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Genetic evidence for direct sensing of phenolic compounds by the VirA protein of *Agrobacterium tumefaciens*

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ABSTRACT **The virulence (*vir*) genes of *Agrobacterium tumefaciens* are induced by low-molecular-weight phenolic compounds and monosaccharides through a two-component regulatory system consisting of the VirA and VirG proteins. However, it is not clear how the phenolic compounds are sensed by the VirA/VirG system. We tested the *vir*-inducing abilities of 15 different phenolic compounds using four wild-type strains of *A. tumefaciens*—KU12, C58, A6, and Bo542. We analyzed the relationship between structures of the phenolic compounds and levels of *vir* gene expression in these strains. In strain KU12, *vir* genes were not induced by phenolic compounds containing 4′-hydroxy, 3′-methoxy, and 5′-methoxy groups, such as acetosyringone, which strongly induced *vir* genes of the other three strains. On the other hand, *vir* genes of strain KU12 were induced by phenolic compounds containing only a 4′-hydroxy group, such as 4-hydroxyacetophenone, which did not induce *vir* genes of the other three strains. The *vir* genes of strains KU12, A6, and Bo542 were all induced by phenolic compounds containing 4′-hydroxy and 3′-methoxy groups, such as acetovanillone. By transferring different Ti plasmids into isogenic chromosomal backgrounds, we showed that the phenolic-sensing determinant is associated with Ti plasmid. Subcloning of Ti plasmid indicates that the *virA* locus determines which phenolic compounds can function as *vir* gene inducers. These results suggest that the VirA protein directly senses the phenolic compounds for *vir* gene activation.**

The Gram-negative soil bacterium *Agrobacterium tumefaciens* causes crown gall tumors after infecting the wound sites of most dicotyledonous and a restricted number of monocotyledonous plants. Virulent strains of *A. tumefaciens* harbor Ti (tumor-inducing) plasmids. A specific segment of the Ti plasmid, the T-DNA, is transferred to host plant cells and is then integrated into the plant nuclear genome. The virulence (*vir*) genes, also located on the Ti plasmid, are specifically involved in the processing and transfer of T-DNA (for reviews, see refs. 1 and 2).

The *vir* genes are transcriptionally regulated by two members, *virA* and *virG*, which belong to a family of two-component regulatory systems (1). VirA protein senses plant signal molecules and then transduces the signal by phosphate transfer to VirG protein, the response regulator. This protein then binds to upstream regions of each of the *vir* genes and transcriptionally activates this regulon. *vir* genes are induced at an acidic pH by phenolic compounds that function in concert with monosaccharides synthesized and exuded from wounded plant cells.

The VirA protein spans the inner membrane with its C-terminal region localized in the cytoplasm and its N-terminal domain in the periplasm. The C-terminal region can be divided into three domains: a linker, a protein kinase, and a phosphoryl receiver (3). The N-terminal region of the VirA protein, including the periplasmic domain, is not essential for *vir* gene induction by phenolic compounds, but the linker domain is required (3, 4). However, these conclusions are based on the inducing properties of modified VirA molecules *in vivo* that lack these specific domains. No reports have appeared that demonstrate that the unmutated VirA protein can bind a phenolic compound. Lee *et al.* (5) reported on experiments using a radiolabeled brominated derivative of the inducing phenolic compound, acetosyringone, which inhibits *vir* gene induction. The VirA protein was not labeled by this inhibitor, but two low-molecular-weight proteins encoded by the bacterial chromosome, p10 and p21, were labeled. These observations suggest that these proteins may interact with phenolic compounds and then presumably interact with VirA protein. However, the chromosomal genes that encode p10 and p21 proteins have not been cloned, nor have noninducible mutants that are unable to bind phenolic compounds been isolated.

In *Rhizobium*, which has been grouped with *Agrobacterium* into the family Rhizobiaceae (6), expressions of the nodulation (*nod*) genes are essential for nodule formation. This expression requires plant signals, generally flavonoids, and the regulatory NodD proteins. In the presence of plant signals, the NodD proteins act as a transcriptional activator of *nod* genes. Like the VirA protein, the NodD proteins are localized in the cytoplasmic membrane and interact with flavonoids in the inner membrane (for review, see ref. 7). Considerable genetic evidence suggests that flavonoid inducers bind directly to NodD proteins, although this binding has not been observed biochemically. Point mutations in NodD can change the specificity of certain flavonoids to induce (8). Exchange of a *nodD* gene between *Rhizobium* strains that differ in their sensitivity to different flavonoids changes the sensitivity of the recipient strain to the flavonoid (9, 10).

