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RESEARCH NOTE

Identification and characterization of an original grapevine cultivar (*Vitis vinifera*) found in Chile

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Abstract

A.S. Gonzalez, F. Massera, D. Moscoso, P. Hinrichsen, G. Montenegro, V. Laucou, T. Lacombe, J.-M. Boursiquot, and Ph. Pszczólkowski. 2016. Identification and characterization of an original grapevine cultivar (*Vitis vinifera*) found in Chile. Cien. Inv. Agr. 43(2):337-345. Currently, many monovarietal vineyards present a minority of mixed cultivars. Some of these cultivars are unknown and could represent an opportunity to discover new and potentially useful genotypes both for research and production purposes. In a 'Carmenère' vineyard planted in 1994 in Palmilla (Colchagua Valley, Chile), a minority presence of other cultivars was found. The present work covers for the first time the identification and characterization of one of these cultivars, which presented a very poor fruit set. Through classic ampelography, it was not possible to associate the studied cultivar with any known cultivar in Chile. However, through a simple sequence repeats (SSR) comparison against the database of the INRA Germplasm Repository "Domaine de Vassal" in France, the cultivar was identified as a triploid accession named 'Folle Blanche Faux' (*Vitis vinifera* L.) that was previously found in Chile during the season of 1950-1951. This cultivar presents earlier bud breaking than 'Carmenère', has morphologically complete flowers, and shows a physiological disorder around the fruit set, leading to partial or complete bunch necrosis under the environmental conditions of Palmilla. This cultivar contributes to the genotypic richness present in Chile and might be an interesting tool for physiological and molecular studies. It also could become productive under other environmental conditions (i.e., environmental conditions that favor a good fruit set) or by the application of adequate vineyard practices, such as cane girdling at bloom time.

Key words: Ampelography, Chile, coulure, molecular markers, poor fruit set, SSR, triploid.

Introduction

The grapevine is one of the oldest perennial crops in the world. Today, it is cultivated to produce wine, juice, table grapes and raisins, making it one of the most important crops around the world. Chilean viticulture dates back to the early 16th-century Spanish colonization (Milla-Tapia *et al*., 2007) and is based on *Vitis vinifera* L. cultivars that were brought from Spain during the 16th century and

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France during the 19th century. The number of *V. vinifera* cultivars referenced around the world was estimated to be approximately 10,000 (Alleweldt and Dettweiler, 1994), but there is still a great number of unreferenced cultivars, whose genotype and characteristics are unknown or barely described.

During the last Chilean wine industry crisis, vineyard plantations dropped from 109,500 hectares in 1979 (Hernández and Pszczólkowski, 1986) to 53,093 hectares in 1994. Plantations in Chile resumed in 1994 and increased in 2014 to an area of 137,592 hectares (SAG, 2015). This last plantation cycle was broadly performed under an old technological paradigm, *i.e.*, the mass selection and obtainment of plant material from renowned vineyards, without a prior sanitary and varietal selection process. These vineyards frequently present a mixture of cultivars and some pathological problems, such as grapevine trunk diseases, viral diseases, and nematodes (Díaz *et al.*, 2013). The mixture of cultivars represents a problem for exporters of varietal labeled wines due to international trade regulations. Therefore, most current growers make efforts to plant genetic and sanitary certified vegetal material and correctly identify the cultivars in their old vineyards. Nevertheless, there are still unidentified cultivars in vineyards around the world and Chile. They represent an interesting germplasm reservoir, an opportunity with potential applications in grapevine research and the wine industry.

The objectives of this investigation were as follows: (1) to identify an unknown cultivar that was found in a minority proportion mixed in a vineyard using classic ampelography and molecular genetic markers; (2) to describe its characteristics and phenology; and (3) to induce fruit setting by cane girdling, a viticultural practice that could be used for this purpose (Gil and Pszczólkowski, 2015).

Materials and methods

Plant material. An unknown cultivar was found inside a commercial vineyard primarily planted with cv. 'Carmenère', located in Palmilla, Colchagua Valley, Chile (34.55ºS 71.40ºW), under a sub-humid Mediterranean climate (Di Castri and Hajek, 1976). Of a total of 112 specimens (1.13% of the complete vineyard) identified as the unknown cultivar and marked in a previous season, 12 plants were selected for this study. The study was performed during the 2012/2013 season. Grapevines were trained as a traditional vertical trellis with cane pruning (two canes per plant with eight to twelve buds per cane). The plants were 18 years old and own-rooted, and the planting density was 3,200 vines per hectare.

