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Assessing the identity of the variety 'Pedro Giménez' grown in Argentina through the use of microsatellite markers

Determinación de la identidad de la variedad 'Pedro Giménez' cultivada en Argentina a través del empleo de marcadores microsatélites

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ABSTRACT

'Pedro Giménez' is a white criolla variety cropped in Argentina, mainly in Mendoza and San Juan, being the most planted white variety destined for wine making in the country. Its origin remains unknown, as well as its relationship with Spanish variety 'Pedro Ximénez', mostly grown in Jerez, Spain. Previous works have probed that most of Criollas varieties existing in America at the moment, are the offspring of 'Muscat of Alexandria' x 'Criolla Chica'. The aim of the present work was to compare 'Pedro Giménez' with the Spanish variety 'Pedro Ximénez', and to establish its degree of relatedness to 'Muscat of Alexandria' and 'Criolla Chica'. Therefore we used a set of 18 nuclear SSR loci and 3 chloroplast SSR loci. 'Pedro Giménez' shared only 38% of the alleles under analysis with 'Pedro Ximénez', indicating that they are indeed two different varieties. In all 18 polymorphic nuclear SSR loci 'Pedro Giménez' shared 50% of its alleles with 'Muscat of Alexandria', while the other 50% of the alleles

RESUMEN

'Pedro Giménez' es una variedad criolla blanca cultivada en Argentina, principalmente en las provincias de Mendoza y San Juan, siendo la variedad con la mayor superficie entre las uvas blancas de vinificación. Su origen es desconocido, como también su relación con la variedad española 'Pedro Ximénez', cultivada especialmente en la región de Jerez, España. En trabajos previos se ha probado que la mayoría de las variedades criollas existentes en América se habrían originado del cruzamiento de 'Moscatel de Alejandría' x 'Criolla Chica'. El presente trabajo tuvo como objetivos comparar las variedades 'Pedro Giménez' y 'Pedro Ximénez', y establecer relaciones de parentesco con 'Moscatel de Alejandría' y 'Criolla Chica'. Se utilizaron 18 loci microsatélites nucleares y 3 loci microsatélites de cloroplasto. 'Pedro Giménez' compartió sólo el 38% de los alelos con 'Pedro Ximénez', por lo que se pudo inferir que se trata de variedades diferentes. En todos los loci polimórficos nucleares analizados 'Pedro Giménez' compartió uno de los alelos con

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present in 'Pedro Giménez' were also present in 'Criolla Chica'. This data, along with those from the chloroplast SSR analysis, strongly suggest that 'Pedro Giménez' is the progeny of 'Muscat of Alexandria' x 'Criolla Chica', being the latest one the most likely female progenitor.

'Moscatel de Alejandría' y el otro con 'Criolla Chica'. Estos datos, junto con el resultado del análisis de SSR de cloroplastos, avalan la hipótesis que, como muchas de las variedades criollas, 'Pedro Giménez' sería fruto del cruzamiento entre estos dos progenitores, siendo 'Criolla Chica' probablemente el progenitor materno.

Keywords

grapevine • *Vitis vinifera* • microsatellites • Pedro Giménez

Palabras clave

vid • *Vitis vinifera* • microsatélites • Pedro Giménez

INTRODUCTION

Argentina is a New World country that has strongly developed its wine industry, with 228,575 ha covered with vineyards, and a wine production of 1,463,000 m^3 per year. The most important wine regions are located in the provinces of Mendoza, San Juan and La Rioja, harboring over 91% of the vineyards in the country. Salta, Catamarca, Neuquén and Río Negro are also wine producing provinces with a relatively important contribution to the country's wine industry, given mostly by high quality wines (19).

Many different varieties of grapes are used for wine making. Among them 'Criollas' occupy approximately 40% of the cultivated area (19). 'Criollas' is a term given to American-born individuals descendant from European parents. They are only grown in the American continent, and there are no records of them being cropped in Europe. They outstand for their vigor, high productivity, and their capability to adapt to unfavorable growing conditions (*i. e.* more tolerant to drought and salinity) compared to European varieties (3, 13). They are considered to have lower oenological quality than European cultivars, and are mostly used to produce table wine, must, grape juice and raisin. 'Torrontés Riojano' represents an exception to this trend, producing a dry aromatic wine that has gained international appreciation in the last decade (5).

