

Research Note

Amplification of different marker sequences for identification of Agrobacterium vitis strains

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Key words: crown gall on grapevine, *Agrobacterium vitis*, latent infection, detection, PCR, T-DNA, IS elements.

Introduction: Crown gall on grapevine caused by *Agrobacterium vitis* (OPHEL and KERR 1990) is reported from vineyards all over the world. Pathogenic *A. vitis* strains harbour large plasmids (pTi) responsible for plant tumor formation. During infection, part of the plasmid (T-DNA) is transferred into the plant cell and integrated into the nuclear plant DNA (CHILTON *et al.* 1977). The expression of T-DNA genes leads to the overproduction of auxins and cytokinins and subsequent tumor formation. The T-DNA also codes for the production of several tumor-specific compounds. These so called opines are excreted and serve as nutrients for *A. vitis* bacteria. Three types of opines are produced by grapevine tumors, so that the inciting *A. vitis* bacterium is assigned to either a nopaline, octopine, or vitopine strain class (SZEGEDI *et al.* 1988).

A. vitis lives saprophytically in the vascular system of the grapevine plant and is transmitted by vegetative propagation of infested cuttings (BURR and KÁTZ 1982, 1984; TARBAH and GOODMAN 1986). Since *A. vitis* cannot survive in soil, the production of pathogen-free propagation stocks has been proposed as an approach for disease control (GOODMAN *et al.* 1986, 1987; TARBAH and GOODMAN 1986; OPHEL *et al.* 1988; JÄGER *et al.* 1989). Rapid and reliable detection methods are needed to recognize and eliminate *A. vitis* infected propagation material.

The objective of this study was to demonstrate the rapid detection of *A. vitis* strains based on the amplification of DNA sequences of the T-DNA oncogenes and species-specific IS elements by means of the polymerase chain reaction (PCR).

Materials and methods: **A m p l i f i e d D N A f r a g m e n t s:** Oligonucleotide primers containing 19–26 nucleotides were selected to amplify specific fragments of the oncogenes of *A. vitis*. The primers are shown in Tab. 1. Physical maps of the selected T-DNA region and the amplified fragments are shown in Fig. 1.

D N A i s o l a t i o n: A loopful of cells from an *A. vitis* colony was suspended in 1 ml PBS, spun down in an Eppendorf microfuge and resuspended in 0.5 ml distilled water. The optical density of the bacterial suspension was determined and aliquots of 0.1 OD were transferred to a 0.5 ml reaction tube containing 50 µl PCR buffer. The tubes were boiled at 95 °C for 10 min to burst the cells.

P o l y m e r a s e c h a i n r e a c t i o n: When the tubes had cooled to room temperature, 200 nM of each primer, 200 µM of each dNTP and 2.5 U *Taq* polymerase was added to the cell lysates and overlaid with a few drops of paraffin oil. Amplification conditions were as follows: 94 °C for 2 min, followed by 30 cycles each consisting of 30 s at 94 °C, 30 s at 50 °C, and 30 s at 72 °C. The extension time was prolonged by 3 s for every cycle to compensate for the gradual decline of the amplification conditions during the course of the experiment.

The amplified PCR products were detected by gel electrophoresis in a 2 % agarose gel containing 0.5 µg/ml ethidium bromide.

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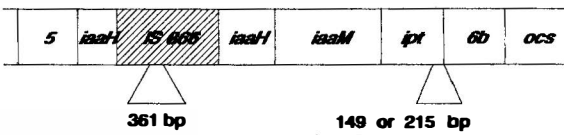
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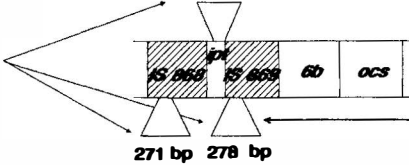
Results and discussion: The localization of *A. vitis* T-DNA fragments used for identification of *A. vitis* are shown in Fig. 1. *A. vitis* octopine/cucumopine strains were detected by amplification of a 215 bp fragment of the Tm4 *ipt* gene. Results are shown in Fig. 2. For identification of *A. vitis* WHR strains (KNAUF *et al.* 1982; PAULUS *et al.* 1991a), the presence of IS866 (BONNARD *et al.* 1989) was tested amplifying a 361 bp fragment of this IS element. This fragment could also be detected in coamplification experiments with primers targeting both the *ipt* gene and the IS866 fragment (Fig. 2). LHR strains were recognized by the absence of IS866, and by the presence of IS868 and IS869 (PAULUS *et al.* 1989, 1991a, 1991b) by means of PCR amplification of 271 bp of the first and 278 bp of the second IS element (Fig. 1).

