonable cost. Currently, many research groups are testing the utility of this type of marker in clonal discrimination and it is plausible that initial publications may appear by the time the present convention is held.