To gain genetic data in *Agrobacterium* on the question of whether or not VirA protein interacts directly with phenolic compounds, four *A. tumefaciens* strains were studied for their ability to be induced by different phenolic compounds. Here we report on *vir* gene induction in different *A. tumefaciens* strains by three groups of phenolic compounds classified according to their chemical structures. We show that the *vir* genes of one *A. tumefaciens* strain (KU12) are induced by phenolic compounds that do not induce other strains. Also, we provide genetic evidence that the abilities of phenolic compounds to induce *vir* genes of *A. tumefaciens* strains are related to the VirA protein. Genetic analysis of KU12 and three other *A. tumefaciens* strains suggests that the VirA protein senses phenolic compounds directly.

MATERIALS AND METHODS

Strains, Plasmids, and Media. All *A. tumefaciens* strains and plasmids used are listed in Table 1. *A. tumefaciens* strains were grown in MG/L medium (23) or AB minimal medium (23) at

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Table 1. Strains of *A. tumefaciens* and plasmids used

Strain and plasmid	Characteristics	Source and refs.
Strain		
KU12	Octopine type pTiKU12	11
C58	Nopline type pTiC58	12
A6	Octopine type pTiA6	12
Bo542	Succinamopine type pTiBo542	12
A136	C58, Ti plasmid cured	12
KU12C3	KU12, Ti plasmid cured	13
A348	A136(pTiA6)	14
KU911	A136(pTiKU12)	15
KU9412	KU13C3(pTiA6)	This study
C58ΔvirA	C58(Δ*virA*)	D. Raineri*
At11068	A348(Δ*virA*)	T. Charles†
Plasmid		
pSM243cd	*virB*::*lacZ* translational fusion (Cb^r)	16
pUFR047	IncW broad-host-range-vector (Cb^r)	17
pTC110	IncW derivative of pUCD2 (Km^r)	18
pTC110BL	IncW derivative of pTC110 (Km^r)	This study
pGP159	*virA, virG, virB*::*lacZ* of pTiA6 (Cb^r,Tc^r) in IncP vector	19
pSG9401	*virA* of pTiA6 in pUCD2 (Cb^r,Tc^r)	This study
pSG9403	*virG* of pTiA6 in pUCD2 (Cb^r,Tc^r)	This study
pYW9503	*virA* of pTiKU12 in pUFR047 (Cb^r)	This study
pKUBF1501	*virG* of pTiKU12 in pTC110BL (Km^r)	This study
pUCD2	IncW broad-host-range-vector (Km^r,Cb^r,Tc^r)	20
pIB50	*virB*::*lacZ, virE*::*cat* of pTiA6 in IncP vector (Km^r)	21
pJD101W	*virA* of pTiC58 in pUCD2 (Cb^r,Tc^r)	J. D. Heath‡
pTB108	*virA* of pTiA6 in pUCD2 (Cb^r,Tc^r)	22

Cb, carbenicillin; Km, kanamycin; and Tc, tetracycline.
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28°C. *Escherichia coli* strain DH5α (24) was grown in LB medium (24) at 37°C and used as the cloning host.

Plasmid Constructions. pTC110BL was constructed by self-ligation of the 8.2-kb *Bam*HI fragment of pTC110 (18), and pKUBF1501 was constructed by introducing a 5.0-kb *Bam*HI fragment of pTiKU12 that contains the *virG* gene into the unique *Bam*HI site of pTC110BL. To construct pSG9401 and pSG9403, pSW169 (25) and pSW167 (26) containing wild-type *virA* and *virG* of pTiA6, respectively, were linearized by digestion with *Eco*RI and ligated with pUCD2 (20) cleaved with *Eco*RI. Plasmids were introduced into *A. tumefaciens* by triparental mating or electroporation using a Bio-Rad electroporator as described (18).