'Pedro Giménez' is the most widely white variety planted in Argentina, representing 38% of the surface cultivated with white grapes cultivars destined for wine making in Mendoza, and 45 % in San Juan (19). It is used to produce most of the country's white table wines, and its varietal wine is mild flavored, unctuous, with white fruit bouquet and golden-greenish colors. Despite being the most cultivated white variety in the country, its origin remains unknown, as well as its relationship with the Spanish cultivar 'Pedro Ximénez' (20). This last variety is cropped in Spain, particularly in Jerez, where it is used, along with variety 'Palomino', to elaborate the Protected Designation of Origin (PDO) 'Jerez'.

Marked ampelographic differences are observed between these two varieties. 'Pedro Giménez' shows hairy shoots and anthocyanic pigmented young leaves. Its mature leaves are tri or penta-lobed with straight teeth and strongly anthocyanic

pigmentation in petiole and main veins. The clusters are big, branchy and slightly compact; the berries are yellow-green, medium to big roundish (Rodríguez and Matus, personal communication; 3). On the other side, Spanish 'Pedro Ximenez' has cottony shoots with yellow young leaves. Mature leaves are penta-lobed without anthocyanic pigmentation, and have convex teeth. The clusters are medium conic and fairly compact with yellow-green elliptic berries (17). Despite these morphological distinctions, so far no reported study has been focused on comparing them through genetic profiling, and it is not clear whether they are homonyms or synonyms.

'Blanca Temprana de Almería' is another Spanish cultivar suspected to be related with 'Pedro Giménez', but regardless of many shared ampelographic characters as cottony sprouts, penta-lobed mature leaves with anthocyanic pigmentation in petiole and yellow-green berries, there are important differences that set them apart like leaves with convex teeth and lack of anthocyanic color in main veins and medium to big ovoid berries (3).

'Pedro Giménez' has been previously grouped morphologically and genetically with several Criollas varieties cultivated in Argentina (*i. e.* 'Torrontes Riojano', 'Torrontes Mendocino', 'Criolla Grande', among others) and separated in the same fashion from some European varieties also cultivated in the country (*i. e.* 'Chardonnay', 'Tempranillo', among others) (20, 21), suggesting that 'Criollas' share a common genetic background. Likewise, Agüero *et al*. (1) and Milla-Tapia *et al.* (24), established, through an SSR (Simple Sequence Repeat) approach, that most of Criollas varieties cultivated in America were likely originated by a crossing between 'Muscat of Alexandria' (MA) and 'Criolla Chica' (CC), two cultivars originally from Northern Africa and the Canary Islands, respectively.

Grapevine microsatellites markers have been successfully applied in cultivar identification (16, 22, 28) and for detecting genetic relationships between grape cultivars (1, 6, 7, 10, 12, 23). Chloroplast SSR polymorphism has proven to be useful in analyses of progeny and maternal inheritance in the *Vitis* genus (2, 4, 15).

In the present study, we have compared 'Pedro Giménez' with both Spanish cultivars 'Pedro Ximénez' and 'Blanca Temprana de Almería' using nuclear SSR fingerprints, in order to determine their degree of relatedness. At the same time, a parent/progeny analysis based on nuclear and chloroplast SSR fingerprints was applied to 'Pedro Giménez', 'Muscat of Alexandria' and 'Criolla Chica', to establish whether 'Pedro Giménez' originated from these two ancient cultivars.

MATERIALS AND METHODS

Young leaves in active growth from the varieties 'Pedro Giménez', 'Muscat of Alexandria', 'Criolla Chica', 'Blanca Temprana de Almería', 'Moscatel Rosado' and 'Palomino', were collected from the grapevine germplasm collection of the Instituto Nacional de Tecnología Agropecuaria (INTA), in Luján de Cuyo, Mendoza.

Cuttings of 2 different clones of the Spanish variety 'Pedro Ximénez' were received from Instituto de Investigación y Formación Agraria y Pesquera (IFAPA) "Rancho de la Merced" grapevine germplasm collection, in Jerez de la Frontera, Spain. After antifungal treatment with Captan-Carbendazim, the cuttings were stored between 4°C and 8°C for 42 days, then basal cuts were restored and treated with a 100 ppm Indolbutiric Acid solution for 24 hours in darkness, and finally planted on plastic pots with a neutral soil. Young leaves were collected after they appeared below the shoot tip, and stored in paper envelopes at -20°C. An additional sample of 'Pedro Ximénez' was obtained from the collection of INTA 'Alto Valle', Río Negro, Argentina.

DNA was extracted as described by Bowers *et al.* (8) from all samples, DNA integrity was assessed by agarose gel electrophoresis and DNA concentration was estimated using a "GeneQuant RNA/DNA calculator" spectrophotometer.

