Effects of phenolic compounds on Agrobacterium vir genes and gene transfer induction—a plausible molecular mechanism of phenol binding protein activation

Philippe Joubert a, Daniel Beaupère b, Philippe Lelièvre b, Anne Wadouachi b, Rajbir S. Sangwan a,*, Brigitte S. Sangwan-Norreel a

a UPRES EA 2084, Laboratoire Androgénèse et Biotechnologie, Faculté des Sciences, Université de Picardie Jules Verne, 33 rue Saint-Leu, 80039 Amiens Cedex, France
b Laboratoire de Chimie Organique, Université de Picardie Jules Verne, 33 rue Saint-Leu, 80039 Amiens Cedex, France

Received 31 October 2001; received in revised form 8 January 2002; accepted 8 January 2002

Abstract

We have studied the effects of nine new phenolic compounds: 4-(4-hydroxy-3-methoxyphenyl)-but-3-en-2-one 4, 4-(4-hydroxy-3-methoxyphenyl)-but-3-en-2-one 5, 4-hydroxy-3,5-dimethoxycinnamyl alcohol or sinapyl alcohol 6, 4-hydroxy-3,5-dimethoxy-N,N-dimethylcinnamamide 7, N-ethyl-4-hydroxy-3,5-dimethoxycinnamamide 8, 1-(4-hydroxy-3,5-dimethoxyphenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-prop-2-en-1-one 11, 1-(4-hydroxyphenyl)-3-(4-hydroxyphenyl)-prop-2-en-1-one 12, 4-(4-hydroxy-3,5-dimethoxyphenyl)-butan-2-one 13, and 1-(4-hydroxy-3,5-dimethoxyphenyl)-3-(4-hydroxyphenyl)-propan-1-one 14, on Agrobacterium virulence gene induction, on Agrobacterium-mediated gene transfer, and on both transient and stable transformation rates on Petunia and tobacco. We confirmed that virulence induction and transformation rates are increased by the use of phenolic vir inducers bearing an unsaturated lateral chain. However, we found that the presence of at least one ortho-methoxy group in the phenolic compounds is required to increase the vir gene induction. Moreover, we synthesized phenolic compounds 13 and 14 with a saturated lateral chain from the corresponding compounds 4-(4-hydroxy-3,5-dimethoxyphenyl)-but-3-en-2-one 2 and 1-(4-hydroxy-3,5-dimethoxyphenyl)-3-(4-hydroxyphenyl)-prop-2-en-1-one 9, in order to stop conjugation between hydroxyl and carbonyl groups in these molecules. These compounds showed a lower efficiency of both vir induction and gene transfer. Furthermore, we also synthesized two amides derivatives 7 and 8 from sinapinic acid. The nature of alkyl groups on nitrogen is essential to the vir induction and gene transfer, and we observed that N-dimethylamide compound 7 is less active than N-ethyl compound 8. This may be due to the difference of the electron-donating effect between methyl and ethyl groups. We present a model for the molecular mechanism of VirA activation by phenols. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Petunia (Petunia hybrida); Tobacco (Nicotiana tabacum); Agrobacterium tumefaciens; Vir gene induction; Transformation induction; VirA; Phenolic compounds

1. Introduction

Among the different techniques used for the introduction of foreign genes in plants, Agrobacterium tumefaciens-mediated transfer remains the most popular and a powerful one [1–4]. It is accepted that Agrobacterium-mediated transfer requires the activation of two Agrobacterium gene families: chv and vir genes. The
hydroxy-AS [8,9], are chemoattractants at very low concentrations, but become \textit{vir} inducers at high concentrations [10–13]. A variety of other phenols have been described and their \textit{vir} gene induction abilities investigated [14–16]. Furthermore, other families of phenolic compound such as hydroxycinnamides are known to act as \textit{vir} gene inducers [17]. It has also been reported that monosaccharides such as D-glucose, D-galactose and D-xylose, which are ‘long distance’ attractors of Agrobacteria, work in synergy with phenolic compounds in order to increase \textit{vir} gene induction [18,19] and enlarge the recognition profiles of sensitive Agrobacteria strains to phenolic compounds [20]. Furthermore, opines [21] and flavonoid compounds [22] may be involved in \textit{vir} gene induction.

It was demonstrated [23] that if the two basic elements required for phenol-mediated induction of virulence gene expression, VirA and VirG are encoded on the Ti plasmid, they are dependent on the chromosomal background for even the very first stage for signal perception.