Enzymes and Reagents. Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories and used according to the supplier's recommendations. All phenolic compounds were purchased from Aldrich. Antibiotics and other reagents were purchased from Sigma.

Cloning of *virA* Gene of pTiKU12. The *virA* gene of KU12 was cloned into pUFR047 (17) by the following procedure. The pTiKU12, isolated from KU12, was partially digested with *Sau*3A1; 5- to 20-kb fragments were isolated by electroelution (24), ligated to the *Bam*HI site of pUFR047, and transformed into *E. coli* DH5α. Recombinant plasmids isolated from transformed *E. coli* DH5α were introduced into *A. tumefaciens* C58ΔvirA containing plasmid pIB50. The *A. tumefaciens* strain containing *virA* of pTiKU12 was selected by plating bacteria on induction medium (27) supplemented with carbenicillin (100 μg/ml), kanamycin (100 μg/ml), chloramphenicol (100 μg/ml), 5-bromo-4-chloro-3-indolyl β-D-galactoside (40 μg/ml), and 100 μM 4-hydroxyacetophenone and then screening for blue colonies. The presence of *virA* was verified by demonstrating that the strain was tumorigenic on leaves of *Kalanchoë diagremontiana.*

β-Galactosidase Assay. β-Galactosidase activities were assayed by the method described by Stachel *et al.* (28).

RESULTS

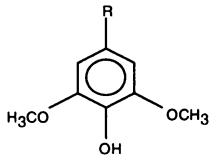
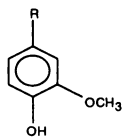
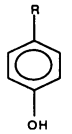
Induction of *vir* Genes in Wild-Type *A. tumefaciens* Strains by Phenolic Compounds. Although a number of studies have been reported on the inducing activity of a wide variety of phenolic compounds, these studies involved only one or two tester strains. To determine whether different wild-type strains of *A. tumefaciens* were similar in their response to different phenolic-inducing compounds, we introduced pSM243cd, which contains a *virB*::*lacZ* fusion, into four different wild-type strains. These four strains were then tested for *vir* gene expression after induction by a variety of possible *vir* gene-inducing phenolic compounds. The compounds differed in the substituents in position 1, as well as in the presence of the methoxy group in positions 3 and 5. The four different wild-type strains of *A. tumefaciens* differ in their opine-utilizing ability. The strains included the widely studied strains A6, C58, Bo542, and a less well-studied strain KU12, a soil isolate from Korea (Table 1). The phenolic compounds were divided into three groups with five phenolic compounds in each group. The results of Table 2 show that the induction response to different phenolic compounds varies among the different strains.

Under our standard induction conditions, *vir* genes in both the octopine-type strain A6 and the succinamopine-type strain Bo542 were strongly induced by all phenolic compounds in group A. The nopaline-type strain C58 showed a moderate level of *vir* gene induction by acetosyringone and sinapinic acid. However, no response to syringaldehyde, syringic acid, and 2,6-dimethoxyphenol was observed. Surprisingly, we found that all phenolic compounds in group A, including acetosyringone, the most commonly used *vir* gene inducer in the laboratory, were unable to induce the *vir* genes of strain KU12. All strains except C58 responded to acetovanillone and ferulic acid of group B, and strains KU12 and Bo542 were both induced by guaiacol. However, only strain Bo542 was induced by vanillin of group B. In our hands, strain A6 was not induced by vanillin, a phenolic compound classified as a strong *vir* gene inducer by Melchers *et al.* (29) using *A. tumefaciens* strain LBA2516, which contains an octopine-type Ti plasmid, pTiB6, that is closely related to pTiA6 (12). The reason for this discrepancy is not clear. Of special interest is the observation that the *vir* genes of strain KU12 were highly induced by a group of compounds in group C that lack both methoxy groups. These compounds included 4-hydroxyacetophenone, *p*-coumaric acid, and phenol, which had previously been reported to be non-*vir* gene inducers (29). In support of this latter report, the three other *A. tumefaciens* strains were indeed not induced by the phenolic compounds in group C.

We tested *vir* gene induction by other phenolic compounds that lacked the hydroxyl group at the R4 position. None of the four strains were induced by any of these phenolic compounds (data not shown), including 3,4,5-trimethoxybenzaldehyde, which has been classified as a weak *vir* gene inducer (29). These results indicate that the *p*-hydroxyl group is absolutely essential for induction in strain KU12, as it is for other strains, in agreement with a published report (30).