DNA was analyzed at 18 nuclear microsatellite loci, including five of the six internationally adopted reference SSR markers (26) and three chloroplast microsatellite loci (table 1).

Table 1. SSR markers used in this study. Indicated allele sizes for chloroplast SSR markers are those previously reported in *Vitis vinifera* (4).

Tabla 1. Marcadores SSR utilizados en este estudio. Los tamaños alélicos indicados para los microsatélites de cloroplasto son aquellos previamente reportados en *Vitis vinifera* (4).

SSR locus	Reference	Chromosome	Allele size range reported (bp)
VVMD5*	Bowers *et al.*, 1996 (9)	16	226 - 246
VVMD6	Bowers *et al.*, 1996 (9)	7	194 - 214
VVMD7*	Bowers *et al.*, 1996 (9)	7	233 - 263
VVMD17	Bowers *et al.*, 1999 (11)	18	212 - 236
VVMD21	Bowers *et al.*, 1999 (11)	6	243 - 266
VVMD24	Bowers *et al.*, 1999 (11)	14	208 - 219
VVMD25	Bowers *et al.*, 1999 (11)	11	243 - 275
VVMD26	Bowers *et al.*, 1999 (11)	1	249 - 265
VVMD27*	Bowers *et al.*, 1999 (11)	5	173 - 194
VVMD28	Bowers *et al.*, 1999 (11)	3	221 - 279
VVMD31	Bowers *et al.*, 1999 (11)	7	196 - 224
VVMD32	Bowers *et al.*, 1999 (11)	4	239 - 273
VVMD36	Bowers *et al.*, 1999 (11)	3	244 - 315
VrZAG62*	Sefc *et al.*, 1999 (25)	7	185 - 203
VrZAG79*	Sefc *et al.*, 1999 (25)	5	236 - 260
VVS29	Thomas and Scott, 1993 (27)	1	169 - 181
VMC2c3	Goto-Yamamoto *et al.*, 2006 (18)	14	158 - 192
VMC2h4	Goto-Yamamoto *et al.*, 2006 (18)	12	197 - 235
cpSSR10	Weising and Gardner, 1999 (30)	Chloroplast	114, 115, 116
ccSSR-14	Chung and Staub, 2003 (15)	Chloroplast	201, 202, 203
ccSSR-23	Chung and Staub, 2003 (15)	Chloroplast	280, 281, 282

* International reference markers (26).

* Marcadores internacionales de referencia (26).

For nuclear microsatellite loci, amplification reactions were performed as described by de Rosas *et al.* (16). Chloroplast microsatellite loci were amplified according to Arroyo *et al.* (4) for cpSSR10, and according to Chung and Staub (14) for ccSSR-14 and ccSSR-23. Annealing temperatures for all three primer pairs, were modified to 53.4°C for cpSSR10, 60.4°C for ccSSR-14 and 61°C for ccSSR-23.

Both nuclear and chloroplast amplified loci were visualized by polyacrylamide gel electrophoresis (PAGE) at 6% and silver staining (16).

Nuclear microsatellite allele sizes were determined using as reference a 100 bp ladder molecular weight marker (Invitrogen) and 2 reference varieties of known allele size for each locus amplified ('Moscatel Rosado' and 'Palomino').

In order to set a relative size reference for chloroplast SSR alleles, 'Torrontés Riojano' was included in this assay due to its proven parent/progeny relationship with both 'Muscat of Alexandria' and 'Criolla Chica' (1).

Likelihood ratios of presumptive and alternative parents were calculated according to Bowers and Meredith (10).

RESULTS AND DISCUSSION

Numerous cultivars have been generated by spontaneous or deliberate crosses since wild grape domestication 6,000 years ago, and up to 10,000 are still in existence to this day. Due to the existence of quality parameters such as PDO and its legal frame, it is of great importance to correctly identify each variety involved in the elaboration of these products.

In the present work a total of 70 different alleles were detected for 18 primers pairs. As expected from the ampelographic differences observed between them, the microsatellite data showed that 'Pedro Giménez', 'Pedro Ximénez' and 'Blanca Temprana de Almería' are indeed three different genotypes (table 2, p. 198). 'Pedro Giménez' and 'Pedro Ximénez' shared 38% of the alleles under analysis, most of those alleles present in less polymorphic loci. Only 12 loci were successfully amplified in 'Blanca Temprana de Almería', sharing 30% of the alleles with 'Pedro Ximénez' and 16% with 'Pedro Giménez', also those alleles present in the less polymorphic loci.