Classical ampelographic description. The unknown cultivar characteristics were defined and measured according to the OIV descriptor list for grapevine cultivars and *Vitis* species (OIV, 2006). Ampelographic observations were made during a single vegetative season for the 12 selected specimens. The shoots were examined on September 27 (BBCH or Einhorn-Lorenz system, E-L 12) when they were approximately 10 to 20 cm in height, and the first five distal leaves of the young shoots were evaluated. Mature leaf descriptions were obtained at the beginning of flowering (E-L 19) and were performed on leaves distal to the inflorescences, in the middle section of the shoot. The berry characteristics were obtained from the few berries left after necrosis took place.

Microsatellite analysis. Samples for molecular marker analyses were collected before flowering (October 11; E-L 15). One shoot was taken from each selected plant. DNA was extracted from the young leaves and stored at -80 °C, as described elsewhere (Narváez *et al.*, 2001). The following fifteen microsatellite loci were used to genotype all accessions: VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32 (Bowers *et al.*, 1996), VVS2 (Thomas and Scott, 1993), VrZAG29, VrZAG62, VrZAG67, VrZAG79, VrZAG83 and VrZAG112 (Sefc *et al.*, 2000). Polymerase chain reaction (PCR) was

carried out as has been described (Mejía *et al*., 2007) in a final volume of 10 µL containing 20 ng total DNA, 10 mM tris HCl, 50 mM KCl (pH 8.3), 2 mM MgCl2, 200 mM of each dNTP, 5% DMSO, 0.2 µM each forward and reverse primers, and 0.4 U Taq DNA polymerase. Amplification was carried out using a touchdown amplification program, as described by Milla-Tapia *et al*. (2007). After separating the amplicons in 6% denaturant polyacrylamide-TBE gels, they were revealed by silver staining, as previously described (Mejía *et al.,* 2007). The simple sequence repeats (SSR) allelic patterns were compared with the available databases at INIA (Santiago, Chile) and the INRA Grape Germplasm Repository at Domaine de Vassal (Marseillan, France; http://www1.montpellier.inra.fr/vassal/).

Histological study. Inflorescence samples were extracted on November 6 and 16, 2012, fixed in formalin/acetic acid/alcohol (FAA) and impregnated in paraffin. Several flower sections were studied under the microscope. The methodology comprises several steps as follows: dehydration with butyric and ethylic alcohols, elution of paraffin in xylol, safranin-fast green staining and finally embedding in Entellán® rapid mounting medium for microscopy (Merck KGaA, Darmstadt, Germany), as described by Calderón-Baltierra *et al*. (2004). A Leitz 1212 rotation microtome (Leica Microsystems GmbH, Wetzlar, Germany) was used to produce transversal, longitudinal, radial and tangential sections of 10 to 25 microns. The microscopic analysis was performed with an Optiphot FX-35/A microscope (Nikon Corp., Tokyo, Japan).

Photographic tracking. Normal shoots were selected and marked from the selected plants. A photographic tracking of each shoot's first inflorescence/bunch was performed on 12 dates between the moment when the inflorescences were clearly visible (E-L 12; September 27) and the beginning of the bunch closure (E-L 32; December 14) using a Lumix DMC-ZS7 camera (Panasonic Corp., Osaka, Japan).

Cane girdling experiment. The twelve selected plants of the unknown cultivar were selected; six were left without cane girdling as a control, and six were girdled. In each plant, two normal shoots (10 to 20 cm long) were labeled, and the basal inflorescence of each of them was selected for photographic tracking to study the bloom characteristics and the effect of cane girdling on the fruit set.

Winemaking. Small-scale winemaking procedures were performed for the grapes of the unknown cultivar (obtained after the cane girdling treatment), without replicates. The wine was made through the traditional red and rosé wine fermentation protocols. The grapes were picked on a single date, April 27, 2013 (corresponding to 25.7° Brix). Two 25-kg grape batches were harvested from the previously identified plants. Fermentation was carried out in 25-L containers in a controlled temperature room (15 °C for rosé and 25 °C for red fermentation) with Rhone 2056 selected yeast (20 g hL^{-1}). Complete spontaneous malolactic fermentation was performed for the red wine. An adjustment to 35 mg L^{-1} free sulfur dioxide was made in all the wines prior to bottling.

