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Research Note

Latent persistence of *Agrobacterium vitis* in micropropagated *Vitis vinifera*

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K e y w o r d s : bacterial contamination, crown gall, detection, grapevine, shoot tip propagation.

Introduction: Crown gall is the most important bacterial disease of grapevine worldwide. Its causal agent, *Agrobacterium vitis* (Ophel and Kerr 1990) exists endophytically in infested plants often without causing symptoms.

Pathogenic *A. vitis* strains harbour Ti-plasmids encoding information for opine synthesis the bacteria need for growth. Crown gall formation is caused by an overexpression of auxins and cytokinins, as a result of stable T-DNA transformation of plant cells by the pathogen (review: Szegedi *et al*. 1988). Since the bacteria are mainly transmitted by vegetative propagation, production of disease-free nursery stock is essential for disease control (Jäger *et al*. 1989).

Approaches to eradicate *A. vitis* in grape include heat therapy and shoot tip propagation (Stover *et al.* 1997). Although micropropagation has been assumed to eliminate pathogens in plants, the problem of latent persistence through *in vitro* culture has been reported for several bacteria, including *Agrobacterium tumefaciens* (Cooke *et al*. 1992). The aim of this study was to investigate shoot tip propagation of *Vitis vinifera* with regard to infection with *A. vitis*.

Material and Methods: S e l e c t i o n o f p l a n t m a t e r i a l : Plant material for micropropagation was collected from field-grown *V. vinifera* cv. Scheurebe (Riesling x Silvaner) and *V. vinifera* cv. Zweigelt (Blaufränkisch x St. Laurent). Ten plants with green, fleshy tumors and 5 symptomless plants were chosen from each variety. To confirm that tumor formation in the positive control plants was due to an infection with *Agrobacterium*, opine tests (Szegedi *et al*. 1988) were conducted with fresh tumor tissue of infested plants.

Cvs Grüner Veltliner, Blaufränkisch, Neuburger, Zweigelt, Pinot noir with a confirmed *A. vitis* infection were propagated *in vitro.*

S h o o t t i p p r o p a g a t i o n : Fifteen to 20 shoot tips (2-4 cm) and nodal cuttings (2-4 cm, 1 bud) were taken from one-year-old, herbaceous shoots of each of the *A. vitis*-positive (visible tumor formation, opines detected) and crown gall-negative control plants (no tumor formation visible). They were washed with tap water and surface-sterilized. *In vitro* propagation was performed on a modified Gelrite-based (2.5 g l^{-1}) culture medium (Brendel 1988) supplemented with 0.13 µM 6-benzylaminopurine (BAP) and 0.05 µM 1-naphthaleneacetic acid (NAA) which was also used for shoot elongation. After 4 weeks of *in vitro* culture the primary explants were transferred to a modified Gelrite-based (2.5 g l^{-1}) MS medium (Murashige and Skoog 1962) containing 0.98 µM indole-3-butyric acid (IBA) to enhance rooting and shoot elongation. The explants were grown at photoperiods of 14 h (fluorescent tubes Sylvania 'Gro Lux', placed 30 cm away from the culture tubes), 30 µmol quanta·m^{-2}·$s^{-1}$and at 25±1 °C.

D e t e c t i o n o f *A g r o b a c t e r i u m v i t i s :* Sample preparation: 14 weeks after *in vitro* introduction the shoot tips of the *in vitro* plants (1 to 2.5 cm, 2-3 leaves) were transferred onto fresh media. The rest of the plants, including callus (developed at the shoot-root junction) and roots, was tested for *A. vitis*. The samples were homogenized with sterile scalpels, incubated in sterile distilled water and dilution-plated on YPG media (5 g yeast extract, 5 g bacto-peptone, 10 g glucose and 15 g agar diluted in 1 l of double-distilled water, pH 7.2). Plates were incubated for 48 h at 28 °C. Colonies with symptoms characteristic for *A. vitis* were subcultured on YPG or 3DG media (Brisbane and Kerr 1983) and identified using PCR and pathogenicity tests.

For identification of *A. vitis* PCR was conducted according to Schulz *et al*. (1993). The primers (obtained from Dr. Schulz, Fa. Vitolab) amplify specific fragments of the oncogenes of *A. vitis*.

Isolated strains were tested for their pathogenicity on *Kalanchoe daigremontiana* and *Kalanchoe tubiflora*. Tumor inducing reference strains that represent major Ti plasmid groups, AB3 (Octopine), AT66 (Nopaline) and S4 (Vitopine) isolated by Szegedi *et al*. (1988) were used for comparison. Tumor tissue of tumor producing test plants was used for opine tests to determine the opine type of the isolated pathogenic strains.

Results: P l a n t d e v e l o p m e n t : After *in vitro* establishment no distinct difference in growth between 'infested' (propagated from plants with visible crown gall symptoms and positive opine tests) and 'healthy' (no symptoms) plantlets was detected after 35 weeks for cv. Scheurebe.

Explants from apparently healthy control plants of cv. Zweigelt had a significant higher number of nodes and greater plant length than explants from infested plant material (Tab. 1).

D e t e c t i o n o f *A. v i t i s :* After 14 weeks of *in vitro* culture the plants were tested for infection with *A. vitis*. While it was not possible to detect *A. vitis* in the 57 explants started from crown gall-positive control plants of cv. Scheurebe, *A. vitis* could be isolated from one of the 35 explants derived from infested plant material of cv. Zweigelt. In addition 25 *in vitro* plants that had been started from *A. vitis*-positive control plants of various other varieties and had been grown *in vitro* for at least 14 weeks were tested for the bacteria. *A. vitis* was detected in one of the 25 samples. None of the *in vitro* plants originating from crown gall-free control plants was tested *A. vitis*-positive.

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Table 1

Growth differences between "healthy" and potentially *Agrobacterium vitis*-infested *in vitro* plants (cv. Zweigelt) after 15, 25 and 35 weeks of shoot tip culture

	Length (cm) after		
	15 weeks	25 weeks	35 weeks
Negative, no visible crown gall symptoms	1.34 a	1.69 a	4.06 a
Positive opine test, visible crown gall symptoms	0.90 a	1.11 a	1.46 b

Table 2

Latent persistence of *Agrobacterium vitis* in micropropagated plants (derived from infested control plants) of *Vitis vinifera* varieties after 14 weeks of *in vitro* culture

Variety	Control plants	Number of *in vitro* plants	*A. vitis*-positive *in vitro* plants	
			number	%
Scheurebe	*A. vitis*-positive	57	0	0
Zweigelt	*A. vitis*-positive	35	1	2.9
Other varieties	*A. vitis*-positive	25	1	4.0
		117	2	1.7

In summary, we detected pathogenic *A. vitis* in 1.7 % of the 117 tested explants that had been started from infested plant materials and grown *in vitro* for at least 14 weeks (Tab. 2).

Discussion and Conclusions: The results of our study show that *A. vitis* persisted in a latent state in at least 1.7 % of *in vitro* propagated vines. The fact that the bacteria were detected in a relatively small number of tested plantlets does not necessarily indicate that only a small number was infested, but is most likely due to a very low *A. vitis* population density in young tissue (BAUER *et al*. 1994), undetectable with current techniques (EASTWELL *et al*. 1995).

The reduced multiplication rate and fresh weight gain of explants taken from infested control plants compared to explants from healthy plant material (cv. Zweigelt) support the assumption that *A. vitis* can persist *in vitro* during shoot tip culture. Therefore the application of shoot tip propagation on it's own is not necessarily a guarantee for *A. vitis*-free plant material.

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