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Agrobacterium vitis strains lack tumorigenic ability on *in vitro* grown grapevine stem segments

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Summary

Grapevine stem segments were cocultivated with three different *Agrobacterium tumefaciens* and three different *A. vitis* strains. *A. tumefaciens* strains induced tumors at variable frequencies, while *A. vitis*-infected stem segments never formed crown galls. The tumorous nature of tissues grown on hormone free medium was confirmed by opine assays. Bioinformatic and PCR analysis of the virulence regions of various *A. tumefaciens* and *A. vitis* Ti plasmids showed that *virH2* and *virK* genes are common in *A. tumefaciens* but they are lacking from *A. vitis*. Thus *virH2* and *virK* genes may be essential for grapevine stem segment transformation, but expression of certain T-DNA genes of *A. vitis* may also prevent the growth of transformed cells. Our data indicate that the tumorigenic ability of *A. vitis* is different on intact plant and on their explants, and that the specific host association of *A. vitis* on grapevine is probably determined by physiological and biochemical factors (e. g., better colonizing ability) rather than by its increased tumorigenic ability. Therefore it is not reasonable to develop „helper" plasmids for grapevine transformation from *A. vitis* pTis, unless their avirulence on *in vitro* explants is determined by T-DNA gene(s). Due to the inability of *A. vitis* to induce tumors on grapevine stem segments, the use of *in vitro* explant assays cannot be reliably used to select *A. vitis* resistant grapevine genotypes or transgenic lines.

K e y w o r d s : crown gall, opines, Ti plasmids, *vir*-region, *Vitis*

Introduction

Tumorigenic agrobacteria (*Agrobacterium tumefaciens*, *A. rubi* and *A. vitis*) cause crown gall or cane gall disease on several, mainly annual crops. Rhizogenic strains (*A. rhizogenes*) cause intensive root formation called hairy root disease. Both diseases are based on the genetic transformation of the host plant leading to elevated hormone level or sensitivity, and opine production. The tumor-inducing (pTi) or root-inducing (pRi) plasmids contain two separate regions coding for this ability of agrobacteria. The *vir*-region carries genes for the DNA transfer from the procaryote bacterium into the eucaryote host plant through a highly sophisticated type IV transport system and directs its integration into the plant chromosome. The second region, called T-DNA, harbours genes that are transferred to the plant cells and are directly responsible for tumor formation. The length of T-DNA transported into the plant cell is determined only by its border sequences. This specific property of agrobacteria led to the development of so called „disarmed" or „helper" pTi plasmid derivatives lacking T-DNA. Such pTi derivatives have been widely used for decades to introduce useful traits into plants (Tzfira and Citovky 2008).

In the nature crown gall symptoms on grapevines are predominantly caused by *A. vitis* (Burr *et al.* 1998, Palacio-Bielsa *et al*. 2009, Filo *et al.* 2013) but the occurrence of *A. tumefaciens* has also been reported (Szegedi *et al*. 2005, Palacio-Bielsa *et al*. 2009, Rouhrazi and Rahimian 2012, Abdellatif *et al*. 2013). In contrast to the predominance of *A. vitis* on grapevine, exclusively *A. tumefaciens* (or sometimes *A. rhizogenes*) derivatives are used for grapevine transformation (Perl and Eshdat 1998, Martinelli and Mandolino 2001, Carimi *et al*. 2012). The potential use of „disarmed" *A. vitis* to introduce foreign genes into grapevine has already been raised (Vivier and Pretorius 2000), but construction of such a plasmid has not been published yet. In a previous study an *A. vitis* strain showed extremely low transformation efficiency compared to *A. tumefaciens* and *A. rhizogenes* strains on grapevine embryogenic calli, thus it was found inappropriate for such purposes (Torregrosa *et al*. 2002). Although it has been shown that the host range pattern (profile) of various agrobacteria differs on various grapevine genotypes (Szegedi *et al*. 1984, Süle *et al*. 1994), transformation experiments are rarely preceeded by such studies.

To test if *A. vitis* can be considered as an efficient gene vector for grapevine transformation we compared the tumor-inducing (transforming ability) of various *A. tumefaciens* and *A. vitis* strains on *in vitro* grapevine stem segments. Such *in vitro* explant assay may also be useful for early selection of resistant *Vitis* genotypes and transgenic lines. Our results showed that *A. vitis* strains are not tumorigenic on *in vitro* stem segments thus their use in grapevine transformation might provide invalid data when assaying genotype susceptibility.