In the \textit{vir} gene induction process, the first stage is the recognition of phenols by a transmembrane VirA protein encoded by the \textit{virA} gene [24–26]. The recognition and autophosphorylation of VirA [27] leads to the phosphorylation of the VirG cytoplasmic protein [28] which, in turn, induces the expression of other \textit{vir} genes. Moreover, a direct molecular mechanism which may explain the action of AS on VirA was put forward [16,29,30]. It was suggested that an acidic VirA residue may protonate the carbonyl group of phenolic compounds. The electron resonance delocalization leads to an electron transfer from the phenol to the acidic residue. Then the phenol can protonate a VirA basic residue, leading to the autophosphorylation of VirA and then to the phosphorylation of VirG.

It was suggested [31] that the VirA protein may not be directly involved in the bonding of phenolic compounds. Indeed, two chromosome-encoded proteins (p10 and p21) were discovered which could act as receptors of phenols and mediate interaction between phenolics and VirA. Turk et al. [32] demonstrated that the periplasmic domain of VirA does not react with AS, whereas its cytoplasmic domain (aa283–aa304) is able to sense the signal whenever AS is present in the medium. Recently, Dyé and Delmotte [33] discovered another \textit{Agrobacterium}-phenol binding protein, Pbp39, but whether this new protein is a \textit{vir} gene inducer has not yet been determined.

In order to understand the molecular interactions between phenolic compounds and the VirA complex protein or any other receptor proteins such as p10 and p21, we have developed new phenolic compounds and tested their effects on \textit{vir} induction. Furthermore, these new phenols were tested on tobacco and \textit{Petunia} transformation and, as the results compared favourably with their \textit{vir} induction, this has enabled us to suggest a mechanism for possible molecular interactions between phenolics and VirA complex protein site.

2. Material and methods

2.1. Chemical structures, synthesis and description

The structures and details of different chemical functions used are reported on Fig. 1 and Table 1. Simple phenolic compounds 1, 3 and 6 were purchased from Aldrich (Strasbourg, France). Typical experimental procedures for synthesis of all other derivatives.

2.1.1. General procedure for synthesis of benzalacetones (2, 4, 5) and chalcones (9–12)

An ether solution of boron trifluoride (1 eq.) and ketone (R–CO–CH3: 2 eq.) were added successively dropwise to a solution of benzaldehyde derivative (HO,R1,R2-PhCHO: 8.2 × 10⁻³ mol) in CH₂Cl₂ (10 ml). The resulting solution was stirred for 24 h at room temperature, then neutralized with NaOAc and extracted with CH₂Cl₂. The combined extracts were washed with water, dried over Na₂SO₄ and filtered. The organic layers were concentrated under reduced pressure and the crude product purified by column chromatography on silica gel (n-hexane-ethyl acetate) for synthesis of \(\alpha,\beta\)-ethylenic ketones were obtained in 60–75% yield. The same aldolisation procedure was applied to acetophenone derivative (HO,R1,R2–PhCOCH₃) for synthesis of \(\alpha,\beta\)-ethylenic ketone 9.

2.1.2. General procedure for ketones 13 and 14

They were obtained by the hydrogenation of \(\alpha,\beta\)-ethylenic ketones 2 and 9 [34]. In this procedure, a toluene solution of unsaturated compound 2 and 9 (0.25 mol⁻¹) and 1-phenylethanol (0.25 mol⁻¹) and hydridotetrazirakis (tris tri-phenylphosphine) rhodium I [35] (RhH(PPh₃)₉; 4.10⁻² eq.) was heated at 50 °C for 2 h. After solvent evaporation, the purification by chromatography on silica gel led to products 13 and 14 in 92 and 96% yield respectively.

2.1.3. General procedure for amides 7 and 8

\(N,N’\)-dicyclohexylcarbodiimide (1.09 eq.) was added to a suspension of 4-hydroxy-3,5-dimethoxycinnamic acid in dry CH₂Cl₂ under N₂ atmosphere. The resulting suspension was stirred for 30 min at room temperature. Then a solution of amine (dimethylamine \(\rightarrow 7\) or ethylamine \(\rightarrow 8\) 2M in THF (1.2 eq.) was added dropwise. The mixture was stirred at room temperature for 12 h. The dicyclohexylurea (DCU) was filtered off, washed with CH₂Cl₂ and the filtrate was concentrated under reduced pressure. The purification of the residue by flash chromatography on silica gel (EtOAc, EtOAc–
EtOH 14:1) led to compounds 7 or 8 as yellow solids in 80 and 82% yield respectively.