Induction of *vir* Genes by Phenolic Compounds Correlates with Their Ti Plasmid. Lee *et al.* (5) reported that proteins not encoded on the Ti plasmid mediate *vir* gene activation by phenolic compounds in a step before the VirA/VirG two-component regulatory system. To explore this possibility further, we constructed, by triparental mating (17), strain KU9412, which contains pTiA6 in the same chromosomal

Table 2. Induction of *virB*::*lacZ* in wild-type *A. tumefaciens* strains by phenolic compounds

Group	Basic structure	Substituents (R)	Name	β-Galactosidase activity,* units			
				KU12	C58	A6	Bo542
A		$COCH_3$	Acetosyringone	3.2	259	910	1864
		CHO	Syringaldehyde	3.3	9.9	722	1373
		COOH	Syringic acid	3.9	5.4	190	823
		CH=CHCOOH	Sinapinic acid	5.6	142	657	1707
		H	2,6-Dimethoxyphenol	4.2	20.0	410	1208
B		$COCH_3$	Acetovanillone	157	8.1	359	1043
		CHO	Vanillin	9.1	3.4	7.0	186
		COOH	Vanillic acid	6.5	3.2	4.5	4.7
		CH=CHCOOH	Ferulic acid	167	10.9	78.5	830
		H	Guaiacol	55.7	4.5	7.4	232
C		$COCH_3$	4-Hydroxyacetophenone	238	4.8	6.5	7.5
		CHO	4-Hydroxybenzaldehyde	14.1	2.2	3.4	4.7
		COOH	4-Hydroxybenzoic acid	3.5	3.1	2.5	3.3
		CH=CHCOOH	*p*-Coumaric acid	295	2.0	1.7	3.9
		H	Phenol	188	2.8	2.7	3.1

*Strains KU12(pSM243cd), C58(pSM243cd), A6(pSM243cd), and Bo542(pSM243cd) were grown for 18 hr in induction medium/10 mM glucose/100 μM phenolic compound at pH 5.5, and β-galactosidase activities (Miller units) were measured as described. pSM243cd contains a *virB*::*lacZ* fusion. Values are averages of three independent experiments; β-galactosidase activities of cultures lacking phenolic compounds were <5 units.

background as KU12. Despite the fact that strain KU9412 has a different chromosomal background than strain A348, the *vir* genes of strain KU9412, like A348, were still induced by acetosyringone and sinapinic acid but were not induced by 4-hydroxyacetophenone and *p*-coumaric acid. Furthermore, strain KU911, which contains pTiKU12 in A136 (which has the same chromosomal background as A348) was not induced by acetosyringone and sinapinic acid but was induced by 4-hydroxyacetophenone and *p*-coumaric acid just like KU12 (Table 3). These results strongly suggest that the molecule that senses phenolic compounds is encoded by the Ti plasmid. However, levels of *vir* gene expression in strains KU9412 and KU12 were consistently ≈2-fold lower than that of strains A348 and KU911, respectively. These results suggest that a factor coded by the C58 chromosome activates *vir* gene induction by phenolic compounds 2-fold. What the nature of this factor is and how it functions is unknown. One possible candidate is the product of the *chvE* gene, which might differ in the two chromosomal backgrounds.

Effect of *virA* and/or *virG* on *vir* Gene Induction by Phenolic Compounds. The most likely gene on the Ti plasmid that senses phenolic compounds is *virA*. To confirm the *virA* and *virG* effect on *vir* gene induction by phenolic compounds, we introduced pGP159 containing *virA*, *virG*, and *virB*::*lacZ* of pTiA6 into A136, KU12C3, KU911, and A348. The *vir* genes of *A. tumefaciens* strain A136(pGP159), KU12C3(pGP159), and A348(pGP159) were induced by acetosyringone and sinapinic acid but were not induced by 4-hydroxyacetophenone and *p*-coumaric acid. However, *vir* genes of KU911(pGP159) were induced by acetosyringone, sinapinic acid, 4-hydroxyacetophenone, and *p*-coumaric acid, although the induction levels were only about one-half the levels when plasmids were present singly in a strain (Table 4). This result is probably explained by the fact that the VirA protein functions as a dimer, and the VirA hybrid of strains A6 and KU12 is not functional (31). Such results show that only *virA* and *virG* are necessary for *vir* gene induction by phenolic compounds and that *virA* and *virG* determine which phenolic compounds can function as *vir* gene inducers. The β-galactosidase activity in strain A136(pGP159) is ≈2-fold higher than that in strain KU12C3(pGP159), similar to strains A348 and KU9412, again demonstrating that *vir* gene induction in the C58 chromosomal background is ≈2-fold higher than in the background of the Korean strain.