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Material and Methods

Plant material: The following five grapevine genotypes were used during this work: *Vitis berlandieri* x *Vitis rupestris* 'Richter 110', the Seyve Villard 12375 x *V. vinifera* interspecific variety 'Fanny' and the *V. vinifera* cvs. 'Kadarka', 'Sauvignon blanc' and 'Ezerjó'. Plants were propagated *in vitro* in 380 ml glass bottles on ½ MS medium (Murashige and Skoog 1962) supplemented with 1.0 % saccharose and 0.25 % phytagel at 14 h photoperiod and at the light intensity of 50 $\mu m \cdot m^{-2} s^{-1}$.

Strains: Bacterial strains used for the experiments and their relevant characteristics are listed in Tab. 1. Cultures for transformation of grapevine explants were grown on glucose/yeast extract medium as previously described (Szegedi *et al*. 2005).

Cocultivation and selection: Stems of *in vitro* grown plants were cut into 5-6 mm pieces in liquid B5 medium containing 1 % (w/v) saccharose (Gamborg *et al*. 1968) to prevent drying and rinsed with bacterial suspensions (approx. 10^7 cfu·mL^{-1}) prepared also in liquid B5 medium. Then stem segments were transferred to solid hormone-free B5 medium containing 1% (w/v) saccharose and 0.6 % (w/v) agar and incubated for 48 hrs at 25-27 °C in dark. After two days of cocultivation the explants were washed in liquid hormone-free B5 medium containing 200 mg·L^{-1} claforan to remove bacteria and transferred to the same solid medium supplemented with 3 % (w/v) saccharose and 0.6 % (w/v) agar. Explants were incubated at 25-27 °C for three weeks at 14 h photoperiod and at the light intensity of 50 μM $m^{-2} \cdot s^{-1}$. Leaves were cut into approx. 6 x 6 mm pieces and transformed similarly as described for stem segments. Embryogenic calli of 'Richter 110' were also cocultivated with agrobacteria using the same protocol. Tumor formation was scored on the basis of hormone independent growth of transformed plant cells following transformation by wild type agrobacteria (Márton *et al*. 1979).

Opine assays: Octopine, nopaline, agropine and mannopine were detected by high voltage paper electrophoresis from the plant samples according to standard protocols (Dessaux *et al*. 1992).

Bioinformatic analysis of the vir genes and Vir proteins: Virulence regions from available full length pTi sequences of *A. tumefaciens* 15955 (NC_002377), C58 (NC_003065) and Bo542 (NC_010929), and *A. vitis* S4 (NC_011982) were obtained from the NCBI RefSeq database. Local alignment of the virulence region was carried out with MultiPipMaker v2011-08-12-01 (Schwartz *et al*. 2000). Homologous sequences of *A. tumefaciens* VirH2 and VirK proteins in the complete pTi sequence of *A. vitis* S4 were searched using tblastn.

PCR conditions: Polymerase chain reactions were carried out with primers designed to amplify the conserved regions of *virH2* (virH2F: 5'-GAT CCC TAT CCG ATT TAT CGC-3' and virH2R: 5'-GGA TTG GTC AGC AAT CCA-3') and *virK* (virKF: 5'-TYA YGG TYG ATT TAA GTT TGT GT-3' and virKR: 5'-GCC AAG CTG GTA CCT TTT C-3') with expected amplified fragment lengths of 701 and 259 bp for *virH2* and *virK*, respectively. Template DNA was prepared as previously described (Szegedi *et al*. 2005). The reactions were carried out in 25 μL volumes containing 1x *Taq* polymerase buffer, 200 μM each of dNTPs, 1.5 mM $MgCl_2$, 5 % (v/v) DMSO, 0.5 μM of each primers, 1.25 units of *Taq* polymerase and 1 μL template DNA. The initial denaturation step (94 °C, 1 min)