2.1.4. Physical data
The physical characteristics of all known compounds were in perfect accordance with the literature values. All unknown compounds were characterised by elementary analysis and NMR Spectroscopy.

2.2. Plant material
Leaves of Petunia hybrida and Nicotiana tabacum were cultured on a basal medium (MS) that contained salts and vitamins of Murashige and Skoog [36], 3% (w/v) sucrose and 0.8% Difco agar, pH adjusted to 5.6 before autoclaving (120 °C for 20 min). The cultures were maintained in a culture room at 27 °C under a light regime of fluorescent light (≈ 32 E m⁻² s⁻¹) 16 h a day.

2.3. Bacterial strains
A. tumefaciens A348 pSM219 virH::*lacZ (pinF::*lacZ) was used to evaluate vir gene induction. It was kindly supplied by B. Hohn (FMI, Basel, Switzerland). A. tumefaciens EHA105 (pG3.3) carrying a binary vector with NPTII and GUS genes was kindly provided by S. Hamon-Poirier (CNRS Gif/Yvette, France). Bacteria

Table 1
Description of various phenolic compounds tested

<table>
<thead>
<tr>
<th>No.</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Usual name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>COCH₃</td>
<td>Acetosyringone (AS)</td>
</tr>
<tr>
<td>2</td>
<td>OCH₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>OCH₃</td>
<td></td>
<td></td>
<td>Sinapinic acid</td>
</tr>
<tr>
<td>4</td>
<td>OCH₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>OCH₃</td>
<td></td>
<td></td>
<td>Sinapyl alcohol</td>
</tr>
<tr>
<td>7</td>
<td>OCH₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>OCH₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>OCH₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>OCH₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Benzalacetones derived

Chalcones derived

<table>
<thead>
<tr>
<th>No.</th>
<th>R₁</th>
<th>R₂</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| 13  | Same 10 with reduced ethylenic bond
| 14  | Same 12 with reduced ethylenic bond

Showing the substitutions at R₁, R₂, R₃, R₅ and R₇. Compounds 1, 2, 3, 9 and 10: Joubert et al. [40], and this work. Compounds 4, 5, 6, 7, 8, 11, 12, 13 and 14: this work.
colonies were picked from selection plates (Luria Broth medium + carbenicillin 100 mg/l (A348) or kanamycin 100 mg/l (EHA105) and grown overnight in Luria Broth liquid medium [37] with the appropriate antibiotics added, at 28 °C, in a rotary shaker (210 rpm).

2.4. Determination of vir gene expression

To test vir gene induction, an overnight colony of Agrobacterium A348 (pSM219) virH::lacZ was centrifuged and suspended in 15 ml of mannitol-phosphate buffer (0.6 M mannitol 0.01 M NaPi, pH 5.2). A 100 µl aliquot of this bacterial suspension was added to a 900 µl MSPS liquid medium (MS medium supplemented with 62.5 mM NaPi and 3% sucrose, pH 5.25), to which the phenolic compound to be tested was also added, and grown for 18 h at 28 °C. The expression of vir genes was monitored through the measurement of β-galactosidase activity of the fusion gene. Bacterial cells were harvested and β-gal activity [37] determined as described by Sambrook et al. [38].

2.5. Inoculation of explants

Young leaves were collected from 2-months-old tobacco and petunia plants. Leaf pieces (1 cm²) were dipped into a suspension EHA105 strain (1 ml overnight culture of bacteria +9 ml of MS medium) for 20 min, blotted dry on sterile paper, and incubated on MS basal medium with the appropriate phenolic compound for 3 days. Cocultured explants were washed in liquid MS medium in the presence of 500 mg/l cefotaxim and blotted dry on sterile paper. They were then plated on MS medium containing 300 mg/l cefotaxim, 250 mg/l kanamycin and: (i) 4.44 µM BAP + 0.57 µM IAA for tobacco plants; and (ii) 4.44 µM BAP + 2.68 µM NAA for petunia plants, for direct selection and regeneration of transformed callus and buds.