To determine whether the phenol-sensing function resulted from differences in *virA* or *virG*, we introduced each of these genes separately into several different strains. We introduced pSG9401 containing *virA* of pTiA6, pYW9503 containing *virA* of pTiKU12, pSG9403 containing *virG* of pTiA6, or pKUBF1501 containing *virG* of pTiKU12 into *A. tumefaciens* strains A136, KU911, and A348, respectively. β-Galactosidase activities of *A. tumefaciens* strains induced by acetosyringone, sinapinic acid, 4-hydroxyacetophenone, and *p*-coumaric acid are presented in Table 5. Because strain A136 containing pSG9401 does not have *virG*, A136(pSG9401) was not induced by phenolic compounds. Strain KU911(pSG9401) has two types of *virA* genes, one derived from pTiA6 and the other from pTiKU12. The *vir* genes of this strain were induced by acetosyringone, sinapinic acid, 4-hydroxyacetophenone, and *p*-coumaric acid. However, for A348(pSG9401), which contains only the *virA* gene from pTiA6, neither 4-hydroxyacetophenone nor *p*-coumaric acid could induce *vir* genes. Because strain KU911(pYW9503) has only the *virA* gene from pTiKU12, *vir* genes in this strain were not induced by acetosyringone and sinapinic acid. In contrast, the *vir* genes of strain A348(pYW9503), which contains *virA* genes derived from both pTiA6 and pTiKU12, were induced by acetosyringone, sinapinic acid, 4-hydroxyacetophenone, and

Table 3. Induction of *virB*::*lacZ* in *A. tumefaciens* strains with isogenic chromosomal background

Strain	Chromosomal background	Ti plasmid	β-Galactosidase activity, units				
			None	AS	SP	HAP	CA
A348(pTiA6, pSM243cd)	C58	pTiA6	6.3	1287	1227	5.7	5.8
KU9412(pTiA6, pSM243cd)	KU12	pTiA6	4.7	716	646	3.4	3.2
KU12(pTiKU12, pSM243cd)	KU12	pTiKU12	2.9	3.2	5.6	505	493
KU911(pTiKU12, pSM243cd)	C58	pTiKU12	4.1	3.6	5.4	925	772

AS, acetosyringone; SP, sinapinic acid; HAP, 4-hydroxyacetophenone; and CA, *p*-coumaric acid.

Table 4. Effect of *virA* and *virG* on induction of *vir* genes of *A. tumefaciens* strains by phenolic compounds

Strain	β-Galactosidase activity, units				
	None	AS	SP	HAP	CA
A136(pGP159)*	3.8	1095	1045	6.6	6.5
KU12C3(pGP159)	8.3	564	456	10.0	9.5
KU911(pGP159)	11.8	619	436	205	154
A348(pGP159)	9.7	1106	912	8.9	10.1

*pGP159 contains *virA*, *virG*, and *virB::lacZ* of pTiA6. AS, acetosyringone; SP, sinapinic acid; HAP, 4-hydroxyacetophenone; and CA, *p*-coumaric acid.

p-coumaric acid. As reported (32), the *virG* of pTiA6 and *virG* of pTiKU12 had little influence on *vir* gene induction by phenolic compounds.