Table 1

Strains used for this study

Strain	Relevant characteristics	Disarmed helper strain/plasmid	Reference
Agrobacterium tumefaciens strains			
A348	pTi A6 in C58 chromosomal background, agropine, mannopine/ octopine pTi	Not available*	Garfinkel *et al*. 1981
C58	Wild type strain, nopaline/ agrocinopine A+B pTi	pMP90, MOG301	Koncz and Schell 1986, Hood *et al*. 1993
A281	pTiBo542 in C58 chromosomal background, agropine, mannopine/ L,L-succinamopine pTi	EHA101, EHA105	Hood *et al*. 1986, Hood *et al*. 1993
GV3101(pTiTm4)	pTiTm4 in C58 chromosomal background	-	Huss *et al*. 1989
II/5-1	Wild type isolate carrying an *A. vitis* octopine/cucumopine type pTi	-	Szegedi *et al*. 2005
Agrobacterium vitis strains			
Tm4	Wild type, octopine/cucumopine pTi	-	Szegedi *et al*. 1988
AT1	Wild type, nopaline pTi	-	Szegedi *et al*. 1988
S4**	Wild type, vitopine pTi	-	Szegedi *et al*. 1988

*Disarmed pTi have been developed from the very similar pTiB6 (MOG101, Hood *et al*. 1993 and GV2260, Deblaere *et al*. 1985) and pTiAch5 (LBA4404, Hoekema *et al*. 1983)

**Identical with the sequenced *A. vitis* S4 (Slater *et al*. 2009).

was followed by 35 cycles of denaturation (94 °C, 40 sec), annealing (50 °C, 40 sec) and synthesis (72 °C, 1 min), and finally terminated (72 °C, 3 mins). Amplification products were analysed after electrophoresis in 1.5 % (w/v) agarose gel and ethidium-bromide staining.

Statistical analysis: Differences between tumor induction ability of different *A. tumefaciens* strains on various grapevine varieties were tested using the chi-square test. Observed frequencies of tumor formation were compared to expected frequencies. Expected frequencies were defined as the average tumor induction ability of all strains on all varieties.

Results

To test the potential suitability of *A. vitis* for grapevine genetic transformation and crown gall resistance assay we have tested the octopine/cucumopine strain Tm4, the nopaline strain AT1 and the vitopine strain S4 on *in vitro* grapevine stem segment explants. The tested strains were tumorigenic on these and/or on several other grapevine cultivars when intact plants were inoculated *in vitro* or in the greenhouse (data not shown). For comparison *A. tumefaciens* A348 (agropine/octopine pTi), C58 (nopaline/agrocinopine A+B pTi) and A281 (agropine/L,L-succinamopine pTi) were used. Disarmed derivatives of these or similar *A. tumefaciens* strains (Tab. 1.) have already been widely used to introduce foreign genes into grapevines. Embryogenic calli are most widely used for genetic transformation of grapevine (Perl and Eshdat 1998, Martinelli and Mandolino 2001, Bouquet *et al*. 2008, Carimi *et al*. 2012), but stem sections or leaf discs are also considered as starting material (Das *et al*. 2002, Maillot *et al.* 2006, Nicholson *et al*. 2012, Nookaraju and Agrawal 2013) thus we included them as well.

Grapevine stem segments collected from *in vitro* grown plants formed tumorous calli on hormone-free B5 medium at various degrees depending on the grapevine genotype after inoculation with *A. tumefaciens* strains (Fig. 1). In contrast to these observations, none of the three *A. vitis* strains, which are the natural agrobacterial pathogens of grapevines, induced tumors on stem segments of any the tested five grapevine cultivars (Fig. 1, Tab. 2). On the rootstock variety 'Richter 110' the A281 strain, on 'Fanny' and *V. vinifera* (European) grapes the C58 strain were the most efficient. C58-induced tissues growing on hormone free medium contained nopaline, while AT1 inoculated stem segments that did not show growth were nopaline negative (Fig. 1.) Similar results were obtained, although tumors were formed at lower frequencies, when 'Richter 110' and 'Ezerjó' leaf discs were cocultivated with *A. tumefaciens* or *A. vitis*. *A. vitis* strains never induced growth (tumor formation) on leaf discs on hormone-free medium (data not shown).

To further confirm the tumorous nature of the calli selected on hormone-free medium 12 independent tumor lines were analysed of each of the 'Richter 110'/*A. tumefaciens* C58, 'Richter 110'/*A. tumefaciens* A281, 'Ezerjó'/*A. tumefaciens* C58 and 'Ezerjó'/*A. tumefaciens* A281 combinations (altogether 48 tumor lines) for the presence of nopaline (C58-induced lines) or agropine and mannopine (A281-induced lines). Each line contained the appropriate opine (nopaline for C58-, and agropine/mannopine for A281-induced tumors) confirming that the selected tissues were true crown galls (Fig. 2).

