First report of the causal agent of vine crown gall in Mendoza, Argentina

Primer reporte del agente causal de la agalla de corona de la vid en Mendoza, Argentina

Sandra Haydeé D'Innocenzo [1*], Georgina Escoriaza [1], Mariano Emanuel Diaz [2, 3]

Originales: *Recepción:* 03/05/2024 - *Aceptación:* 04/09/2024 *Nota científica*

Abstract

Crown gall is one widespread grapevine disease worldwide, caused by *Allorhizobium vitis* (syn. *Agrobacterium vitis*) and *Agrobacterium tumefaciens* (syn. *Rhizobium radiobacter*). *All. Vitis*, is the predominant species and primary cause of the disease. This study aimed to identify and characterize molecularly the agrobacteria in plants with crown gall symptoms in Mendoza vineyards. Diseased plants with trunk-based galls were sampled from different areas of Mendoza. Two multiplex PCRs were performed for bacterial identification and characterization, demonstrating that 91.6% of the strains obtained were agrobacteria (77% *A. tumefaciens* and 23% *All. vitis*). Fifty percent of *All. vitis* and 16% of *A. tumefaciens* identified strains were pathogenic. Pathogenicity tests were also conducted on *Kalanchoe daigremontiana*, with resulting tumorigenic symptoms.

Keywords
Allorhizobium • *Agrobacterium* • *Vitis vinifera* • crown gall • Mendoza

1 INTA EEA Mendoza. Laboratorio de Fitopatología. San Martín 3853. Luján de Cuyo. C. P. 5507. * dinnocenzo.sandra@inta.gob.ar
2 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Cátedra de Industrias Agrarias. Almirante Brown 500. M5528AHB. Chacras de Coria. Mendoza. Argentina.
3 INTA EEA La Consulta. Laboratorio de Fitopatología.

Resumen

Una de las enfermedades de la vid ampliamente distribuida en el mundo es la agalla de corona, que tiene como agente causal a *Allorhizobium vitis* (syn. *Agrobacterium vitis*) y *Agrobacterium tumefaciens* (syn. *Rhizobium radiobacter*), siendo la primera especie la que predomina como agente causal de la enfermedad en vid. El objetivo de este estudio fue identificar mediante técnicas moleculares las agrobacterias patógenas presentes en plantas con síntomas y determinar cuál de ellas predomina en viñedos de la provincia de Mendoza. Plantas de vid con agallas en el tronco provenientes de diversas zonas de la provincia de Mendoza se utilizaron para realizar los aislamientos. Para la identificación y caracterización molecular de los aislados se realizaron dos reacciones múltiples de PCR. Se identificó el 91,6% de las cepas obtenidas como agrobacterias (77% *A. tumefaciens* y 23% *All. vitis*). Se determinó que el 50% del total identificado como *All. vitis* son cepas patógenas, mientras que para *A. tumefaciens* sólo el 16% de los aislados dio patogenicidad positiva. También se realizaron pruebas de patogenicidad en *Kalanchoe daigremontiana*, donde se observó el desarrollo de los síntomas típicos de tumorigénesis.

Palabras clave
Allorhizobium • *Agrobacterium* • *Vitis vinifera* • agalla de corona • Mendoza

Introduction

With 207,047 hectares cultivated with grapevines (*Vitis vinífera*), Argentina leads the international wine industry. The province of Mendoza produces 70% of Argentinian wine (11) and is considered one of the Wine Capitals Worldwide. This industry, including grape growing, wine and must production, and tourism, is fundamental to the economic development of the province.

Various pests and diseases significantly reducing production quantity and quality affect grapevine cultivation. Crown gall, a disease caused by *Allorhizobium vitis* (15) and *Agrobacterium tumefaciens* (14), is among the most important and widespread vine diseases globally. These bacteria were first isolated in the United States in 1907 and were later reported in China, Japan, South Africa, and some countries in Europe and South America (4).

In grapevine, *All. vitis* is the predominant species causing the disease, while *A. tumefaciens* is found less frequently and in smaller proportions. *A. tumefaciens* is polyphagous and can affect several dicotyledon species, including *Solanaceae* and various *Asteraceae* (4, 6). Currently, rrs analysis and constitutive genes have described new species of *Agrobacterium* initially identified as *A. tumefaciens* in various hosts (7).

