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Incidence of *Grapevine Leafroll Associated Viruses -1, -2, and -3* in Mendoza vineyards

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ABSTRACT

Viticulture is important in Argentina's economy, especially in the province of Mendoza, which is responsible for more than 75% of the crop cultivated area. In this work, we evaluated the incidence of *Grapevine leafroll-associated viruses* (GLRaV) -1, -2, and -3 in *Vitis vinifera* clones of cultivars Cabernet Sauvignon, Cabernet Franc, and Sauvignon Blanc, planted in different zones of Mendoza. The selected clones were previously reported as putatively infected by GLRaV-2. All selected samples were analyzed by DAS-ELISA for GLRaV-1, -2 and -3. GLRaV-2 was the only virus identified in all the analyzed clones. The overall infection rates were 0.6%, 18.8% and 1.2% for GLRaV-1, 2 and 3 respectively. For the clone Cabernet Sauvignon 337, the infection rate was very high (68.3%).

Key words: *Vitis vinifera*, virus, Argentina.

RESUMO

Incidência do *Grapevine Leafroll Associated Viruses -1, -2, e -3* em vinhedos de Mendoza

A viticultura é importante para a economia da Argentina, especialmente na província de Mendoza, que abrange mais de 75% da área cultivada do país. Neste trabalho, nós avaliamos a incidência de *Grapevine leafroll associated virus* (GLRaV) -1, -2 e -3 em clones de *Vitis vinifera* das cultivares Cabernet Sauvignon, Cabernet Franc e Sauvignon Blanc, cultivadas em diferentes zonas de Mendoza. Os clones selecionados foram previamente relatados como provavelmente infectados por GLRaV-2. Todas as amostras selecionadas foram analisadas por DAS-ELISA para GLRaV-1, -2 e -3. GLRaV-2 foi o único vírus identificado em todos os clones analisados. As incidências das infecções globais foram 0,6%, 18,8% e 1,2% para GLRaV-1, 2 e 3, respectivamente. No Cabernet Sauvignon clone 337 a incidência da infecção foi muito elevado (68,3%).

Palavras-chave: *Vitis vinifera*, vírus, Argentina.

In Argentina, about 225,000 hectares are devoted to viticulture, 93% of which involve wine production. The main varieties used are red-berry cultivars such as Malbec, Bonarda, Cabernet Sauvignon and Syrah, and white-berry cultivars such as Pedro Giménez, Torrontés Riojano, Chardonnay, Muscat of Alexandria, Chenín, and Torrontés Sanjuanino. The vineyards are mainly distributed in Mendoza, San Juan, La Rioja, Salta and Río Negro, although Mendoza has 75% of the cultivated vineyards (INV, 2008).

Leafroll disease (LD) is a complex pathology that affects grapevines in many countries around the world (Martelli et al., 2002). LD reduces both bunch quantity and fruit quality, followed by a decline in growth and plant development. At least 11 different species of viruses belonging to the family *Closteroviridae*, known as *Grapevine leafroll-associated virus* (GLRaV) 1-11 are associated with the disease (Alkowni et al., 2004; Martelli et al., 2005; Maliogka et al., 2008). These viruses are phloem-restricted and their dispersion is mainly caused by propagation of infected material and by insect vectors such as mealybugs. The most successful control method is propagating virus-free certified material for the new vines.

Previous studies have reported the occurrence of GLRaV-1, -2, -3, -5 and -6 in the vineyards of Mendoza (Gracia et al., 1997; Gomez Talquenca et al., 2003a, 2006, 2009). The occurrence of leafroll symptoms in Mendoza vineyards has witnessed a remarkable increase during the last decade, exhibiting not only the typical symptoms (as described by Boudon-Padieu (2000) and Martelli and Boudon-Padieu (2006)) but also an increase in graft incompatibility syndrome. This latter problem is frequent in the imported Cabernet Sauvignon clones 191, 337, and 341 (Soto 2006). The ONIVINS report (2002) identified the following clones as being infected with GLRaV-2: Cabernet Sauvignon 191, 336, 337, 340, and 341; Cabernet Franc 312, 326, 330, and 331; Sauvignon Blanc 316 and 317, among others. In Argentina, the cultivated area with these last three cultivars increased remarkably (by around 500%) in the 1990 -2005 period (INV, 2008). *Grapevine leafroll associated virus 2* has also been associated with graft incompatibility syndrome (Greif et al., 1995; Uyemoto et al., 2001; Borgo et al., 2006). In this study, we evaluated the presence of GLRaV-1, -2 and -3 in vineyards planted with some of the previously

mentioned clones, and we compared them with an old vineyard without sanitary selection.