Just as in human paternity analysis, DNA typing can reveal unexpected parentage of grape cultivars. Microsatellite analysis has been successfully used to assess and confirm the parentage relationships in *Vitis vinifera* such as 'Cabernet Sauvignon' a progeny from the cross between Cabernet Franc and Sauvignon Blanc (10). Others cultivars as 'Chardonnay', 'Gamay noir', 'Aligote', and 'Melon', are proved to be the progeny of a single pair of parents, 'Pinot' and 'Gouais blanc' (12). Vouillamoz *et al.* (29) reported that 'Sangiovese' was a progeny of 'Ciliegiolo' and 'Calabrese di Montenuovo', a red grapevine from Campania, Italy.

Table 2. SSR fingerprints for the cultivars under study. Molecular weight (bp) of alleles found in each variety under study. 'Pedro Giménez' is between its putative parents. NA- No amplification.

Tabla 2. Peso molecular de los alelos (en pares de bases) encontrados en cada variedad. 'Pedro Giménez' se encuentra entre sus posibles progenitores. NA- No amplificó.

	Parent	Progeny	Parent		
	Criolla Chica	Pedro Giménez	Muscat of Alexandria	Pedro Ximénez	Blanca T. Almería
VVMD5	240 - 228	228 - 228	228 - 232	234 - 238	238 - 238
VVMD7	239 - 249	239 - 249	249 - 249	239 - 239	241 - 249
VVMD17	220 - 220	220 - 220	220 - 220	220 - 220	221 - 221
VVMD25	243 - 245	245 - 253	253 - 253	247 - 253	243 - 247
VVMD27	185 - 189	185 - 194	179 - 194	181 - 185	181 - 181
VVMD28	237 - 247	237 - 271	247 - 271	259 - 265	259 - 261
VVMD32	257 - 259	259 - 265	265 - 273	249 - 273	NA
VVS29	171 - 171	171 - 181	171 - 181	171 - 171	171 - 171
VrZAG62	197 - 197	187 - 197	187 - 205	189 - 189	189 - 205
VrZAG79	243 - 251	247 - 251	247 - 255	243 - 247	257 - 259
VMC2c3	165 - 165	165 - 170	165 - 170	165 - 192	165 - 165
VMC2h4	206 - 210	206 - 210	206 - 218	210 - 218	210 - 218
VVMD31	212 - 212	212 - 216	216 - 224	208 - 214	206 - 220
VVMD6	205 - 214	214 - 214	194 - 214	212 - 214	
VVMD21	243 - 249	243 - 256	256 - 266	243 - 249	
VVMD24	210 - 210	210 - 214	214 - 214	210 - 214	
VVMD26	249 - 249	249 - 249	249 - 251	249 - 249	
VVMD36	270 - 288	254 - 270	254 - 264	NA	

The comparison between the microsatellite data obtained for 'Pedro Giménez' with those obtained for 'Criolla Chica' and 'Muscat of Alexandria' clearly shows that 50% of the alleles present in 'Pedro Giménez' are also present in 'Criolla Chica', while the other 50% of the alleles from 'Pedro Giménez' are found in 'Muscat of Alexandria'. In other words, for each microsatellite locus successfully amplified and analyzed in this study, one of the alleles in 'Pedro Giménez' is shared with 'Criolla Chica', while the other one is shared with 'Muscat of Alexandria'. Likelihood ratios supporting this hypothesis are presented in table 3 (p. 199).

These results were similar to those obtained by Milla Tapia *et al.* (24), who demonstrated that 23 accessions were a hybrid progeny of 'Listán Prieto' (synonym with 'Criolla Chica') and 'Muscat of Alexandria'.

In the same article, Milla Tapia *et al.* (24) mentioned that the ancient American cultivar known as 'País' in Chile showed identical microsatellite patterns to 'Rosa del Perú' type 1, 'Negra Corriente' both from Perú, and also to 'Mission', name received in the USA. All these varieties displayed a common microsatellite pattern with known European and American cultivars, and were also shown to be identical to the Spanish cultivar 'Listán Prieto'.

Table 3. Parentage analysis of 'Pedro Giménez' and its presumptive parents 'Muscat of Alexandria' (MA) and 'Criolla Chica' (Cc) based on alleles at 18 microsatellite loci.

Tabla 3. Análisis de paternidad de 'Pedro Giménez' y sus presuntos progenitores 'Moscatel de Alejandría' (MA) y 'Criolla Chica' (Cc), basado en los alelos presentes en 18 loci microsatélites.

