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**MOLECULAR CHARACTERIZATION  
OF GRAPEVINE CULTIVARS  
FROM ARAGON GENE BANK (SPAIN)  
USING SEQUENCE-TAGGED  
MICROSATELLITE MARKERS**

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Zaragoza, June 2005

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FROM ARAGON GENE BANK (SPAIN)  
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*"Mine is the first step and therefore a small one, though worked out with much thought and hard labor. You, my readers, if you think I have done as much as can fairly be expected of an initial start, will acknowledge what I have achieved and will pardon what I have left for others to accomplish".*

*Aristotle*

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## ABBREVIATIONS

A.O.C	Appellation d'Origine Controlée
AF	Allele Frequency
AFLP	Amplified Fragment Length Polymorphisms
AS	Allele Size
ASAPs	Allele-Specific Associated Primers
bp	base pairs
CAPs	Cleaved Amplified Polymorphic sequences
cM	centiMorgans
CTAB	Cetyltrimethylammonium Bromide
D	Discrimination power
D.O	Denominación de Origen
ENA	Effective Number of Alleles
ESTs	Expressed Sequence Tags
hl	hectolitres
I.N.D.O	Instituto Nacional de Denominación de Origen
IGGP	International Grape Genome Program
IPGRI	International Plant Genetic Resources Institute
MAS	Marker Assisted Selection
OIV	Office International de la Vigne et du Vin
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
PVP	Polyvinylpyrrolidone
QWPDR	Quality Wines Produced in Demarked Region
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SCARs	Sequence Characterized Amplified Regions
SSR	Simple Sequence Repeat marker
STMS	Sequence-Tagged Microsatellite Site
STS	Sequence Tagged Site markers
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
UPGMA	Unweighted Pair-Group Method with Arithmetic Average
VMC	<i>Vitis</i> Microsatellite Consortium

## Resumen

El objetivo del estudio fue la caracterización molecular y la determinación de la diversidad genética presente en el banco de germoplasma de Movera (Zaragoza). Para este fin, 100 cultivares de vinificación de esta colección han sido estudiados por medio de marcadores STMS. Seis STMS loci fueron analizados: VVS2, VVMD5, VVMD7, *ssrVrZAG47*, *ssrVrZAG62*, y *ssrVrZAG79*.

El número total de alelos detectados fue 54 alelos, y varió entre 7 para el locus *ssrVrZAG62* y 10 para los loci VVS2, VVMD7 y *ssrVrZAG79*; los genotipos observados por locus variaron entre 13 en VVMD7 y 22 en VVMD5. La heterocigosidad observada osciló entre 61% en VVMD7 y 86% en *ssrVrZAG79*. El microsatélite VVMD5 fue el más informativo.

Entre las 100 accesiones, 88 han sido caracterizadas, y todas, excepto 14, fueron discriminadas. Los resultados han permitido la confirmación del nombre asignado en 26 accesiones, la corrección de la denominación de 19 accesiones mal catalogadas, y la identificación de 14 accesiones que no tenían ninguna denominación. Dos cultivares denominados como Ambrosina (4 accesiones) y Cadrete / Parrel (11 accesiones) han sido caracterizados por primera vez. Estas accesiones junto con las que no han podido ser identificadas van a ser el objeto de un estudio más extendido porque algunas podrían corresponder a variedades autóctonas de Aragón y estar en peligro de extinción.

Los marcadores STMS han resultado ser un instrumento eficiente para la caracterización de los cultivares y realizar estudios de diversidad genética en vid.

Los resultados de este estudio son de uso directo para la correcta catalogación de la colección de germoplasma de Aragón, pues permiten un mejor conocimiento de la variabilidad genética existente en la colección y por lo tanto mejores estrategias de gestión de la misma.

**Palabras clave:** *Vitis vinifera* L., Microsatélites, Identificación de variedades, Recursos genéticos.

## Résumé

Dans le but de la caractérisation moléculaire et de l'analyse de la diversité génétique au sein de la collection de ressources génétiques de vigne conservées à Movera (Zaragoza), cent accessions faisant partie de cette collection ont été examinées au moyen de marqueurs microsatellite (STMS). Six locus STMS (Séquence Tagged Microsatellite Site) ont été employées: VVS2, VVMD5, VVMD7, ssrVrZAG47, ssrVrZAG62 et ssrVrZAG79.

Le nombre total d'allèles observés lors de notre caractérisation était de 54 allèles, qui variait entre 7 (au locus ssrVrZAG62) et 10 (au locus VVS2, VVMD7 et ssrVrZAG79) et le nombre de génotypes observés par locus variait entre 13 (VVMD7) et 22 (VVMD5). L'hétérozygotie observée variait entre 61% (pour le locus VVMD7) et 86% (pour le locus ssrVrZAG79). Le locus VVMD5 était le plus informatif.

Entre les cent accessions étudiées, 88 ont été caractérisées. Toutes les accessions, sauf 14, ont pu être identifiées. Les résultats de ce travail ont permis la confirmation de l'appellation de 26 accessions, la correction de 19 accessions et l'identification de 14 accessions qui ne portaient aucune appellation au niveau de la collection. Deux variétés ont été caractérisées pour la première fois, qui sont Ambrosina (4 accessions) et Cadrete / Parrel (11 accessions). Les accessions qui n'ont pas pu être identifiées vont être l'objet d'une analyse plus poussée afin de savoir s'il s'agit de variétés autochtones de la région d'Aragón.

Les microsatellites se sont avérés des outils efficaces pour l'identification variétale et pour la réalisation d'études de la diversité génétique chez la vigne.

Les résultats de ce travail peuvent être d'une utilité directe pour la collection de Movera dans la mesure qu'ils permettent l'élimination des cépages répétées et la connaissance de la diversité génétique qui existe réellement au sein de cette collection. .

**Mots clés:** *Vitis vinifera* L., Microsatellites, Identification génotypique, Ressources génétiques.

## Summary

The aim of the study was the molecular characterization and the determination of the genetic diversity present in the *Vitis* germplasm collection of Movera (Zaragoza). For this purpose, a set of 100 red grapevine cultivars belonging to this collection were studied using STMS markers.

Six sequence tagged microsatellite site (STMS) loci were assayed: VVS2, VVMD5, VVMD7, *ssrVrZAG47*, *ssrVrZAG62*, and *ssrVrZAG79*.

The total number of alleles detected was 54 alleles, which ranged from 7 for the locus *ssrVrZAG62* to 10 for the loci VVS2, VVMD7 and *ssrVrZAG79*, and the observed genotypes per locus varied between 13 (VVMD7) and 22 (VVMD5). The observed heterozygosity varied between 61% (VVMD7) and 86% (*VrZAG79*). The VVMD5 was the most informative microsatellite.

Among the 100 accessions studied, 88 grapevine accessions have been characterized. All the accessions, except 14, were discriminated. The results allowed to confirm the trueness-to-type of 26 accessions, to correct the misnaming among 19 accessions, and to identify 14 of the unknown accessions. Two cultivars denominated as Ambrosina (4 accessions) and Cadrete / Parrel (11 accessions) were genotyped for the first time. The accessions that were not be identified, will be further investigated because some of them could be autochthonous varieties from Aragón.

The STMS markers have proved to be an efficient tool for cultivar identification and for conducting genetic diversity studies in the grapevine collection of Movera.

The results of this study can be of a direct use for the germplasm collection, in the sense where it allows a better knowledge of the existing genetic variability in the collection and therefore a better management of this one.

**Key words:** *Vitis vinifera* L., Microsatellites, Cultivar identification, Genetic resources.