A. tumefaciens and *All. vitis* exist in nature as pathogenic and non-pathogenic strains. Pathogenic strains contain a non-essential tumor-inducing plasmid (pTi) involved in disease triggering (16). *All. vitis* genomic organization is characterized by two circular chromosomes. The smaller, chromosome II later classified as a chromid, is essential for disease development. *A. tumefaciens* carries one circular chromosome and a secondary linear chromid (16).

Typically, the process begins with a wound in the trunk or roots. The wound releases chemical signals that, perceived by bacteria, induce virulence (2). The disease is triggered when certain genes from the Ti plasmid are transferred to the host genome, encoding overexpression of phytohormone synthesis. This overexpression augments cell division (hyperplasia) and cell size (hypertrophy), leading to the characteristic tumor. This plasmid also contains genes encoding opine synthesis. Opines are low-molecular-weight compounds, used by agrobacteria as carbon and nitrogen source (18). According to Kuzmanović *et al.* (2020). Ti plasmids are classified into three major groups: octopine, nopaline and vitopine. Genes coding for octopine are present in 60% of the strains. About 30% of strains carry the *nos* genes (nopaline synthase), and only 10% of strains have vitopine type (4).

The development of one or more tumors around a diseased organ alters sap movement, causing chlorosis, vigor loss and decreased production. In extreme cases, it may lead to plant death, including nursery young plants or cuttings (4).

Several chromosomal genes aid in accurate identifications of pathogenic agrobacteria species. Plasmid genes determine the presence of pathogenicity-related oncogenes. Due to the importance of viticulture in Mendoza, this study aimed to define the main molecular traits of the causal agent of crown gall identifying pathogenic species. We finally aimed to determine the predominant species in Mendoza vineyards.

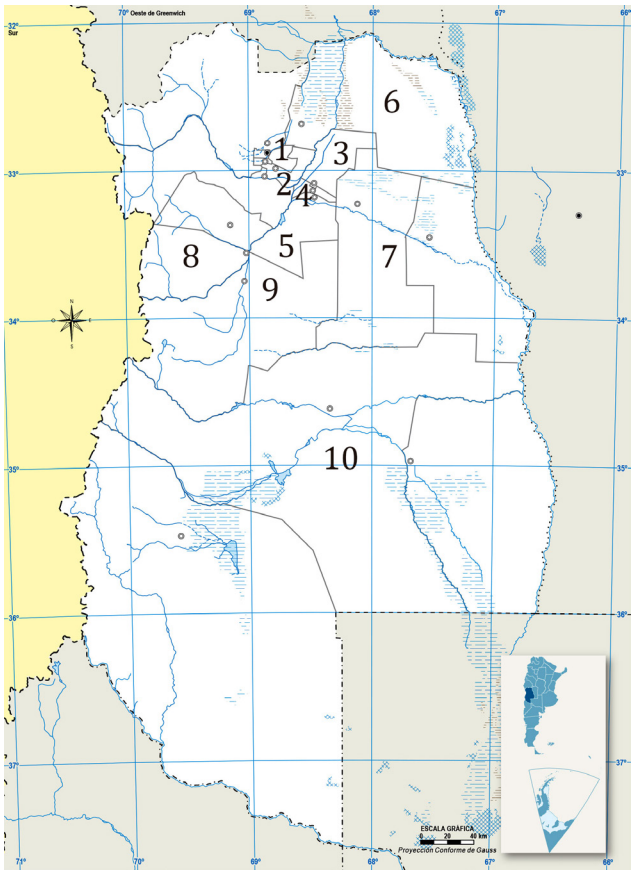
Materials and methods

Plant samples and Bacterial strains

One hundred and forty-eight symptomatic plants (figure 1) were collected from various vine-growing areas of Mendoza (figure 2). Composite samples were taken from plants within the same vineyard, resulting in 86 samples to be analyzed (table 1, page 90-91).

Figure 1. Symptoms of crown gall on Mendoza grapevines.

Figura 1. Síntomas de agalla de corona en vides de Mendoza.



1- Guaymallén
2- Maipú
3- San Martín
4- Junín
5- Rivadavia
6- Lavalle
7- Santa Rosa
8- Tunuyán
9- San Carlos
10- San Rafael

Figure 2. Mendoza, sampled departments.

Figura 2. Mendoza, departamentos muestreados.

Table 1. Strain identification, geographical origin, plant age, cultivar, number of plants, number of analyzed samples, isolation.

Tabla 1. Identificación de la cepa, origen geográfico, edad de las plantas, cultivar, número de plantas, número de muestras analizadas y aislamiento.