Plant samples were collected from vineyards located in different regions of Mendoza district. Cabernet Sauvignon clones 341 and Sauvignon Blanc 316 and 317 were collected from a private vineyard in Agrelo (33°10' S, 68°55' W); Cabernet Franc clone 326 in La Consulta (33° 45' S, 69° 08' W), and the Cabernet Sauvignon clone 337 was collected from two different regions, La Consulta and Barrancas (33°05' S, 68°42' W). Finally, an old bulk-selected Cabernet Sauvignon, located at the experimental field of the Estación Experimental Agropecuaria INTA Mendoza, Luján de Cuyo (33°00' S, 68° 51' W), from a vineyard without sanitary control was sampled for purposes of comparison with other imported and certified clones.

Mature canes were collected from April to July of 2007 from a randomly delimited area. The material was stored at 4°C until processing. A total of 1,172 samples were analyzed. The samples were evaluated by DAS-ELISA and TAS-ELISA techniques with specific antibodies. To evaluate the GLRaV-2 virus, DAS-ELISA was used following the Bioreba protocol; to evaluate the GLRaV-1 and -3 viruses, TAS-ELISA kits were used (Engel et al., 2008). The different phloem tissues obtained from the cortical scrapings were macerated in a mortar with grapevine extraction buffer (500 mM Tris, 138 mM NaCl, 2% PVP, 1% polyethylene glycol, 0.02% Na₃N, 0.05% Tween 20, pH 8.2, adjusted with HCl). The presence of GLRaV-1 and -3 was confirmed by measuring absorbance at 405 nm after 2 h; for GLRaV-2, this was done after 12 h (overnight) of incubation at 4°C. Samples were considered 'positive' when the values of absorbance at 405 nm were at least two times higher than the two negative controls (healthy grapevine) included in the same plate.

The ELISA results were confirmed by testing by RT-PCR specific for each virus as described for GLRaV-1 by Good and Monis (2001), for GLRaV-2 by Zhu et al., (1998) and for GLRaV-3 by Minafra et al., (1994). Two negative

samples and up to three positive samples were randomly selected for each clone and virus combination. Total RNA was extracted according to the method described by Chang et al., (1993).

From a total of 1,172 samples tested, 241 were considered positive for virus infection; from these, 220 were positive for GLRaV-2. This means that approximately 92% of the infected plants were infected with this virus. It should be noted that, from all the plants analyzed, only one presented a mixed infection of GLRaV-2 and GLRaV-3, and another one was infected with both GLRaV-1 and GLRaV-2 (Table 1).

The presence of each virus in all positive samples tested was confirmed by RT-PCR, where none of the negative controls produced the expected fragment for the corresponding virus. GLRaV-1 was absent in most of the clones analyzed, except for Sauvignon Blanc clone 316: five positive plants were found, and in the old bulk-selected vineyard, where two more positive samples were identified. GLRaV-3 also showed a low rate of infection, affecting only 6% of the infected plants. In the bulk-selected vineyard tested, GLRaV-3 was not found. As expected, GLRaV-2 was the most widespread among the selected clones and varieties sampled. GLRaV-2 affected mainly the Cabernet Sauvignon clones 341 and 337, with 170 positive plants out of 220 virus-infected ones. The viral infection rates from the different locations screened are shown in Table 1.

The plants presented symptom differences in all four geographic regions analyzed. The plants cultivated in Agrelo (clone 341) showed small spots on the leaves with reddish coloration typical of the disease. The other clones planted in this zone (Sauvignon Blanc clones 316 and 317) were white cultivars, and the symptoms were very difficult to visualize. Cabernet Franc clone 326 and Cabernet Sauvignon 337, grown in La Consulta, showed similar symptoms to the those described above, but the expression was milder. The old vineyard located in Luján de Cuyo showed typical leafroll symptoms in all virus-infected plants. The 337 clone planted in Barrancas showed poorly

TABLE 1 - Samples that tested positive for the ELISA and RT-PCR assays in every clone tested. BS=bulk selection