	Likelihood ratios of Cc x MA vs alternative parents (observed allele frequencies)				
	X x Y[a]	**Cc x X[b]**	**MA x X[b]**	**Cc x MA relative[c]**	**MA x Cc relative[d]**
Pedro Giménez	$3.95x10^{14}$	$3.52x10^{7}$	$1.14x10^{8}$	$5.27x10^{2}$	$3.81x10^{2}$
	Likelihood ratios of Cc x MA vs alternative parents (upper 95% confidence limits of allele frequencies)				
	X x Y[a]	**Cc x X[b]**	**MA x X[b]**	**Cc x MA relative[c]**	**MA x Cc relative[d]**
Pedro Giménez	$3.33x10^{12}$	$4.06x10^{6}$	$1.37x10^{7}$	$3.32x10^{2}$	$2.46x10^{2}$

a X and Y are random unrelated cultivars.
b The identity of one parent is assumed and the other parent is a random unrelated cultivar.
c The identity of one parent is assumed and the other is a close relative of 'Muscat of Alexandria'.
d The identity of one parent is assumed and the other is a close relative of 'Criolla Chica'.

As it was mentioned before, previous studies have grouped 'Pedro Giménez' in both genetical and morphological ways with many other Criollas cultivars planted in Argentina, that were also demonstrated to be progeny of 'Muscat of Alexandria' x 'Criolla Chica' (1, 20, 21, 24). These two cultivars have also been identified as parents of others Argentinean, Peruvian and Chilean varieties like 'Torrontés Riojano', 'Torrontés Sanjuanino', 'Moscatel Amarillo', 'Criolla Grande', 'Criolla Mediana', 'Cereza', 'Torontel', 'Rosa del Perú', and 'Huasquina Pisquera'. All these data led us to hypothesize the same origin for 'Pedro Giménez', and the results of our markers approach confirmed our thoughts.

The three chloroplast microsatellite loci chosen for this study were successfully amplified and visualized by polyacrylamide gel electrophoresis and silver staining, however the exact molecular weight of the alleles couldn't be estimated due to the lack of chloroplast SSR alleles size standards (figure, p. 200).

No differences were observed in loci cpSSR10 and ccSSR-23. Locus ccSSR-14 showed a different allele size for 'Muscat of Alexandria', suggesting that 'Pedro Giménez' share the allele in this particular locus with 'Criolla Chica'. This finding suggests that 'Criolla Chica' would have acted as the female progenitor in its cross with 'Muscat of Alexandria', thus being the 'mother' of 'Pedro Giménez'. 'Torrontés Riojano' displayed the same pattern.

'Criolla Chica' and 'Muscat of Alexandria' are two varieties widely extended along the American continent, and with a long history of cultivation in Argentina. These facts, along with our data, suggest a local origin for 'Pedro Giménez', like 'Torrontés Riojano',

since it is not cultivated anywhere else but in Argentina and Chile, and there are no synonyms known for this cultivar either.

Disclosing the relationships among studied and non-studied Criollas varieties (*i. e*. whether they have ancestors and/or progenies in the New World) would be the first step towards a better understanding of viticultural migrations in America.

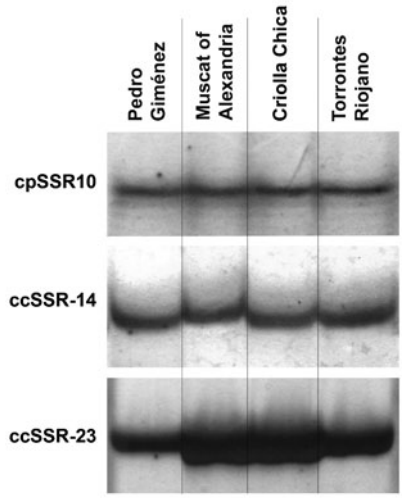

Figure 1. Chloroplast SSR loci alleles distribution in poliacrylamide gel electrophoresis (PAGE) 6%.

Figura 1. Distribución de los alelos de microsatélites de cloroplasto en gel de poliacrilamida (PAGE) 6%.

CONCLUSIONS

SSR marker analysis allowed to solve the homonyms confusion between 'Pedro Giménez' and 'Pedro Ximénez', as well as demonstrate the lack of relatedness between these two cultivars and 'Blanca Temprana de Almería'. At the same time, we established a parent/offspring relationship between 'Pedro Giménez' and the two cultivars 'Muscat of Alexandria' and 'Criolla Chica', being the latest one the most likely female progenitor. Finally, our data strongly suggest an Argentinean origin for 'Pedro Giménez'.

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