Strain	Geographical Origin	Age	Cultivar	Plants	Samples	Isolation
A	San Martín	2	Malbec	1	1	+
B	San Martín	8	Tannat	1	1	+
D	San Martín	4	Cereza	1	1	+
E	San Martín	2	Tannat	1	1	+
F	Guaymallén	2	Cereza	1	1	+
G	Lavalle	4	Aspirant Bouschet	1	1	+
I	San Carlos	3	Malbec	1	1	
J	Junín	3	Sauvignon Blanc	1	1	+
L	San Martín	3	Malbec	1	1	+
M	Rivadavia	2	Aspirant Bouschet	1	1	+
N	Tunuyán	2	Malbec	1	1	
Q	Rivadavia	3	Cereza	1	1	+
R	Junín	UN	UN	1	1	+
S	San Rafael	UN	UN	2	1	+
T	San Carlos	UN	UN	1	1	+
U	San Carlos	UN	UN	1	1	+
V	Tunuyán	6	Tempranillo	1	1	+
W	Tunuyán	2	Malbec	1	1	+
X	San Carlos	UN	Sauvignon Blanc	2	1	+
10	San Carlos	4	Torrontés riojano	1	1	+
19	San Carlos	2	Torrontés riojano	1	1	
21	Maipú	1	Malbec	2	1	+
23	Maipú	1	Malbec	3	1	+
37	Santa Rosa	UN	Bonarda	4	2	+
51	San Rafael	UN	Malbec	2	2	+
52	San Martín	3	Torrontés riojano	2	0	
53	San Rafael	2	Torrontés riojano	2	0	
54	San Carlos	UN	Tempranillo	3	1	+
58	San Carlos	10	Malbec	3	1	+
64	Maipú	UN	UN	5	0	
65	San Rafael	UN	Syrah	1	1	+
87	Rivadavia	4	Cereza	9	3	+
90	San Martín	3	Tempranillo	1	0	
92	San Martín	UN	Cereza/Torrontés	2	2	+
111	San Martín	UN	Cereza	5	1	+
113	Lavalle	UN	Bonarda	1	1	+
115	San Martín	UN	Cereza	3	1	+
145	Maipú	12	Syrah	1	0	
148	Maipú	1	Malbec	3	1	+
153	Tunuyán	15	Aspirant Bouschet	1	1	+
163	Santa Rosa	1	Malbec	10	1	+
165	Junín	3	Cereza	2	1	+
201A	San Martín	17	Syrah	2	1	+
201B	San Martín	6	Cereza	3	1	+
Sant2	San Martín	6	UN	1	1	+
Sant3	San Martín	6	UN	1	1	+
Sant4	San Martín	6	UN	1	1	+
Sant5	San Martín	6	UN	1	1	+
219	San Rafael	UN	UN	1	1	+
275	San Martín	1	Chardonnay	7	1	+
295	San Martín	1	Cabernet franc	8	1	+
A1	Lavalle	5	Cereza	1	1	+
A2	Lavalle	5	Cereza	1	1	+

UN: unknown/desconocido.

Strain	Geographical Origin	Age	Cultivar	Plants	Samples	Isolation
A3	Lavalle	5	Cereza	1	1	+
A4	Lavalle	5	Cereza	1	1	+
A5	Lavalle	5	Cereza	1	1	+
A6	Lavalle	5	Cereza	1	1	+
A7	Lavalle	5	Cereza	1	1	+
A8	Lavalle	5	Cereza	1	1	+
A9	Lavalle	5	Cereza	1	1	+
A10	Lavalle	5	Cereza	1	1	+
A11	Lavalle	5	Cereza	1	1	+
A12	Lavalle	5	Cereza	1	1	+
A13	Lavalle	5	Cereza	1	1	+
A14	Lavalle	5	Cereza	1	1	
L1	Lavalle	5	Cereza	1	1	
L4	Lavalle	5	Cereza	1	1	+
L5	Lavalle	5	Cereza	1	1	
L5b	Lavalle	5	Cereza	1	1	
L6	Lavalle	5	Cereza	1	1	
L7	Lavalle	5	Cereza	1	1	+
L8	Lavalle	5	Cereza	1	1	
L9	Lavalle	5	Cereza	1	1	+
L10	Lavalle	5	Cereza	1	1	
L11	Lavalle	5	Cereza	1	1	+
L12	Lavalle	5	Cereza	1	1	+
L13	Lavalle	5	Cereza	1	1	
L14	Lavalle	5	Cereza	1	1	
L15	Lavalle	5	Cereza	1	1	+
L16	Lavalle	5	Cereza	1	1	+
L17	Lavalle	5	Cereza	1	1	+
L21	Lavalle	5	Cereza	1	1	+
L22	Lavalle	5	Cereza	1	1	+
L23	Lavalle	5	Cereza	1	1	+
L25	Lavalle	5	Cereza	1	1	+
L26	Lavalle	5	Cereza	1	1	+

UN: unknown/desconocido.