Further Evidence That VirA Protein Is the Phenolic-Sensing Molecule. Although the *vir* genes of strains A6 and Bo542 were induced by two members of group A, sinapinic acid and 2,6-dimethoxyphenol, the *vir* genes of strain C58 were not induced by these same two members of group A (Table 1). To confirm that these differences in strains A6 and C58 were due to differences in their *virA* genes, we introduced pTB108, containing the *virA* gene of pTiA6, or pJD101W, containing the *virA* gene of pTiC58, into *A. tumefaciens* strain C58ΔvirA, which contains pTiC58 deleted of *virA* or At11068, which contains pTiA6 deleted of *virA*, respectively. Strains transformed by pJD101W containing the *virA* gene of pTiC58 were not induced by sinapinic acid and 2,6-dimethoxyphenol, the same as found for wild-type C58 (Table 6). However, both C58virA and At11068 strains transformed by pTB108 were induced by sinapinic acid and 2,6-dimethoxyphenol. These data support our previous observations that differences in induction by phenolic compounds track with the *virA* gene.

DISCUSSION

The results reported in this paper provide the best genetic evidence available on the question of whether the phenolic-inducing compounds are recognized directly by the VirA protein or whether they first bind to a protein that then interacts with VirA. This latter model is similar to the situation for the inducing activity of monosaccharides. In this case the monosaccharides first interact with the glucose-galactose binding protein, which in turn interacts with the periplasmic domain of the VirA protein. Whereas the importance of the monosaccharides in *vir* gene induction was recognized after the isolation of a mutant defective in the glucose-galactose binding protein, no noninducible mutants have been isolated that lack the ability to bind acetosyringone, although numerous attempts have been made to isolate such mutants (S.J., unpublished data). The present genetic data suggest that our inability to isolate such mutants can be explained by the hypothesis that such proteins are not required for the induction of *vir* genes. If such binding proteins exist, there must be different ones for those phenolic compounds, such as acetosyringone and 4-hydroxyacetophenone, that differ in their inducing activity for strains KU12 and C58. These different phenolic-binding proteins must be present in strain KU12, as well as strain C58, even though the strain is not induced by that phenolic compound. We have looked for other functions of these inducing compounds in the physiology of the cell, such as serving as a carbon and energy source or a chemoattractant (or repellent). Such activities could very well require a binding protein. None of the phenolic compounds that induce *vir* gene expression in these studies could serve as a carbon source or chemoattractant for any of the four strains of *Agrobacterium* (Y.-W.L., unpublished observations). Thus, why a strain should synthesize proteins that have no obvious function is not clear. This result contrasts markedly with the situation for the glucose-galactose binding protein, which plays a role in chemoattraction, intracellular transport, as well as *vir* gene induction.

In all studied cases, the inducing activity of a particular phenolic compound is directly related to the *virA* gene in the strain. Because these *vir* gene inducers have no apparent function in the physiology of *Agrobacterium*, except as inducers of *vir* genes, it seems likely that the differences in the VirA protein in the various strains relate to differences in their ability to interact with the phenolic compound directly rather than with a protein that has bound the phenolic compound. A similar conclusion has been reached for *nod* gene induction in *Rhizobium*, also based on genetic data (9, 10).

These studies compare a large number of different phenolic compounds in their ability to induce *vir* genes of several unrelated strains of *Agrobacterium*. Previous studies reported testing of one or two strains as the assay organism to identify compounds that induce *vir* genes. Stachel *et al.* (30) used the octopine-utilizing strain A348 to identify acetosyringone and hydroxyacetosyringone as natural inducing compounds in cultured tobacco cells. Messens *et al.* (33) isolated ethyl ferulate as the major inducing compound in wheat, again using strain A348 for assay. Obviously quite different answers would have been obtained had strain KU12, which is not induced by acetosyringone, been used to identify inducing compounds from tobacco and had strain C58, which is not induced by ferulic acid, been used as the assay organism for identification of inducing compounds from wheat. Because strain C58 can transfer T-DNA into wheat as measured by agroinfection (34), there must be other inducing compounds not yet identified in this plant.

