



BMT/11/8

ORIGINAL: English

DATE: August 20, 2008

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR
TECHNIQUES AND DNA PROFILING IN PARTICULAR**

Eleventh Session
Madrid, September 16 to 18, 2008

USE OF A MICROSATELLITE-BASED SYSTEM FOR THE CONSTRUCTION OF A
REFERENCE COLLECTION IN GRAPEVINE

Document prepared by experts from Spain

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SUMMARY

1. A grapevine variety collection is under construction by the *Oficina Española de Variedades Vegetales* (OEVV) and the *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA). This collection is used as a reference for the technical examination needed for granting breeders' rights and for the Register of Commercial Varieties. A collaboration agreement between the *Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario* (IMIDRA) and the OEVV started in 2004, with the aim of supporting them in grapevine variety characterization, by using 9 microsatellite markers. The microsatellite system is being used for different purposes: in the management of the reference collection, through the identification of the different plants to detect mistakes, to help confirming the true-to-type status of certain varieties, and to confirm or reject synonymies and homonymies; and in the technical examination for Distinctness, Uniformity and Stability (DUS), to detect mistakes, and to determine which will be the most appropriate varieties to compare with.

INTRODUCTION

2. Grapevine is one of the oldest cultures in the world. Their plants are woody, and asexually multiplied through cuttings. There are a lot of varieties in the world (between 5,000 and 10,000) and many of them have been cultured for several centuries. Most are local varieties, and there are numerous synonyms (one variety having different names) and homonyms (different varieties having the same name) within and between countries.

3. The natural phenotypic variation found among (and within) individual plants within a variety and the existence of synonyms and homonyms (see above) have made the identification of grapevine varieties through morphology a difficult process. Competent organisations, particularly the International Organisation of Vine and Wine (OIV), IPGRI (Biodiversity), and UPOV have been working on identification based on morphology. Today, the use of molecular markers is contributing to an improvement of such systems. OIV has recently incorporated in its descriptor list the use of 6 DNA markers (microsatellites) studied during the GENRES 081 project (This et al. 2004) for the purpose of variety identification (OIV 2007).

4. In Spain, there are 2 main grapevine variety collections, one in the south (Rancho de la Merced, in Jerez, Cadiz), and the other placed at the Finca El Encín, in Alcalá de Henares, Madrid which belongs to IMIDRA. In addition, there are a number of smaller collections,

some of which are of great importance: for example, OEVV/INIA in Murcia (reference for DUS testing), OEVV in Aranjuez and OEVV/ETSIA, which comprises virus-free plants, and is used for certification, and the clone collection in Colmenar de Oreja (Madrid). The El Encín collection has been the most extensively studied (more than 1,200 accessions) using a microsatellite-based system previously described in BMT meetings (Ibáñez and Vélez 2005; Ibáñez et al. 2006).

5. The aim of this paper is to show how that system of 9 microsatellites is being used for the identification of grapevine varieties in the reference collection used by the Spanish Plant Variety Office (OEVV), and managed by the Centre of Trials for Variety Assessment in Murcia.

System of 9 microsatellites

6. A set of 9 microsatellite markers was chosen based on public availability, map position (genetically independent), high level of polymorphism, size of alleles (to allow multiplexing), and 'quality' on the basis of ease of amplification, absence of known null alleles, absence of alleles differing only in 1 bp.

7. The following 9 microsatellite markers were chosen: VVS2 (Thomas and Scott 1993), VVMD5, VVMD27, VVMD28 (Bowers et al. 1996; Bowers et al. 1999), ssrVrZAG29, ssrVrZAG62, ssrVrZAG67, ssrVrZAG83 and ssrVrZAG112 (Sefc et al. 1999). A system was optimized, including the design of a multiplex PCR with the 9 markers and capillary electrophoresis and fluorescence analysis in an automatic sequencer. Simplex PCRs were done when necessary.

8. Approximately 6,000 plants were studied using this system, mainly within two different projects: one to characterize the collection of grapevine varieties of "El Encín", in Madrid; and the other to study the uniformity and stability of those markers using 19 varieties (Ibáñez et al. 2006).