Galls were washed with running water. Subsequently, they were disinfected with 1.1% sodium hypochlorite for 5 minutes in a laminar flow cabinet. After disinfection, they were rinsed twice with sterile distilled water, completely removing sodium hypochlorite. Galls were then cut into small pieces, discarding the external part to minimize contaminating microorganisms. The resulting pieces were placed in 5 ml sterile distilled water for one hour allowing diffusion of bacteria in the sample.

Each bacterial suspension was streaked onto Roy and Sasser (RS) semiselective culture medium for *All. vitis* and Schroth culture medium for *A. tumefaciens.* Culture plates were incubated at 27 °C in darkness. Colony development was observed after seven days. *All. vitis* colonies on RS medium had a dark red center with transparent or white edges. The red center is not always evident (Schaad *et al*., 2001 and Burr, T. J. personal communication, June 28, 2016). *A. tumefaciens* colonies acquire a reddish color in Schroth culture medium. Colonies with these characteristics were then transferred to Luria Bertrani (LB) culture medium.

DNA extraction and specific PCR amplification

DNA was extracted according to Khlaif, H. and Al-Karablieh (2002). *All. vitis* and *A. tumefaciens*, were differentiated according to differences in the 23S rDNA gene (20). A universal forward and two specific reverse primers were used: B1R for *A. tumefaciens* and AvR for *All. vitis* (table 2, page 92).

Table 2. Primers for identification and molecular characterization of *All. vitis* and *A. tumefaciens* strains.

Tabla 2. *Primers* usados para identificación y caracterización molecular de cepas de *All. vitis y A. tumefaciens*.

Primers	Sequence 5'-3'	Length of the Amplified Fragment	Gene	Reference
UF/B1R	GTAAGAAGCGAACGCAGGGAACT / GACAATGACTGTTCTACGCGTAA	184 bp	23S ADNr	(20)
UF/AvR	GTAAGAAGCGAACGCAGGGAACT / AACTAACTCAATCGCGCTATTAAC	478 bp	23S ADNr	(20)
iaaHF2/ iaaHR1	ACATGCATGAGTTATCGTTTGGAAT/ GCATCAAGGTCATCGTAAAAGTAGGT	420 bp	*iaaH* gene of *A. tumefaciens* and *All. vitis octopine and nopaline strains*	(3)
S4iaaM5/ S4iaaM3	CGCGTCCCCGTTTACACTA/ CGAGATCGCGCTTCAAGAT	800 bp	*iaaM* gene of *All. vitis* vitopine type	(3)

Multiplex PCR (polymerase chain reaction) used the following reagents: 1X PCR buffer, 1.5 mM MgCl2, 200 mM dNTP, 1 mM of each primer and 1U of Recombinant DNA polymerase (Invitrogen) and 5 µl of template DNA for a final reaction volume of 25 µl. The PCR consisted of initial denaturation at 94°C 1 min, 35 cycles at 94°C 1 min, 67°C 1 min, 72°C 1.5 min and 72°C 10 min, using an Eppendorf thermocycler.

Multiplex PCR with specific primers for oncogenes allowed for pathogenic strain detection. The reaction combined the primers iaaHF2/iaaHR1 and S4iaaM5/S4iaaM3 for the auxin-biosynthesis genes iaaH and iaaM, respectively. The reaction was carried out in a final volume of 25 µl, with 1X Buffer, 1.5 mM MgCl2, 0.5 µM of each primer, 200 µM of dNTP, 1.25 U of polymerase (Invitrogen Platinum DNA polymerase) and 1 µl of DNA. Amplification began with initial denaturation at 94°C for 1 min, followed by 30 cycles at 92°C 1 min, 54°C 1 min, 72°C 1.5 min and 72°C 3 min (3).