Must and wine analyses. The total soluble solids was determined by direct reading with a digital refractometer (Pocket PAL-1, Atago, Japan), the pH using a pH meter (Orion 5-Star, Thermo Scientific, Singapore) and the titratable acidity using a pH meter and 0.1 N NaOH. The alcoholic strength was determined by the use of an "Alcodest" distillation unit (JP SELECTA SA, Barcelona, Spain). The phenolic composition and color of the wines were determined using a UV/Vis spectrophotometer model Spectronic Genesys 2 (Milton Roy, Rochester, NY). All the analyses were performed according to the methods described by Iland *et al.* (2004).

Statistical analysis. The data were analyzed and compared using Excel (Microsoft, Redmond, Washington, USA).

Results and discussion

Identification by molecular markers. The SSR allelic patterns of the unknown cultivar and the other cultivars analyzed for comparison (Cabernet Franc, Cabernet-Sauvignon, Carmenère, Merlot, Côt, Pinot Noir, Syrah, País, Perlette, Centennial and Black Seedless) are shown in Table 1. The SSR pattern of the unknown cultivar was different from all the cultivars used for comparison. Four of these cultivars ('Merlot', 'Carmenère', 'Cabernet Franc' and 'Cabernet-Sauvignon') shared at least one allele with every SSR marker studied, suggesting a close parentage. The fractions of shared alleles with the unknown cultivar ranged from 0.58 to 0.67 as shown in Table 1, including three table grape cultivars that ranged from 0.33 to 0.36. The SSR pattern of the unknown cultivar was different from the over 200 genotypes registered at the INIA database (Chile), but it fully matched an accession found in the INRA - Domaine de Vassal database as follows: a triploid accession coming from Chile named 'Folle Blanche Faux' (European *Vitis* Database accession number: FRA139-0Mtp384). The accession was introduced in the Vassal Collection in the winter season of 1951-1952 from Chile, as wrong (*i.e.,* "faux" in French) 'Folle Blanche', a known old cultivar from the west side of France that has white grapes and is traditionally used in the Cognac and Armagnac regions (Boidron *et al*., 1995). The similarities between the morphologies and SSR allelic patterns of 'Folle Blanche Faux' and the other wine grape genotypes (Table 1) is presumably related to the origin of this cultivar in the south-western region of France.

As a future task, the triploid character of this cultivar needs confirmation. This cultivar could also be a cytochimera, which is a type of genetic mosaic or a mixture of genetically different tissues having various ploidy levels (Pelsy, 2010), as was first observed by Einset and Pratt (1954) in the cell layers of grapevine shoot apical meristems. As a confirmation of the triploid nature of this cultivar, the analysis of different grapevine organs will be needed.

Ampelography and phenology. The major ampelographic characteristics are shown in Figure 1 and Table 2. The shoot tip (Figure 1B) has a piping distribution, a medium intensity of anthocyanin coloration on the prostrate hairs and a medium density of erect hairs. The shoot growth habit is semi-erect (Figure 1A). The mature leaf (Figure 1D) has five lobes and a convex teeth shape. The flower (Figure 1C) has fully developed stamens and gynoecium. The flowers were found to be morphologically complete (Figure 1C), but the inflorescences/bunches showed a severe physiological disorder at the moment of fruit set, beginning with stigma necrosis and followed by complete bunch necrosis under the existing local plant and environmental conditions. All these characteristics (Table 2) together do not agree with those of any of the cultivars present in the Chilean legislation or previously known by the authors (Gil and Pszczólkowski, 2015). On the other hand, the unknown cultivar showed an earlier development of the phenological stages when compared with cv. 'Carmenère' from budburst until harvest-ripe berries (Table 3).

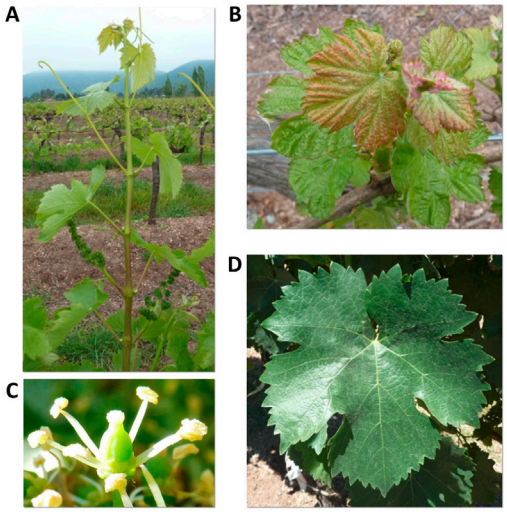

Figure 1. Ampelographic characteristics of the unknown cultivar: shoot (A), young leaves (B), flower (C) and mature leaf (D).