Surprisingly, neither *A. tumefaciens* nor *A. vitis* strains transformed embryogenic calli of 'Richter 110'. We could not select any lines growing on hormone-free MS or B5 media. Altogether 72 callus lines, 12 for each of the six strains, were assayed for the presence of the appropriate opines. All lines were opine negative confirming that transformation did not take place.

Next, stem segments of 'Kadarka', 'Ezerjó' and 'Sauvignon blanc' were coinoculated with 1:1 mixtures of *A. tumefaciens* C58 and *A. vitis* Tm4 cells. Thirty-six tumors,

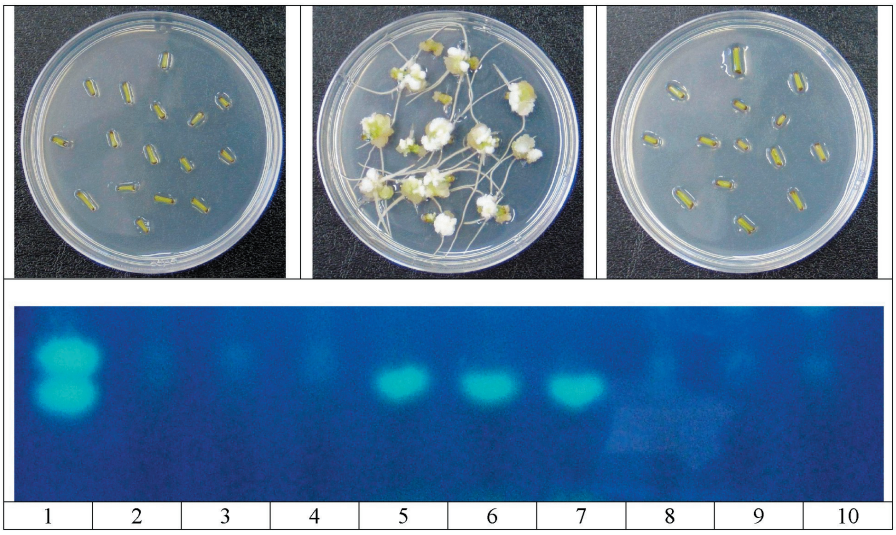

Fig. 1: *In vitro* tumor induction assay using *V. vinifera* cv. 'Fanny' stem segments. Upper panel: Non-inoculated control (left), C58-inoculated explants (middle) and AT1-inoculated explants (right). Lower panel: Nopaline assay of the above shown samples. Lane 1: 0.6 µg each of synthetic octopine (upper spot) and nopaline (lower spot), lanes 2-4 are three independent control samples, lanes 5-7 are three independent C58 inoculated samples and lanes 8-10 are three independent AT1-inoculated samples.

Table 2

Transformation efficiency of various *Agrobacterium tumefaciens* and *Agrobacterium vitis* strains on grapevine stem segment assays. Asterisks denote significant difference in tumor formation ability of different *A. tumefaciens* strains on the given variety (* p = 0.05, ** = 0.01), while + denotes significant difference between the response of different varieties to a given *A. tumefaciens* strain ($^{++}$ p = 0.01)

Strain	'Richter 110'*		'Fanny'**		'Sauvignon blanc'**		'Kadarka'**		'Ezerjó'*	
	No of tumorous/No of total stem segments	% of tumorous stem segments	No of tumorous/No of total stem segments	% of tumorous stem segments	No of tumorous/No of total stem segments	% of tumorous stem segments	No of tumorous/No of total stem segments	% of tumorous stem segments	No of tumorous/No of total stem segments	% of tumorous stem segments
Control	0/37	0	0/33	0	0/28	0	0/26	0	0/29	0
A348^{++}	14/32	43.7	0/31	0	3/31	9.6	10/30	33.3	22/33	33.3
C58^{++}	18/32	56.2	30/32	93.7	21/31	67.7	28/29	96.5	30/33	90.9
A281^{++}	26/33	78.8	2/35	5.7	20/30	66.6	17/28	60.7	28/33	84.8
Tm4	0/37	0	0/32	0	0/28	0	0/25	0	0/31	0
AT1	0/38	0	0/32	0	0/29	0	0/26	0	0/34	0
S4	0/38	0	0/32	0	0/27	0	0/26	0	0/32	0