PCR-generated amplicons were detected by electrophoresis using 1% agarose gel, run at 90 volts for 1 hour and stained with ethidium bromide. The gels were visualized under UV light and photo-documented using Bio-Rad equipment and Quantity One software. Band size was compared with a 100 bp ladder molecular marker (Invitrogen).

Pathogenicity tests

PCR results were confirmed via biological tests performed on *Kalanchoe daigremontiana* plants to evaluate isolate-pathogenicity. Inoculation was carried out through punctures on the stem with a micropipette tip and 2.5 µl of bacterial suspension 10^9 cfu/ml of each strain. Each strain was inoculated in three plants via 5 stem wounds per plant. Sterile distilled water was the negative control and *All. vitis* and *A. tumefaciens* reference strains were positive controls.

The plants were kept in the laboratory at room temperature, and covered with plastic bags to maintain approximately 90 % humidity for three days. Then, bags were removed and plants were taken to the greenhouse. Observations were made every 15 days for two months (23). Isolations from tissues developed in the inoculation zone were carried out in a semiselective culture medium, using the same method as with vine galls.

Results

Molecular analysis

Sixty-nine isolates out of 86 samples analyzed resulted in 91.6% identified as agrobacteria, among which 77% were *A. tumefaciens* and 23%. *All. vitis*. Figure 3 presents a PCR with 3 isolates where A1 was *A. tumefaciens* and A2 and A3 were *All. vitis*. The multiplex PCR was performed with the combination of primers iaaHF2/iaaHR1, while S4iaaM5/S4iaaM3 determined pathogenicity. The iaaH gene was only amplified on 16% of *A. tumefaciens* strains, molecularly identified as pathogenic, and 50% of *All. vitis* isolates proved to be pathogenic. The iaaM gene did not amplify. Figure 4 shows amplification of the pathogenicity gene present in both species. Isolates A1 of *A. tumefaciens* and A2 and A3 of *All. vitis* present positive pathogenicity.

From left to right: M: marker 100 bp (PROMEGA); Bco: water; At: *A. tumefaciens* reference strain; Av: *All. vitis* reference strain.; gall isolates: A1, A2, A3.

De izquierda a derecha: M: marcador 100 pb (PROMEGA); Blanco: agua; At: cepa de referencia de *A. tumefaciens*; Av: cepa de referencia de *All. vitis*; aislados de agalla: A1, A2, A3.

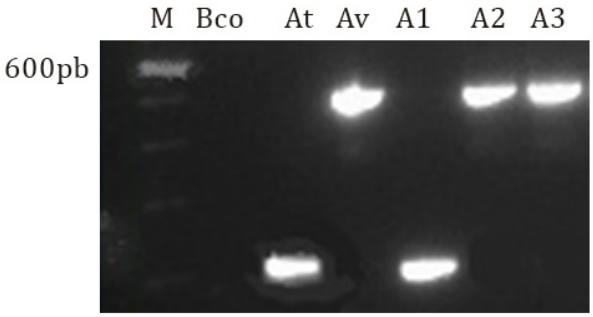


Figure 3. Multiplex PCR with primer pairs UF/B1R (184 bp) and UF/AvR (478 bp).

Figura 3. PCR múltiple con los pares de *primers* UF/B1R (184 pb) y UF/AvR (478 pb).

From left to right: M: 100bp marker (Promega), Bco: water; At: *A. tumefaciens* reference strain; Av: *All. vitis* reference strain; gall isolates: A1, A2 and A3.

De izquierda a derecha: M: marcador 100 pb (Promega), Blanco: agua; At: cepa de referencia de *A. tumefaciens*; Av: cepa de referencia de *All. vitis;* aislados de agalla: A1, A2 y A3.