Octopine strains :

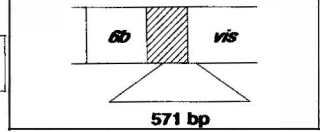
Tm4 - like ("large TA")



AB3 - like ("small TA")



Vitopine strains :



**Nopaline strains :
(chrom. DNA)**

Fig. 1: Physical maps of the T regions of *A. vitis* Ti plasmids and amplified regions reproduced from PAULUS *et al.* 1989, 1991; CANADAY *et al.* 1992.

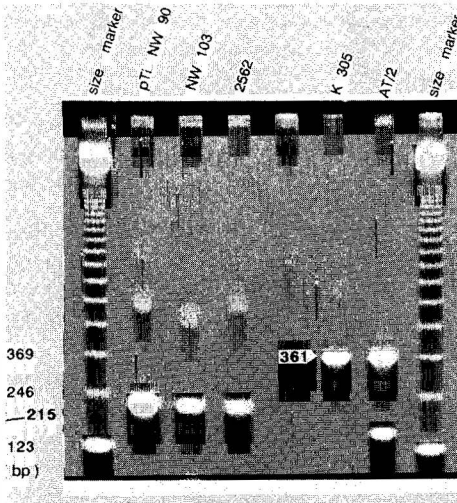


Fig. 2 (left): Detection of *A. vitis* octopine strains by Tm4 *ipt* (NW 90, NW 103, 2562) and/or IS866 (K 305, AT-2).

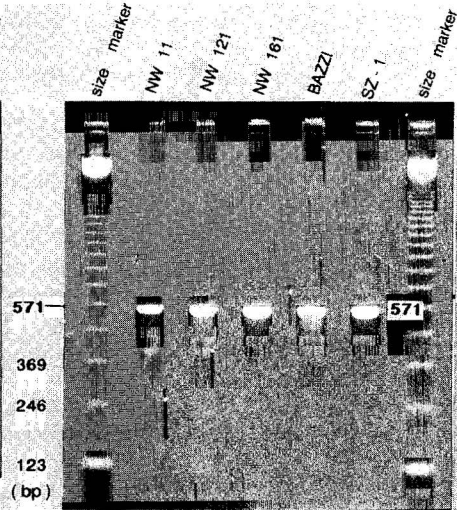


Fig. 3 (right): Detection of *A. vitis* vitopine strains by S4 *6b/vis*.

A. vitis vitopine strains were identified amplifying 571 bp between the *6b* and *vis* gene (CANADAY *et al.* 1992) (shown in Figs. 1 and 3). Nopaline strains were distinguished by the presence of IS869 and the absence of the other molecular markers in the examined strains (Figs. 1 and 4).

All *A. vitis* strains examined could be identified by the occurrence of distinct IS elements. However, since insertion elements are instable genetic structures, conserved *A. vitis* DNA sequences seem to be more suitable for indexing latently infected grapevines by PCR. The DNA fragments presented here are very useful molecular markers for the characterization of an *A. vitis* type detected in a distinct grapevine clone. When the grapevine clones that serve as hosts for certain *A. vitis* strains have been identified it might be possible to trace back infection pathways and to forecast future ways of progression.

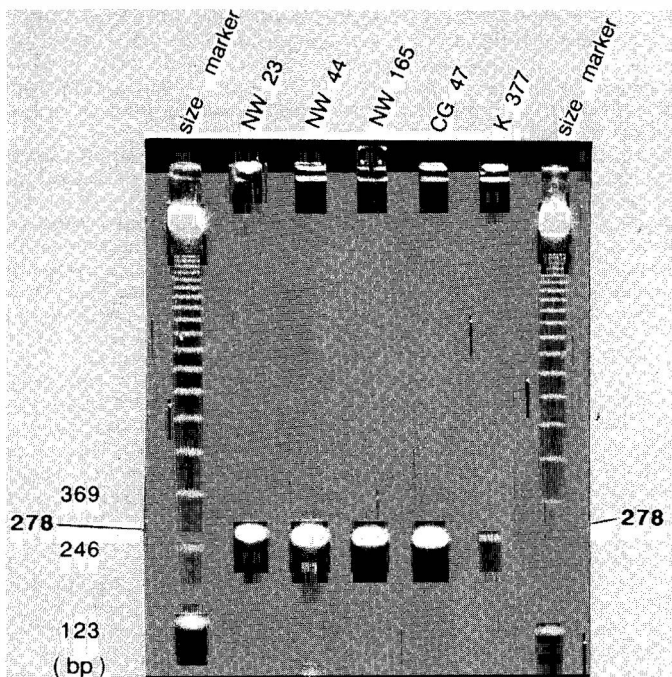


Fig. 4: Identification of *A. vitis* nopaline strains by the presence of IS869 and absence of the other molecular markers.

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