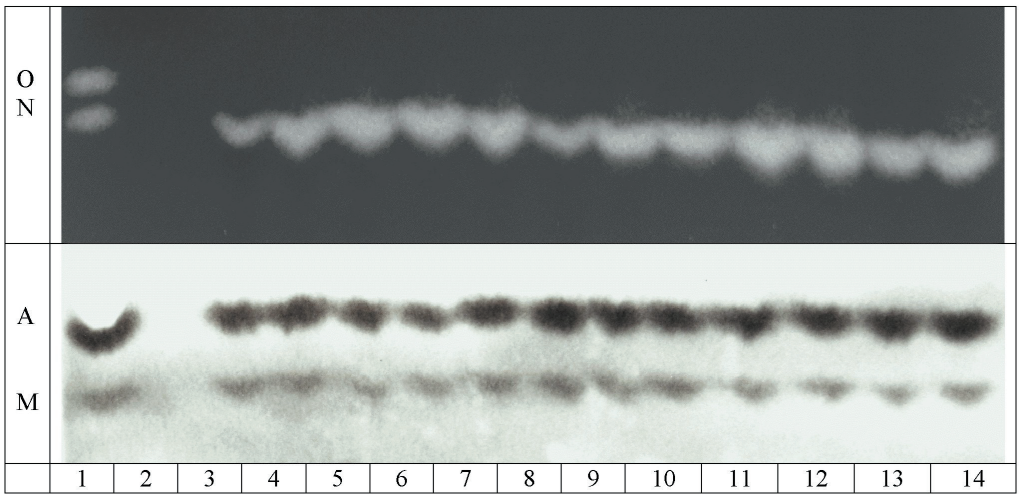

Fig. 2: Detection of the appropriate opines from 'Richter 110' tumors induced on *in vitro* stem segments with *Agrobacterium tumefaciens* C58 (upper panel) and *A. tumefaciens* A281 (lower panel). Lane 1: Pure octopine (**O**) and nopaline (**N**) or agropine (**A**) and mannopine (**M**), lane 2: non-transformed 'Richter 110' stem extract, lanes 3-14: 12 independent tumor lines.

12 for each combination, were analysed for the presence of nopaline and octopine. All samples contained only nopaline, but not octopine indicating that the tumors were exclusively induced by *A. tumefaciens* C58. Thus C58 did not complement the lacking avirulence of Tm4.

To test if this avirulence of *A. vitis* on *in vitro* grapevine stem segments is due to chromosomal or Ti plasmid differences between *A. tumefaciens* and *A. vitis*, we tested also *A. tumefaciens* GV3101 (pTiTm4) and *A. tumefaciens* II/5-1 strains, both carrying *A. vitis* type pTis (Tab. 1.), on 'Kadarka' explants. They showed the same negative results as the wild type *A. vitis* strains.

The results described above suggested that lack of certain virulence genes located on the pTis may be responsible for the different tumorigenic ability of *A. tumefaciens* and *A. vitis* on grapevine stem segments. Alignment of the virulence regions of *A. tumefaciens* 15955, C58 and Bo542, and *A. vitis* S4 showed that most of the well characterized *vir* genes are shared among the virulence regions of different *Agrobacterium spp.* strains (Fig. 3). Two virulence genes, *virH2* and *virK* commonly occurred in all *A. tumefaciens* strains, but they were lacking from *A. vitis* S4. *A. tumefaciens* C58 harbours two copies of *virE3*, which explains the scattered alignment of *virE3* sequences in different *Agrobacterium spp.* strains. While the majority of virulence genes shows a relatively high sequence homology in different strains, *virD3* shows peculiar distribution of gap-free alignments, where only the N and C terminal sequences seem to be conserved among *Agrobacterium spp.* strains. *VirF* genes from different *A. tumefaciens* show a low sequence similarity, the applied local alignment algorithm was unable to detect significant gap-free alignments for *virF* from strains C58 and 15955 (Fig. 3), although the latter also carries *virF*.