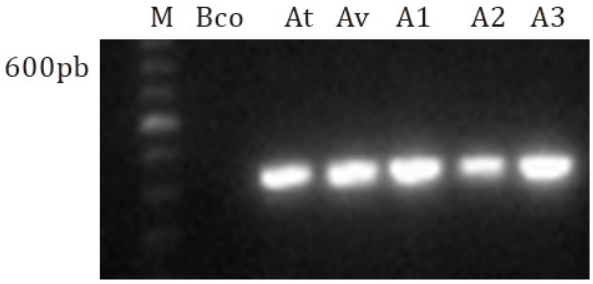
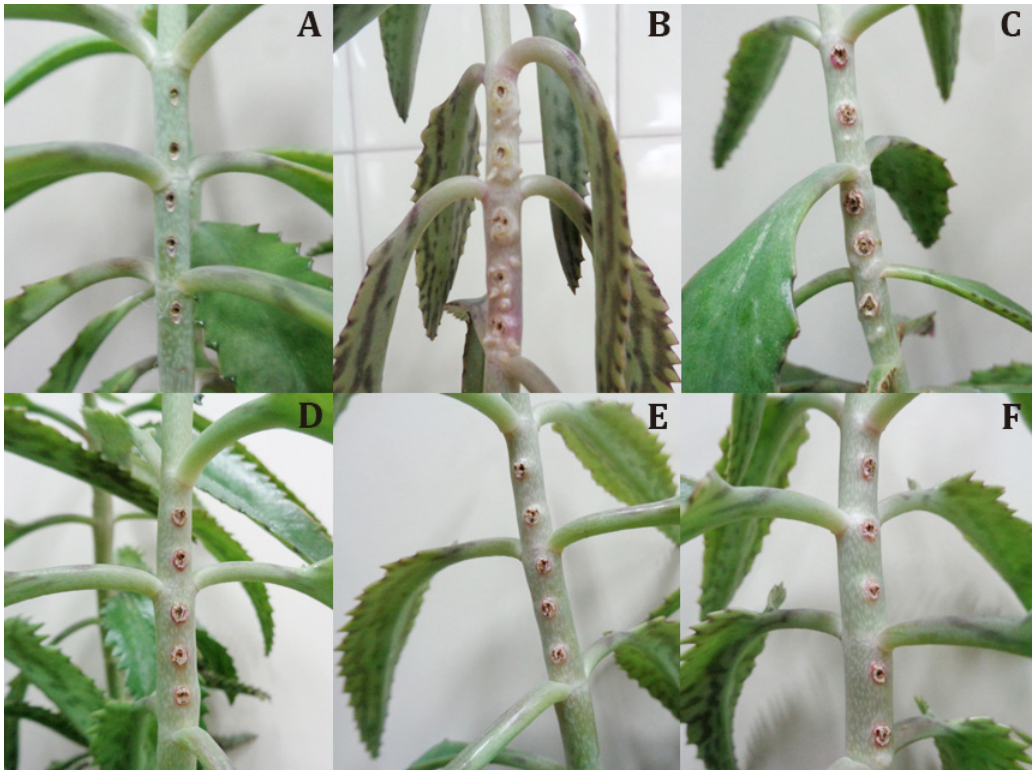


Figure 4. Multiplex PCR with primer pairs iaaHF/iaaHR (420 bp) and S4iaaM5/S4iaaM3 (800 bp).

Figura 4. PCR múltiple con los pares de *primers* iaaHF/iaaHR (420 pb) y S4iaaM5/S4iaaM3 (800 pb).

Pathogenicity Test

Two weeks after inoculation, positive results were observed in *Kalanchoe* plants. Abnormal growth and color change (redness) were similar to those in plants inoculated with reference strains. In some cases, corky tissue developed at the inoculation site (figure 5). These results became more pronounced two months after inoculation.



A: Negative control (water), B: reference strain of *A. tumefaciens*, C: reference strain *of All. vitis*, D, E and F: gall isolates: A1, A2 and A3 with positive pathogenicity result (molecular analysis).

A: Control negativo (agua), B: cepa de referencia de *A. tumefaciens,* C: cepa de referencia de *All. vitis,* D, E y F: aislado muestra con resultado de patogenicidad (análisis molecular) positivo.

Figure 5. Symptoms observed in *Kalanchoe* stems 2 weeks after inoculation.

Figura 5. Síntomas observados en tallos de Kalanchoe a las 2 semanas de la inoculación.

Bacteria isolated from inoculated plants developed typical agrobacteria colonies on semiselective culture medium, fulfilling Koch's postulates. These results align with those obtained through molecular analysis. In 79% of cases, pathogenic isolates by PCR also tested positive in the biological test.