Inflorescence and flower necrosis. The unknown cultivar presents severe flower coulure, leading

Table 1. SSR allelic patterns for the unknown cultivar and its comparison to reference genotypes of wine and table grapes.

SSR marker	Unknown cultivar	'Cabernet franc'	'Cabernet Sauvignon'	'Merlot'	'Carmenère'	'Côt'	'Pinot noir'	'Syrah'	'País'	'Perlette'	'Centennial'	'Black Seedless'
VVMD5	240-238-226	*240-226*	240-232	236-226	*238-226*	238-228	238-228	232-226	240-228	236-234	238-236	234
VVMD7	261-243	265-243	243	251-243	267/269-243	267-243	247-243	243	253-243	257-251	243	257-253
VVMD21	257-249	***257-249***	***257-249***	249-243	***257-249***	249-243	249	265-249	249-243	255-249	249-243	255-249
VVMD24	218-208	***218-208***	***218-208***	212-208	212-208	208	216-214	214-208	208	208	***218-208***	220-208
VVMD25	253-243	***253-243***	***253-243***	***253-243***	259-243	***253-243***	***253-243***	245	245-243	245-243	259-243	253-249
VVMD27	189-181	***189-181***	189-175	191-189	189-175	191-189	189-185	191-189	189-185	181-179	195-179	195-181
VVMD28	251-237-227	237-235	237-235	235-227	*251-237*	271-235	237-221	227-221	247-237	247-237	237-221	249-221
VVMD32	254-238	238	238	238	238	250-238	270-238	270-238	254	270-248	270-262	248
VVS2	148-136	144-136	***148-136***	***148-136***	144-136	148-130	148-134	130	132-130	142-130	132	152-148
VrZaG29	109	***109***	***109***	***109***	***109***	113-109	113-109	113-109	***109***	111-109	***109***	***109***
VrZaG62	193-187	203-193	***193-187***	193	203-187	201-187	***193-187***	***193-187***	195-193	203-187	187	201-187
VrZaG67	137	137-123	137-123	137-129	151-137	137-123	151-123	149-123	149-129	153-137	137-129	129-123
VrZaG79	258-246-244	*258-246*	246	258	246	*258-244*	244-238	250-244	250-242	254-246	254-246	250-246
VrZaG83	201-195-191	201	201	*201-195*	201	*195-191*	201-189	*201-195*	*195-191*	195-189	195-189	195-189
VrZaG112	241-233-227	*233-227*	*233-227*	*241-227*	*241-227*	239	241-239	*241-227*	231-227	*241-227*	245-239	259-227
Total allelic matching		22	21	19	19	16	15	15	13	12	12	11
SSR perfect matching		5	6	3	2	1	2	1	1	0	2	1
Fraction of shared alleles	33	0.67	0.64	0.58	0.58	0.48	0.45	0.45	0.39	0.36	0.36	0.33
Excluding alleles		0	0	0	0	2	2	3	3	4	4	4
Possible filial relationship		Yes	Yes	Yes	Yes	No	No	No	No	No	No	No

Notes: The unknown cultivar and 'Folle blanche faux' shared the exact same allelic pattern. The cultivars used for comparison where ordered according to the "Fraction of shared alleles" compared to the genotype under study (FBF). SSR patterns that shared all the alleles with the FBF pattern are in italics; those that were identical to FBF ("SSR perfect matching") are in bolded italics. The patterns shaded ("Excluding alleles") correspond to SSRs that did not match any allele of FBF and so can be considered as indicators of unrelatedness.

Table 2. Primary OIV descriptor priority list (OIV, 2006), for a quick characterization of the unknown cultivar.

OIV Code	Organ	Characteristic	Notes
1	Young shoot	Opening of the shoot tip	Fully open
4		Density of prostrate hairs on the shoot tip	High
16	Shoot	Number of consecutive tendrils	Two or fewer
51	Young leaf	Color of upper side of blade (4th leaf)	Bronze
67	Mature leaf	Shape of blade	Circular
68		Number of lobes	Five
70		Area of anthocyanin coloration of main veins on upper side of blade	Absent
76		Shape of teeth	Both sides convex
79		Degree of opening / overlapping of petiole sinus	Overlapped
81-2		Petiole sinus base limited by vein	Not limited
84		Density of prostrate hairs between main veins on lower side of blade	Low
87		Density of erect hairs on main veins on lower side of blade	None or very low
223	Berry	Shape	Globose
225		Color of skin	Red

Table 3. Dates of occurrence of phenological grapevine growth stages for the unknown cultivar and cv. 'Carmenère', according to the adapted BBCH (E-L) system.