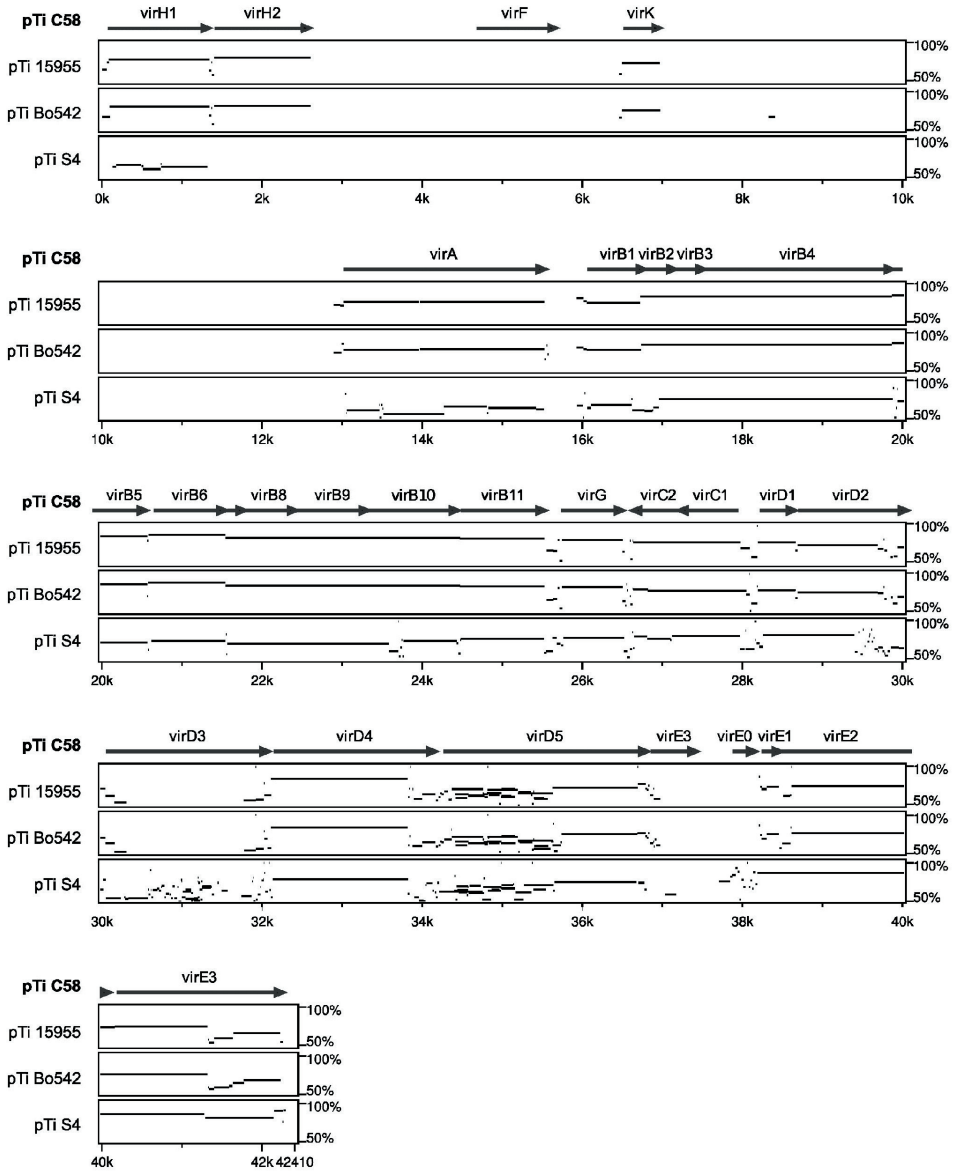

Fig. 3: Local alignments of available *Agrobacterium spp.* virulence regions. The sequences of *A. tumefaciens* 15955 (NC_002377), *A. tumefaciens* Bo542 (NC_010929) and *A. vitis* S4 (NC_011982) have been aligned to the reference sequence of the virulence region of *A. tumefaciens* C58 (NC_003065). Each line shows gap-free local alignments between the query sequence and the C58 reference. Vertical position of the lines inside each box indicate percentage nucleotide similarity of the alignment.