2.6. Transient and stable transformation results

Transient transformation was assessed 3 days after coculture. Leaf explants were ground and extracts used to measure β-glucuronidase activity, as described by Jefferson [39]. Four or five weeks after coculture, regenerated transformed (kanamycin resistant) callus and buds of tobacco and petunia, respectively, were counted. The average number of regenerates per explant was then established. Forty five explants were cocultured per series and all experiments repeated three times.

2.7. Statistical studies

All experiments were controlled through statistical analysis carried out with the STAT-ITCF computer program provided by the Institut Technique des Céréal et des Fourrages (Paris, France). In each case, we proceeded to a variance analysis. This test gave us probable differences between phenolic treatments. Additional investigations with a Newman–Kuels test classified these treatments in decreasing order with a 95% safety margin.

3. Results

3.1. Action of benzalacetones and chalcones on vir gene expression and transformation rates

Benzalacetone derivatives 2, 4 and 5, which differ by the number of methoxy groups, have been tested (Table 1). As reported in our previous work [40], we noted (Fig. 2) that a compound without methoxy groups, benzalacetone 5, does not induce vir functions, whereas the presence of one of them, in benzalacetone 4, leads to less active (2/5, 32) vir inducers. Similarly, the chalcone derivative 12, lacking methoxy group as compared to the structure of compound 11, does not induce vir genes, whereas 11 had an effect on the vir induction similar to AS effect.

However, the tetra-methoxylated compound 11 showed a significant decrease of vir induction ability compared to its analogue derivatives 9 and 10. We suggest that the presence of four methoxy groups increases the molecular bulk of the phenolic molecule and consequently reduce its affinity to the VirA receptor site.

In all cases, we noted that activity of 2, 9 and 10 on vir induction was very high and greater than AS. From the hypothesis of Hess et al. [16], the conjugated structures led to a delocalization of electrons by mesomeric effect on the whole molecule (Fig. 3). In comparison with AS, the additional double bond on R₃ alkyl chain should contribute to a better stabilisation of the mesomeric form, thereby allowing the phenolic proton to leave more easily and then activate phenol binding protein before speeding up the VirA.

Following tests of these compounds as vir inducers in Agrobacterium, we tested them for tobacco transformation efficiency. Most compounds displayed positive effects on transformation results (Fig. 4). Phenolics 2, 9 and 1 (AS) appeared to be the strongest vir inducers with the production of twice as many transformed callus than in control experiment (control: leaf explants cocultured without any phenolic) while compound 3, of a limited vir induction ability, did not enhance the transformation rate. These results confirm the importance of these compounds and their effects, on the virulence of Agrobacterium, and hence on T-DNA transfer.

We also checked the GUS gene expression by quantification of β-glucuronidase in order to evaluate
the relationship between vir gene induction, and transient and stable gene transfer. This experiment was aimed at linking vir induction results (Fig. 2) to stable transformation occurrences (Fig. 4) generated by the same molecules. Fig. 5 shows that the most efficient vir inducers molecules (2, 9, 1 (AS), 10 and 11) also result in a high gene transfer frequency in Petunia and tobacco. The gradient of gene transfer and stable transformation seems to follow vir gene induction. Nevertheless, we noted the particularly high level of gene transfer induced by compound 9 on Petunia. Conversely, we tested a non vir-inducing compound in order to find out whether it may also induce a low frequency of gene transfer on Petunia and tobacco. The molecule 5 tested showed a
total inability to induce gene transfer and, furthermore, a low inhibition of this transfer, a feature presumably related to the general toxicity of phenols.

3.2. Action of hydrogenated benzalacetones and chalcones on vir gene and transformation induction

In relation to the hypothesis of Hess et al. [16], we synthesized molecules 13 and 14 and tested them on vir gene induction. The double bonds of compounds 2 and 9 were treated under catalytic hydrogenation conditions to give products 13 and 14. According to the above mentioned hypothesis, the electron transfer between the carbonyl and phenolic groups of molecules 13 and 14 was not possible. The acidity of the hydroxyl group was only enhanced by attractive effect of the phenyl group (Fig. 6). As regards vir gene induction, compounds 13 and 14 showed a limited efficiency compared to compounds 2 and 9, respectively (Fig. 7). Such reduced activities accounted for 1/2 and 1/3 of that of the original products, but remained much higher than the activities observed in the control experiment.
We tested these four molecules 2, 13, 9 and 14 in the presence of Agrobacteria throughout cocultivation with explants of Tobacco and Petunia. After a few weeks on selection medium, we calculated that explants cocultivated with 13 and 14 showed a significant decrease in the regenerated callus number. Therefore, compound 14 did not enhance the transformation induction. However, explants cocultivated with 13 maintained a higher level of transformation than the control.