9. Within the former project a large grapevine collection was studied, using 996 accessions, and determined:

a) the highest number of different alleles within a variety (including "sports"): 1

b) the lowest number of different alleles between different varieties (excluding "sports"): 2

As a consequence, the theoretical minimum distance could be established in 2 alleles for these microsatellites in grapevine. A database of 489 different genotypes was created from these data.

10. In the second project, plants from 10 different plots of 19 varieties from 7 countries were studied. In summary, for each allele of each microsatellite of each variety about 185 individual values were studied. In the study, differences that could not be explained as technical variations were not detected, with the exception of several putative chimeras. This phenomenon was detected in Merlot (VVMD27), and Cardinal (VVMD5), and had been previously described in grapevine (Franks et al. 2002; Crespan 2004; Bertsch et al. 2005).

11. The conclusion of the study is that the 9 microsatellites are very uniform and stable.

A reference grapevine collection

12. The Spanish Plant Variety Office (OEVV) relies on the Centre of Trials for Variety Assessment for the technical examination of grapevine varieties to be included in the Register of Commercial Varieties (RCV) and for the granting of plant breeders' rights. This Centre of Trials started to build its reference collection (OEVV/INIA, Murcia) in 2002, and now has 331 grapevine varieties grafted on Richter 110 as rootstock, in trellis cultivation, and with drip irrigation. In addition, there are 4 varieties under evaluation for plant breeders' rights, and 20 for the Register of Commercial Varieties. There is also a collection of 39 rootstock varieties, including the 24 listed in the RCV.

13. The material for the reference collection comes from different sources: OEVV, IMIDRA, ETSIA (Madrid), IMIDA (Murcia), Estación Biológica de Galicia (CSIC), Estación Ecológica de Leiro, Viveros Provedo, Agrovolcan, and different research centres at the Comunidades Autónomas de Cataluña, Navarra, Castilla y León, Castilla-La Mancha, Extremadura, Aragón etc.

14. One of the first priorities was to check the true-to-type status of the varieties being incorporated in the collection. As explained above, grapevine is a woody plant, and so, there is a long time (1-3 years) before the grafted scions can be described using the official descriptors, and even then the fruit has to be described. A complete description takes at least 2 years to be completed.

15. A collaboration agreement between IMIDRA and the OEVV started in 2004, with the aim of supporting the Centre of Trials for Variety Assessment in their grapevine variety characterization, by using the 9 microsatellite system. It is being used for different purposes; and

- a) in the management of the reference collection, through the identification of the different plants to detect mistakes, and to help to confirm the true-to-type status of certain varieties that have been fully characterized in El Encín collection. In addition it is used to confirm or reject synonymies and homonymies.
- b) in the technical examination of DUS, to detect mistakes as well, and to determine which will be the most appropriate varieties to compare with. Distinctness is always based on morphological characteristics, but if the genetic distance between a candidate variety and any of the collection, measured through the 9 microsatellite markers, is large enough (> 3 alleles), then Distinctness can be ensured. Until now, and as far as we know, there have not been any cases where two grapevine varieties that were different by means of microsatellites were non-distinct by means of morphology.

16. Since 2004, a total of 910 samples have been sent to IMIDRA, including 115 in July 2008, which are now under analysis. A total of 731 samples have been reported, of which 645 were completely genotyped. The remainder correspond mostly to material that could not be analyzed; new material was claimed and studied under a different entry register.

17. Of the 645 samples completed, 375 could be identified using as a reference the El Encín collection database, and corresponded to 159 different genotypes. The other 270 samples could not be identified in a first attempt, because the microsatellite genotype did not match with any of the El Encín collection. As the El Encín database is continuously growing, those samples that were not identified might be identified later.

18. Most of the troubles we have encountered have been technical problems. The problems with the allele binning (the assignment of an integer value to the real value obtained from the analysis) have been especially relevant. These have been caused, in part, by a change of the automatic sequencer.

19. Due to these technical issues, in practice it would be convenient to re-analyze the samples when finding only between one and three different alleles and to analyze more microsatellites if such a difference persists.

20. The collaboration contract between IMIDRA and OEVV ended in 2007, and a new contract is under negotiation for the next 4 years.

Acknowledgements

21. This work was financially supported by the projects VIN01-025 and RF99-009, INIA, Agriculture Ministry of Spain, and a contract with OEVV (Agriculture Ministry of Spain).

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