Table 5. Effect of *virA* or *virG* on induction of *vir* genes of *A. tumefaciens* strains by phenolic compounds

Strain*	Complementing *vir* gene	β-Galactosidase activity, units				
		None	AS	SP	HAP	CA
A136(pSG9401)	*virA* of pTiA6	8.2	8.7	12.0	8.4	7.7
KU911(pSG9401)	*virA* of pTiA6	7.8	501	430	103	70
A348(pSG9401)	*virA* of pTiA6	11.0	1424	1208	11.6	13.2
KU911(pYW9503)	*virA* of pTiKU12	10.5	20.4	8.1	650	592
A348(pYW9503)	*virA* of pTiKU12	5.3	689	387	722	668
A136(pSG9403)	*virG* of pTiA6	4.8	6.6	6.1	5.7	3.6
KU911(pSG9403)	*virG* of pTiA6	2.5	3.9	6.9	392	209
A348(pSG9403)	*virG* of pTiA6	4.6	1097	831	5.9	9.1
KU911(pKUBF1501)	*virG* of pTiKU12	13.3	22.7	11.1	622	536
A348(pKUBF1501)	*virG* of pTiKU12	3.1	656	279	4.5	4.0

*All strains contained pSM243cd. AS, acetosyringone; SP, sinapinic acid; HAP, 4-hydroxyacetophenone; and CA, *p*-coumaric acid.

Table 6. Effect of *virA* on induction of *vir* genes of *A. tumefaciens* strains

Strain*	Chromosome/Ti plasmid/origin of *virA*	β-Galactosidase activity, units				
		None	AS	SP	SA	DP
C58ΔvirA(pUCD2)	C58/pTiC58(Δ*virA*)/none	2.4	2.0	6.0	2.9	2.5
C58ΔvirA(pTB108)	C58/pTiC58(Δ*virA*)/pTiA6	4.7	306	284	185	240
C58ΔvirA(pJD101W)	C58/pTiC58(Δ*virA*)/pTiC58	2.9	247	147	6.7	39.0
At11068(pUCD2)	C58/pTiA6(Δ*virA*)/none	2.5	2.6	6.6	4.2	2.8
At11068(pTB108)	C58/pTiA6(Δ*virA*)/pTiA6	3.4	925	710	260	502
At11068(pJD101W)	C58/pTiA6(Δ*virA*)/pTiC58	2.8	787	581	4.0	22.0

*All strains contained pIB50. AS, acetosyringone; SP, sinapinic acid; SA, syringic acid; DP, 2,6-dimethoxyphenol.

Inducing compounds were identified in grapevine plants, using a limited host-range *Agrobacterium* strain as the assay organism (35). This strain was induced by syringic acid methyl ester and by acetosyringone but only at very high concentrations. The question arises as to whether the acetosyringone and syringic acid methyl ester concentrations reach high enough levels in wounded plants that infected by this *Agrobacterium* strain to allow plant infection. The present studies raise the possibility that another inducer(s), as yet unidentified, is present in these plants.

Although the phenolic compounds chosen for study have not all been isolated from plants, many of the most active inducers have been identified as naturally occurring in plants, often as a component of the phenolic cell-wall-polymer lignin or its precursors. The cell walls of all plants contain various amounts of lignin, which is synthesized by the oxidative coupling of three major phenylpropanoid units, *p*-coumaryl alcohol, coniferyl alcohol, and syringyl alcohol. Ferulic acid is an important constituent of lignin in the monocots. Our study shows that each of these compounds or a close derivative is a *vir* gene inducer in one or more strains tested. Coniferyl alcohol is a strong inducer of all strains tested here (ref. 36; Y.-W.L., unpublished observations). Considering the range of naturally occurring compounds that induce and the ubiquity of lignin in all plants, it is not surprising that two strains that respond to different signal molecules are nevertheless virulent on the same plants and that all four strains can infect a broad range of plants. Interestingly, strain KU12 has been reported to have a broader host range than strain A348 (37).

The structural requirements for *vir* gene inducers have been expanded with these studies on strain KU12. Previously, several groups noted that one or two methoxy groups in the ortho position are required, and a wide variety of substituents are permitted in the position para to the hydroxyl group of the phenol (29, 38). However, the *vir* genes of strain KU12 are induced by several compounds, including 4-hydroxyacetophenone, *p*-coumaric acid, and phenol, that lack any methoxy groups. In agreement with previous studies, none of the common laboratory strains, such as C58, A6, and Bo542, were induced by these compounds.

One obvious question raised by these studies is whether or not the *vir* gene-induction properties of the common laboratory strains A6 and C58 can be altered, so that these strains gain the ability to recognize new inducers and perhaps lose the ability to be induced by compounds that previously served as inducers.

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