

Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers

Liliana Martínez*

Laboratorio de Biología Molecular
Departamento de Ciencias Biológicas
Facultad de Ciencias Agrarias
Universidad Nacional de Cuyo
Almirante Brown 500, Chacras de Coria, M5528AHB
Mendoza, Argentina
Tel: 0054 261 496004 (ext 2032)
Fax: 0054-261-4960469
E-mail: lmartinez@fca.uncu.edu.ar

Pablo Cavagnaro

Laboratorio de Biología Molecular
Departamento de Ciencias Biológicas
Facultad de Ciencias Agrarias
Universidad Nacional de Cuyo
Almirante Brown 500, Chacras de Coria, M5528AHB
Mendoza, Argentina
Tel: 0054 261 496004 (ext 2032)
Fax: 0054 261 4960469
E-mail: pfcavagnaro@hotmail.com

Ricardo Masuelli

Laboratorio de Biología Molecular
Departamento de Ciencias Biológicas
Facultad de Ciencias Agrarias
Universidad Nacional de Cuyo
Almirante Brown 500, Chacras de Coria, M5528AHB
Mendoza, Argentina
Tel: 0054 261 496004 (ext 2032)
Fax: 0054 261 4960469
E-mail: rmasuelli@fca.uncu.edu.ar

José Rodríguez

Cátedra de Viticultura
Departamento de Producción Agropecuaria
Facultad de Ciencias Agrarias
Universidad Nacional de Cuyo
Almirante Brown 500, Chacras de Coria, M5528AHB
Mendoza, Argentina
Tel: 0054 261 496004 (ext 2032)
Fax: 0054 261 4960469
E-mail: jrodriguez@fca.uncu.edu.ar

Financial support: The present work has been done in the framework of a research programme (1998-2002) funded by SECYT (Secretaría de Ciencia y Técnica) of the National University of Cuyo.

Keywords: ampelography, Criollas, genetic variability, molecular markers.

Abbreviations:

AFLP: Amplified Fragment Length Polymorphism;
NTSYS: Numerical Taxonomy and Multivariate Analysis System;
O.I.V.: Office International du Vin;
PCR: Polymerase Chain Reaction;
RAPD: Random Amplified Polymorphic DNA;
RFLP: Restriction Fragment Length Polymorphism;
SSR: Simple Sequence Repeats.

Half of the Argentine grapevine growing area is cultivated with local varieties generically called

*Corresponding author

“Criollas”. These materials differ in morphology, physiology and enological aptitudes from traditional European varieties. To discriminate among them, we used morphological markers to evaluate the genetic diversity and phenetic relatedness of 9 Criollas, 6 European and 1 American varieties and compared with AFLP markers. Three AFLP primer combinations generated a total of 111 scorable fragments. Dendrograms obtained with morphologic and AFLP markers agreed in clustering the “Criollas” separately from the European and American varieties assayed, except for Muscat d’ Alexandrie and Tempranillo which clustered with Criollas in the case of AFLP. Comparison of UPGMA dendrograms of morphological and AFLP markers using the Mantel test indicated a not significant correlation of $r = 0.33$. Nevertheless, AFLP and selected morphological characters appear as useful and complementary techniques for grapevine identification and for evaluation of genetic diversity. Among the “Criollas”, AFLP similarities ranged from 76 to 98% (Dice coefficient), indicating an important source of genetic diversity that can be exploited in future breeding programs. To our knowledge, this is the first report using AFLP markers to assess genetic variability on these materials.

Argentina is one of the largest grape and wine producing countries in South America. In the last decades its viticulture and enology industries have acquired great importance from an economical point of view. Currently, 45% of the grapevine cultivated area is covered with a group of varieties generically called “Criollas”, a term given to American-born individuals descendant from European parents, although the possibility that some of these varieties arrived as seeds cannot be excluded. It is likely, that these varieties were introduced in Argentina soon after the Spaniard conquerors arrived to the New World. Settlers began planting vines as early as 1556, at Santiago del Estero province (Maurín-Navarro, 1967) and later in the foothills of the Andes Mountains, in Mendoza and San Juan provinces. Currently, around 70% of the total area cultivated with “Criollas” is in Mendoza, whereas San Juan accounts for 20% of the surface (INV, 2001).

Due to their rusticity, the “Criollas” have called the attention of local plant physiologists, who have noticed significantly higher tolerance to some environmental stresses when compared with European traditional varieties (Kaiser and Cavagnaro, 2001). These varieties can grow in soils with low water availability and high salt concentration, and still maintain their characteristic high yield and vigour. Characterizing the diversity of local populations would allow a more useful application of these materials in breeding programs.

Some “Criollas” varieties such as Moscatel Amarillo, Criolla Chica, Torrontés Mendocino and Torrontés Riojano, give raise to valuable regional wines. Torrontés Riojano has been internationally recognized for originating a dry wine

with a Muscat taste (Agüero et al. 2001). The rest of the “Criollas” shows relatively low enology quality, only appropriate as table wines.

Molecular markers like RFLP (Bowers and Meredith, 1996), RAPD (Gogorcena et al. 1995; Grando et al. 1995), AFLP (Sensi et al. 1996; Cervera et al. 1998) and SSR (Bowers et al. 1996; Sefc et al. 2000) have been used for genetic studies in grapevine. These studies have increased the understanding of the relatedness of cultivars within and among regions. The high level of heterozygosis that present vegetatively propagated grapevines (Reisch, 2000) allows the distinction of the most important cultivars by using almost any molecular marker technique. The recent availability of these molecular markers has facilitated research in *Vitis* genetics (Reisch, 2000). AFLP (Vos et al. 1995) is a PCR-based fingerprinting technique that is particularly useful for this purpose, since it can detect a large number of polymorphism in a single reaction. It presents a good repeatability generating primarily dominant markers which are distributed throughout the genome (Cervera et al. 1998). The goals of this work were to i- evaluate the genetic diversity of Argentine grapevine germplasm using AFLP and morphological data, ii- compare the phenetic relationships obtained by both systems of analysis and iii- compare European with Criollas varieties.

MATERIALS AND METHODS

Plant material

Nine “Criollas” varieties: Cereza, Criolla Grande, Criolla Chica, Pedro Giménez, Moscatel Rosado, Moscatel Amarillo, Torrontés Riojano, Torrontés Sanjuanino and Torrontés Mendocino, 6 European varieties: Chardonnay, Syrah, Cabernet Sauvignon, Malbec, Muscat d’ Alexandrie and Tempranillo, and 1 American hybrid rootstock: SO4 (*Vitis berlandieri* x *Vitis riparia*), were assayed. All accessions were taken from the collection vineyard at the INTA Luján de Cuyo and the Agricultural College, Universidad Nacional de Cuyo, Mendoza, Argentina.

Morphological characters analysis

Fifty-three characters (Table 1), analyzed and described by Alcalde, 1989, using “Criollas” varieties, were numerically codified using a qualitative multi-status criterion (from 0 to 8, depending on the variables of each character) (Sneath and Sokal, 1973) and used to design a numbered-data matrix. The corresponding morphological characters of the European varieties, described by O.I.V. were also included in the analysis. Modal values of morphological descriptors from 15 vines per European and Criollas varieties were analyzed in 20 consecutive years.

DNA extraction

For each variety, young leaves from 5 vines, were independently collected and used for DNA isolation as