Clone	ELISA samples tested (no.)	GLRaV-1				GLRaV-2				GLRaV-3			
		ELISA		RT-PCR		ELISA		RT-PCR		ELISA		RT-PCR	
		positive	Pos.	Neg.	Pos.	positive	Pos.	Neg.	positive	Pos.	Neg.		
316 - Agrelo	185	5	2.7%	3/3	2/2	24	13.0%	3/3	2/2	7	5.2%	3/3	2/2
317 - Agrelo	164	-	0%	-	2/2	7	4.3%	3/3	2/2	1	0.6%	1/1	2/2
326 - La Consulta	180	-	0%	-	2/2	8	4.4%	3/3	2/2	3	1.7%	3/3	2/2
341 - Agrelo	175	-	0%	-	2/2	45	25.7%	3/3	2/2	-	0%	-	2/2
337 - La Consulta	180	-	0%	-	2/2	2	1.1%	2/2	2/2	-	0%	-	2/2
337 - Barrancas	180	-	0%	-	2/2	123	68.3%	3/3	2/2	3	1.7%	3/3	2/2
BS - Luján de Cuyo	108	2	1.8%	2/2	2/2	11	10.2%	3/3	2/2	0	0%	-	2/2
Total	1,172	7	0.6%			220	18.77%			14	1.2%		

developed plants, with down-rolled and reddish leaves, including the vein area. This last observation differed from the typical leafroll symptoms (Boudon-Padieu 2000; Martelli & Boudon Padieu, 2006), but was similar to the foliar manifestation of graft incompatibility previously recorded in Argentina (Gómez Talquenca et al., 2003b; Soto et al., 2006). It has been reported that the symptom expression due to GLRaV-2 infection is variable depending on viral strain and kind of rootstock used (Bertazzon et al., 2010). However, as the grapevine is prone to multiple virus infections, the influence of other related filamentous viruses cannot be discarded in the observed symptomatology.

In all cases, the red-berried cultivars infected with GLRaV-1 or GLRaV-3, alone or combined with GLRaV-2, showed typical leafroll symptoms. LD is one of the most deleterious viral diseases in grapevine. Eleven different viral species have so far been associated with the disease, but none has fulfilled Koch's postulates. Consequently, no single virus can be definitively assigned as the causal agent of the disease. GLRaV-2 is the only member of the *Closterovirus* genus involved in the etiology of this disease and in graft incompatibility syndrome (Borgo et al., 2006; Pirolo et al., 2006; Soto et al., 2006). Several studies in different countries have been done to elucidate the mechanism of GLRaV occurrence. In this study, we report that the incidence of the three GLRaVs analyzed is quite variable in Mendoza, reaching up to 70%. The occurrence of GLRaV-2 described in many countries across the world ranged between 0.2 and 14.8%, although, in Argentina, we previously found a higher incidence of GLRaV-2 (36%) (Gómez Talquenca et al., 2003a). The results obtained in the present work for GLRaV-1 and -3 are quite similar to other reports. Notably, the variability of GLRaV-2 infection rates among the tested clones (1.1% up to 68.7%) is high. Cabernet Sauvignon clone 337 from Barrancas county was implanted in 2003, two years after the ONIVINS communication concerning Cabernet Sauvignon clone 337 infection with GLRaV-2. The vineyard implantation year can be a possible explanation for this, as the La Consulta vineyard was established prior to 1995. In consequence, a putative infection of the original mother plants of 337 clones could occur after this stock distribution. The Barrancas vineyard was established after 2002, increasing the probability of occurrence of GLRaV-2 infected propagation stocks coming from these putative infected mother plants in the late 90's. It should be noted that all the presented results were obtained from a survey addressed to find high infection rates, as the analyzed plants are the clones reported by ONIVINS communication. Also, high infection rates of GLRaV-2 were reported from different regions and cultivars, as stated by Angelini et al., (2003) in Italy for cv. Red Globe (87% of GLRaV-2 infection) and Crimson Seedless (100% infection rate), and by Digiario et al., (2006) for Crimson Seedless from Chile (56%) and Italy (100%). This agreed with the fact that all the clones analyzed were infected with this virus (ONIVINS 2001). These results

also confirmed the expression of the disease symptoms in Argentina.

Another important fact is the variability of the symptoms displayed in different geographic regions. In the Barrancas zone, in addition to typical leafroll symptoms, a high proportion of the infected Cabernet Sauvignon 337 grafted on Paulsen 1103 plants showed graft incompatibility symptoms. The latter suggests that some environmental factors (climatic factors and probably the soil type) together with the use of rootstock affect the behavior of the plant against the pathogen and, in consequence, the plant's response to the infection. In other regions where samples were collected, symptoms were milder, with small variation between clones, except for clones 316 and 317 (these are white cultivars, in which symptoms are difficult to visualize).

The absence of an efficient certification program of plant-propagating material can explain, in part, the widespread occurrence of leafroll viruses, especially GLRaV-2. This virus has no record of natural dispersion in Mendoza vineyards until now, but GLRaV-3 has been proved to be naturally dispersed by *Planococcus ficus* in local vineyards (de Borbón et al., 2004), and the existence of a natural vector of GLRaV-1 and 2 cannot be fully discarded. The results confirm the high prevalence of GLRaV-2 in the clones reported as putatively infected with GLRaV-2 by ONIVINS.

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