Discussion

Bacterial genetic diversity of both species limits detection efficiency in grapevines (3). *All. vitis* strains are genetically diverse (4, 8, 9, 16, 23). Our data suggest *All. vitis* could be a species complex comprising several genomic species (16). In this study, after obtaining pure and simple isolates, successful species identification followed the molecular protocol described by Pulawska *et al*. (2006). However, since this PCR does not identify pathogenicity genes, the analysis must be complemented with additional PCR determining gene presence (1, 3, 8, 17, 19, 22). This research used specific primers iaaHF2/iaaHR1 and S4iaaM5/S4iaaM3 for iaaH and iaaM genes, respectively, showing non-pathogenic *A. tumefaciens* strains predominated over *All. vitis* strains in the analyzed grapevine samples. However, 50% of *All. vitis* isolates were pathogenic. This finding indicates that *All. vitis* is the predominant pathogenic species and main disease cause in grapevines studied in Mendoza, in agreement with prior studies (2, 5, 10, 14, 22).

The same PCR determining pathogenicity, determined opine type. We found absent vitopina type in all *All*. *vitis* isolates and presence of the octopine/nopaline types in Mendoza,

Seventy-nine percent of pathogenicity tests in inoculated *Kalanchoe* showed disease symptoms. This value is within the expected range (78-94%) (21). These data also align with Kuzmanović *et al.* (2016), who observed that some strains did not demonstrate their tumorigenic capacity in inoculated plants despite possessing pathogenicity-associated genes molecularly identified. This suggests that such isolates remain potentially tumorigenic. However, pathogenicity is influenced by plant age and environmental conditions. Absent Crown gall symptoms do not imply absent tumorigenesis genes (18), probably because no single host is infected by more than 81% of pathogenic strains and not all strains produce tumors in every host (13). According to Lamovšek *et al*. (2014), determining pathogenicity through molecular tests might replace biological tests. The PCR are less time-consuming and labor-intensive. However, given the occurrence of false negatives, pathogenicity tests remain a valuable tool in plant bacteriology.

Conclusions

This study successfully identified and characterized the causal agents of Crown gall in Mendoza vineyards using molecular methods. Our methodology enables the characterization of agrobacteria in Argentina and provides a quick and precise diagnostic tool, even for evaluating asexually propagated grapevines.

This information will help develop management strategies to reduce disease spread and incidence in our vineyards and nurseries and improve the health and productivity of their vineyards.

Finally, our results aiding bacterial identification in plant material allow for protocols to detect bacteria in asymptomatic material ensuring propagation of healthy plants from health-controlled material.

To the best of our knowledge, this is the first study identifying uncited crown gall species in Argentina.

References

1. Alippi, A. M.; López, A.; Balatti, P. 2011. Métodos para la detección de *Agrobacterium* a partir de muestras de material vegetal, suelo y agua. Revista Argentina de Microbiología. 43: 278-286.
2. Argun, N.; Maden, S. Momol, M. T.; Momol, E. A.; Reid, C. L.; Celik, H.; Burr, T. J. 2002. Characterization of *Agrobacterium vitis* strains isolated from Turkish grape cultivars in the Central Anatolia Region. Plant Disease. 86(2): 162-166.
3. Bini, F.; Kuczmog, A.; Putnoky, P.; Otten, L.; Bazzi, C.; Burr, T. J.; Szegedi, E. 2008. Novel pathogenspecific primers for the detection of *Agrobacterium vitis* and *Agrobacterium tumefaciens*. Vitis. 47(3): 181-189.
4. Burr, T. J.; Bazzi, C.; Süle, S.; Otten, L. 1998. Biology of *Agrobacterium vitis* and the development of disease control strategies. Plant Disease. 82(12): 1288-1297.
5. Canik Orel, D.; Karagoz, A.; Durmaz, R.; Ertunc, F. 2016. Phenotypic and molecular characterization of *Rhizobium vitis* strains from vineyards in Turkey. Phytopathologia Mediterranea. 55(1): 41-53. DOI: 10.14601/Phytopathol_Mediterr-15776
6. Chen, F; Hseu, S.; Hung, S.; Chen, M.; Lin, C. 1999. Leaf, stem and crown galls on perennial asters caused by *Agrobacterium tumefaciens* in Taiwan. Bt. Bull. Acad. Sin. 40: 237-242.
7. Flores-Félix, J.; Menéndez, E.; Peix, A.; García-Fraile, P.; Velázquez, E. 2020. History and current taxonomic status of genus Agrobacterium. Systematic and Applied Microbiology. 43(1):126046. https://doi.org/10.1016/j.syapm.2019.126046
8. Genov, I.; Atanassov, I.; Tsvetkov, I.; Atanassov, A. 2006a. Isolation and characterization of *Agrobacterium* strains from grapevines in Bulgarian vineyards and wild grapes, *V. vinifera* ssp. Silvestris. Vitis. 45(2): 97-101.
9. Genov, I.; Atanassov, I.; Yordanov, Y.; Tsvetkov, I.; Atanassov, A. 2006b. Genetic diversity of *Agrobacterium vitis* strains, isolated from grapevines and wild grapes in Bulgaria, assessed by Cleaved Amplified Polymorphic Sequences analysis of 16S-23S rDNA. Vitis. 45: 125-130.
10. Genov, N.; Llop, P.; López, M.; Bobev, S.; Álvarez, B. 2015. Molecular and phenotypic characterization of *Agrobacterium* species from vineyards allows identification of typical *Agrobacterium vitis* and atypical biovar 1 strains. Journal of Applied Microbiology. 118(6): 1465-1477. DOI: 10.1111/jam.12791