We also searched for homologous sequences of *A. tumefaciens virK* and *virH2* proteins in the complete pTi sequence of *A. vitis* S4 using tblastn. Significant similarity to VirK was not found at e-value threshold of 0.1, while *A. tumefacines* queries showed significant similarity to *A. vitis* S4 VirH1. Phylogenetic analysis of *A. tumefaciens* and *A. vitis* VirH1 and VirH2 proteins orders *A. vitis* S4 VirH1 to the VirH1 protein sequences of different *A. tumefaciens* strains and not to VirH2 sequences (data not shown). Based on these results, *virK* and *virH2* genes are indeed missing from *A. vitis* S4.

PCR analysis of the tested wild type agrobacteria, as expected, detected *virH2*- and *virK*-specific sequences in all *A. tumefaciens* strains. In contrast to these results we could not amplify any *virH2*- and *virK*-specific fragments with the primers used from *A. vitis* octopine (Tm4), nopaline (AT1) or vitopine (S4) strains (Fig. 4).

Discussion

An *in vitro* stem segment assay was expected to provide a simple method to test the tumorigenicity of various agrobacteria or susceptibility of various grapevine genotypes. We have shown that the natural grapevine pathogen *A. vitis* does not induce tumors on grapevine stem segments, while *A. tumefaciens* strains, although at variable frequencies depending on the grapevine cultivar, were tumorigenic in this assay. The reason of negative transformation results of embryogenic cell line with wild type agrobacteria is unknown. It may be due to the inappropriate media we used or due to the sensitivity of embryogenic cells to the hormone overproduction caused by *Agrobacterium*-transformation. Our data are not in agreement with some previous observations. Huss and coworkers (1990) successfully induced tumors on *V. vinifera* 'Chardonnay'

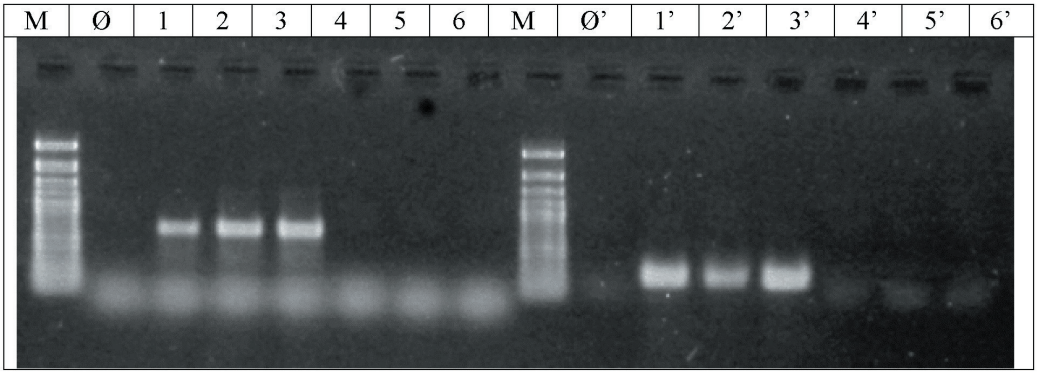

Fig. 4: PCR analysis of *Agrobacterium tumefaciens* and *A. vitis* strains for the presence of *virH2* (1-6) and *virK* (1'-6') genes. **M**: size marker (Fermentas SM0328), **Ø** and **Ø'**: DNA-free samples with *virH2*-, and *virK*-specific primers, respectively. **1** and **1'**: *A. tumefaciens* A348, **2** and **2'**: *A. tumefaciens* C58, **3** and **3'**: *A. tumefaciens* A281, **4** and **4'**: *A. vitis* Tm4, **5** and **5'**: *A. vitis* AT1 and **6** and **6'**: *A. vitis* S4.

stem segments with *A. vitis* Tm4 and AB3. In another study *A. vitis* CG450 induced tumors *in vitro* on 'Richter 110' stem segments, when this assay was used to select crown gall resistant transgenic lines (Krastanova *et al*. 2010), although in both cases plant samples were collected from the greenhouse and not from *in vitro* plants.

Torregrosa *et al.* (2002) found that the frequency of transformation is determined both by the grapevine genotype and *Agrobacterium* strains. Our results confirm these observations. *A. tumefaciens* strain A281 transformed the rootstock 'Richter 110' more efficiently than A348 or C58, while C58 was more efficient on certain European grapevine cultivars than A281. These data may be considered for the selection of the appropriate disarmed („helper") strain for introducing foreign genes into grapevine. Host range differences among various agrobacteria within *Vitis* spp. have also been observed earlier (Szegedi *et al*. 1984, Süle *et al.* 1994).