E-L Nº	Phenological stage	Unknown cultivar	'Carmenère'
2	Budswell		01-Sep
3	Woolly bud—brown wool visible	01-Sep	
7	First leaf separated from shoot tip		27-Sep
12	5 leaves separated; shoots approximately 10 cm long; inflorescence clear	27-Sep	11-Oct
15	8 leaves separated, shoot elongating rapidly; single flowers in compact groups	11-Oct	27-Oct
17	12 leaves separated; inflorescence well developed, single flowers separated	27-Oct	03-Nov
19	Approximately 16 leaves separated; beginning of flowering (first flower caps loosening)	03-Nov	06-Nov
21	30% caps off	06-Nov	09-Nov
23	17-20 leaves separated; 50% caps off (= full-bloom)	09-Nov	13-Nov
25	80% caps off	13-Nov	16-Nov
26	Cap-fall complete	16-Nov	19-Nov
27	Setting; young berries enlarging (>2 mm diam.), bunch at right angles to stem	19-Nov	22-Nov
29	Berries peppercorn size (4 mm diam.); bunches tending downwards	22-Nov	29-Nov
31	Berries pea-size (7 mm diam.)	29-Nov	
32	Beginning of bunch closure, berries touching (if bunches are tight)	14-Dec	
38	Berries harvest-ripe	13-Mar	05-Apr

to an extremely poor fruit set (0-10%). In agreement with our observations, the grape breeder and ampelographer Paul Truel, in unpublished data from the INRA Domaine de Vassal archives (personal communication, 1959), described this cultivar as having morphologically hermaphrodite flowers but being non-fertile because of a very important coulure every year. Exceptionally, a few flowers were pollinated and fertilized, giving rise to large seeded berries. In our present work, the study of the few berries left after fruit set shows that they are able to grow and ripen. Between September 27 and November 6, a normal development of inflorescences was observed. The unknown cultivar showed a physiological disorder in its inflorescences, initially characterized by the necrosis of the stigma tissue on November 9 (E-L 23) followed by the full necrosis of the inflorescence (Figure 2 A and C), which could be associated with the triploid nature (Park *et al*., 2002; Staudt, 1995) and/or a high sensitivity to early bunch stem necrosis (EBSN) (Jackson, 1991). The flower tissues, particularly the ovary wall, showed high accumulations of phenolic compounds (Figure 3) at the end of the cap-fall stage (E-L 26). After fruit set, it is possible to observe complete necrosis in the inflorescences and some inflorescences that could set one seeded berry (Figure 2 B and D). Only one inflorescence (out of twelve) did not show complete necrosis, presumably because of the presence of a single seeded berry in the cluster.

Effects of cane girdling. Using cane girdling, this cultivar did not experience bunch necrosis (which normally leads to no production at all), setting berries that fully developed and ripened in a cluster with one large shoulder and producing grapes for the first time, as shown in Figure 4. The developed berries on the treated canes were mostly seedless. In the future, a thorough knowledge of the inflorescence and flower development is needed to understand the poor fruit setting behavior of this cultivar and the nature of bunch necrosis. Must from the harvested berries presented low pH and high acidity. Rosé and red wines presented normal to high alcohol content and normal color (Table 4).

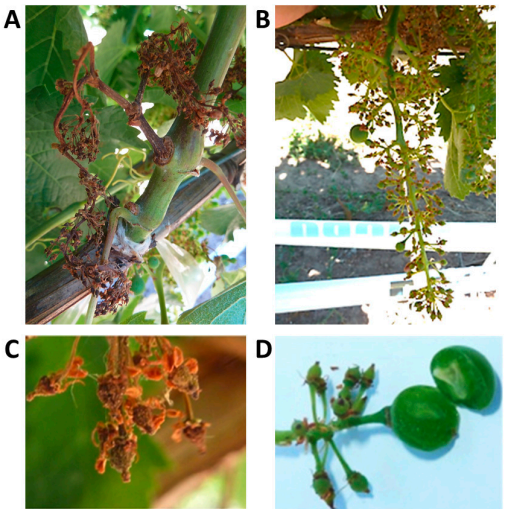

Figure 2. Necrotic inflorescence (A), non-fertilized flowers after bloom on December 14 (C), unfertilized flowers, seeded berries and very small seedless berries on November 29 (B, D).