11. INV. 2022. Informes anuales. https://www.argentina.gob.ar/inv/vinos/estadisticas/superficie/anuarios
12. Khlaif, H.; Al-Karablieh, N. 2002. Occurrence and distribution of crown gall disease in Jordan. Phytopathology Mediterranean. 41: 226-234.
13. Kuzmanović, N.; Gašić, K.; Ivanović, M.; Prokić, A.; Obradović, A. 2012. Identification of *Agrobacterium vitis* as a causal agent of grapevine crown gall in Serbia. Archives of Biological Sciences. 64(4): 1487-1494. DOI:10.2298/ABS1204487K
14. Kuzmanović, N.; Biondi, E.; Ivanović, M.; Prokić, A.; Zlatković, N.; Bertaccini, A.; Obradović, A. 2016. Evaluation of different PCR primers for identification of tumorigenic bacteria associated with grapevine crown gall. Journal of Plant Pathology. 98(2): 311-319.
15. Kuzmanović, N.; Biondi, E.; Overmann, J.; Puławska, P.; Verbarg, S.; Smalla, K.; Lassalle, F. 2020. Revisiting the taxonomy of *Allorhizobium vitis* (*i.e. Agrobacterium vitis*) using genomics -emended description of *All. vitis* sensu stricto and description of *Allorhizobium ampelinum* sp. nov. bioRxiv. https://doi.org/10.1101/2020.12.19.423612
16. Kuzmanović, N.; Biondi, E.; Overmann, J.; Puławska, P.; Verbarg, S.; Smalla, K.; Lassalle, F. 2022. Genomic analysis provides novel insights diversification and taxonomy of *Allorhizobium vitis* (*i.e. Agrobacterium vitis*). BMC Genomic 23: 462. https://doi.org/10.1186/s12864-022-08662-x
17. Lamovšek, J.; Zidarič, I.; Mavrič Pleško, I.; Urek, G.; Trdan, S. 2014. Comparative study of diagnostic methods used for monitoring of common grape vine (*Vitis vinifera* L.) crown gall (*Agrobacterium vitis* Ophel & Kerr*)* in Slovenia. Acta Agriculturae Slovenica. 103.
18. Llop, P. P. 2003. Caracterización molecular de la pérdida del poder patógeno en *Agrobacterium tumefaciens.* Tesis doctoral. Facultad de Ciencias Biológicas. Universidad de Valencia. España.
19. Peduto, F.; Marchi, G.; Surico, G. 2010. Indexing Agrobacterium vitis in asymptomatic grapevine propagation material by two nested PCR Assays. American Journal of Enology and Viticulture. 61(1): 102-112.
20. Pulawska, J.; Willems, A.; Sobiczewski, P. 2006. Rapid and specific identification of four *Agrobacterium* species and biovars using multiplex PCR. Systematic and Applied Microbiology. 29: 470-479.
21. Schaad, N. W.; Jones, J. B.; Chun, W. 2001. Laboratory guide for identification of plant pathogenic bacteria, 3° Edición. Minnesota.
22. Szegedi, E.; Bottka, S. 2002. Detection of *Agrobacterium vitis* by polymerase chain reaction in grapevine bleeding sap after isolation on a semiselective medium. Vitis. 41: 37-42.
23. Tolba, I.; Zaki, M. 2011. Characterization of *Agrobacterium vitis* isolates obtained from galled grapevine plants in Egypt. Annals of Agricultural Science. 56: 113-119. doi:10.1016/j.aoas.2011.06.001

Acknowledgment

Dr. Ibrahim Tolba (Plant Pathologist at the Faculty of Agriculture, Ain Shams University, Egypt) for supplying positive controls.