To get an insight into the posssible role of the genetic background we tested two *A. tumefaciens* strains carrying *A. vitis* type pTis. Since neither GV3101 (pTiTm4) nor II/5-1 were tumorigenic, this property of *A. vitis* is probably determined by pTi-encoded virulence or T-DNA genes. Until now data are available only for pTiS4 (Slater *et al*. 2009) that does not allow us a comprehensive comparison of these regions of *A. tumefaciens* and *A. vitis*.

The bioinformatic and PCR analysis of virulence regions suggested us that the presence (in *A. tumefaciens*) or absence (in *A. vitis*) of *virH2* and *virK* genes are common and basic differences between *A. tumefaciens* and *A. vitis* pTis. The *virH2* protein detoxifies the phenolic compounds formed after wounding plant tissues (Brencic *et al*. 2004). It looks unlikely that a small piece of stem segments produces sufficient amounts of phenolics to prevent transformation. The second gene, *virK*, also does not seem to be a basic virulence factor (Kalogeraki and Winans 1998). It is also possible that the T-DNA genes of *A. vitis* are transferred but, under the used circumstances, their expression prevents the growth of transformed cells. Thus further studies should be carried out to find which genes contribute to, or prevent tumor formation on *in vitro* grapevine stem segments.

Besides the bacterial virulence factors, genetic transformation of plants by *Agrobacterium* involves several host genes (proteins) as well (Gelvin 2010, Magori and Citovsky 2012, Tzfira and Citovsky 2008). We should also consider that these contributing plant proteins are not produced in grapevine explants like stem segments or leaf discs. Manipulating such host factors may help us to understand grapevine-*A. vitis* interaction as well as to design strategies for crown gall resistance.

Another possibility is that competent cells of the stem segments and leaf discs (embryogenic calli) do not survive cocultivation with *A. vitis*. *A. vitis* produces polygalacturonase (*pehA*), a cell wall degrading enzyme encoded by a chromosomal gene. The *pehA* minus mutant strain CG50 derived from the *A. vitis* nopaline strain CG49 (Rodriguez-Palenzuela *et al*. 1991) showed the same negative reaction on grapevine stem segments as its wild type parent (Szegedi and Burr, unpublished observations). Besides polygalacturonase production *A. vitis* also induces tissue necrosis by a quorum-sensing regulated manner (Zheng *et al*. 2003). The necrosis-minus (*aviR*-) mutant of *A. vitis* S4 was also non-tumorigenic on grapevine stem segments like its wild type S4 (Szegedi and Burr, unpublished observations). Thus the chromosomally encoded tissue necrosis induced by *A. vitis* probably is not the key factor in the determination of non-tumorigenic response of grapevine explants to *A. vitis*.

Taken together, the susceptibility of intact grapevines and explants to *A. vitis* differs. A similar phenomenon was described for *Kalanchoe daigremontiana* stem segments inoculated with the *A. tumefaciens* octopine strain B6S3 and nopaline strain C58. Both strains induce tumors on intact *Kalanchoe* plants, but only B6S3 transformed its stem segments as shown by LpDH activity (octopine production). Additionally, B6S3 complemented the lacking avirulence of C58 (Otten 1982). This difference between the transforming ability of B6S3 and C58 was shown due to their different *virF* functions (Otten *et al*. 1985).

Our results suggest that specific adaptation of *A. vitis* to grapevine is primarily determined by physiological and metabolic factors, e. g. the ability of tartrate utilization from the bacterial side (Kado 1998, Salomone *et al*. 1998)

and the production of tartrate from the host side (Ruffner 1982) rather than by the host-specific virulence properties of the pathogen. Additionally, we confirm the previous observations (Torregrosa *et al*. 2002) showing that *A. vitis* cannot be efficiently used as a tool for introduction foreign genes into grapevines, unless the avirulence of *A. vitis* on *in vitro* explants is determined by T-DNA genes. The method described previously (Huss *et al*. 1990, Krastanova *et al*. 2010) and here may provide an easy assay to test various helper plasmids for their utility for gene introduction into a given grapevine genotype. On the other hand, we show that stem segment assays cannot be routinely used to select *A. vitis*-resistant genotypes from natural or transgenic populations.

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