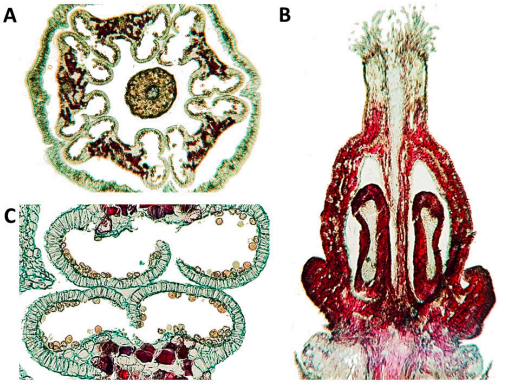

Figure 3. Microscopic images of the unknown cultivar flower tissue on November 16 (E-L 26; cap-fall complete): cross section (A), longitudinal section (B), and anther section (C).

The main conclusions are as follows. An unknown cultivar that was found mixed in a 'Carmenère' vineyard from Chile was successfully identified as cv. 'Folle Blanch Faux' by its SSR allelic pattern. This cultivar was characterized by classic ampelography using the OIV codes, and its phenology was described. The flowers are morphologically complete, but its inflorescences/bunches showed a severe physiological disorder

at the moment of fruit set that begins with stigma necrosis and then proceeds into complete bunch necrosis. Cane girdling at bloom time completely overcame the necrosis and induced berry set, growth and the final ripening. This cultivar could become an interesting tool for physiological and molecular studies.

Figure 4. Unknown cultivar ripe grape cluster at harvest, after cane girdling treatment.

Table 4. Chemical analyses of rosé and red wines made from grapes of the unknown cultivar.

Parameter	Rosé winemaking	Red winemaking
Alcohol (%v/v)	14.6	14.2
Total acidity (g H_2SO_4 L^{-1})	8.66	8.03
pH	2.96	2.95
Total anthocyanins (mg L^{-1})	31.1	231. 5
Total polyphenols (DO280)	0.95	36.26
Wine color index	0.03	5.23
Hue	1.73	0.59

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Resumen

A.S. Gonzalez, F. Massera, D. Moscoso, P. Hinrichsen, G. Montenegro, V. Laucou, T. Lacombe, J-M. Boursiquot y Ph. Pszczólkowski. 2016. Identificación y caracterización de un cultivar de vid (*Vitis vinifera*) original, encontrado en Chile. Cien. Inv. Agr. 43(2):337-345. Actualmente, muchos viñedos monovarietales presentan mezclas de otros cultivares en proporciones menores. Algunos de estos cultivares son desconocidos y podrían representar una oportunidad para descubrir genotipos nuevos y potencialmente útiles en investigación y/o producción. En un viñedo cv. 'Carmenère', plantado en 1994 en la comuna de Palmilla (Valle de Colchagua, Chile), se determinó la presencia minoritaria de otros cultivares. El presente trabajo cubre por primera vez la identificación y caracterización de uno de estos cultivares, el cual presentó una muy pobre fructificación. A través de ampelografía clásica, no fue posible asociar el cultivar en estudio a ningún cultivar conocido en Chile. Sin embargo, a través de una comparación de marcadores moleculares microsatélites con la base de datos del Repositorio de Germoplasma de Vid del INRA "Domaine de Vassal" en Francia, fue identificado como un acceso triploide llamado 'Folle Blanche Faux' (*Vitis vinifera* L.), el cual había sido descubierto en Chile previamente durante la temporada 1950-1951. Este cultivar presenta una brotación más precoz que la del cv. 'Carmenère', posee flores morfológicamente completas y muestra un notorio desorden fisiológico en su fructificación, el cual se manifiesta a través de una necrosis de racimo parcial o completa, bajo las condiciones ambientales de Palmilla. Este cultivar contribuye a la riqueza genotípica presente en Chile y eventualmente podría llegar a ser una herramienta interesante para estudios fisiológicos y moleculares. Adicionalmente, podría volverse productiva bajo otras condiciones ambientales (*i.e.* condiciones ambientales que favorezcan una buena cuaja frutal) o por la aplicación de prácticas vitícolas adecuadas, como el anillamiento de cargadores durante la floración.

Palabras clave: Ampelografía, Chile, corrimiento, cuaja, marcadores moleculares, SSR, triploide.

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