From both the molecular mechanism suggested by Hess et al. [16] and the above results, we believe that the following hypothesis is well-founded. On the one hand, a non-conjugated lateral chain R₃ might explain the relative decrease in the vir and transformation induction (Fig. 7 and Fig. 8). On the other hand, the hydrogenation of this chain changes the initial planar structure of the molecule and induces, in comparison with 2 and 9, a new freedom of movement for R₃. Therefore, the

![Fig. 7. Action of hydrogenated compounds—13 and 14—on virH gene induction. Compounds were tested at 100 µM. Values are average (n = 9) ± s.e. Probability showing differences between media was equal to 1.00 and Newman–Kuels test classified medium effects in five classes noted A to E.](image)

![Fig. 8. Influence of phenolics 13 and 14 on Petunia and Tobacco transformation. The number of callus regenerated per leaf explant after 3 weeks' culture on selection medium for Tobacco and 4 weeks for Petunia is represented. Explants had been cocultured with bacteria for 3 days in the presence of 100 µM tested phenolic compound. The control was a cocultured medium without phenolic. Values are average (n = 6) ± s.e. Probability showing differences between media was > 0.95 and Newman–Kuels test classified phenolic effect in three homogeneous groups noted A, B and C.](image)
probability of simultaneous recognition of both the chemical phenol and carbonyl functions in the VirA site decreased.

3.3. Action of amino compounds on vir and transformation induction

It was reported by Dyé et al. [41,42] that different syringamides are new vir inducers. These compounds appeared to have 2–20 times stronger action than the syringic acid, depending on the Agrobacterium lacZ fusion strains. We decided to test amides of the sinapinic acid as vir inducers. So, we synthesized two amide derivatives of 3: the 4-hydroxy-3,5-dimethoxy-N,N-dimethylcinnamamide 7 and the N-ethyl-4-hydroxy-3,5-dimethoxycinnamamide 8.

Fig. 9 shows the vir induction of these two products (7 and 8) compared with the analogous cinnamic derivatives: 2, 3 (sinapinic acid) and 6 (sinapyl alcohol) and with the two controls (negative control without any phenolic, and positive control with AS in the same concentration as all other phenolics, 100 μM). In both cases the induction ratios of 3 (sinapinic acid) and 6 (sinapyl alcohol) compared with 2 were equal to 0.6. Compound 8 had the same effect as compound 3 (90% of it). Compound 7 showed a lower vir induction activity: 43% that of compound 3 and 26% that of compound 2. The activity of the two amides did not reach that of 3 as expected, and as reported by Dyé et al. [41] for the ethyl amide of syringic acid. However, we noted that the replacement of the two methyl groups of the nitrogen atom (7) by only one ethyl chain (8) induced an increase in the vir gene induction (8/7 = 2).

The spatial size of the dimethyl groups on nitrogen (7) is higher than in the ethyl group (8). This may explain why it is so difficult for the carbonyl group to reach the VirA acidic receptor site.

We noted that compound 6 has a higher vir action while its R3 chain has an alcohol function and not a carbonyl function. This molecule could be oxidized by the oxygen in the medium and transformed into aldehyde, then acid, which are reliable vir inducers.

4. Discussion

Hess et al. [16] first suggested a hypothetical direct mechanism of VirA activation, when the phenolic molecule is placed in the receptor protein site VirA. Another hypothetical mechanism implies mediated-protein such as p10 and p21 or even the Pbp39 [31,33], which leads to an indirect mechanism of VirA activation. Then, transfer of phenolic proton to the protein basic residue and transfer to the phenol carbonyl group from the protein acidic residue proton, are possible through the conjugation of the molecules.

Here we confirmed that the induction of the Agrobacterium vir genes was essential for an effective gene

![Fig. 9. Action of nitrogenated compounds—7 and 8—on virH gene induction, compared to the corresponding benzalacetones. Compounds were tested at 100 μM. Values are average (n = 6) ± s.e. Probability showing differences between media was equal to 1.00 and Newman–Kuels test classified medium effects in five classes noted A, B, C, D and E.](chart)

Table 2
Comparison of phenolic pKₐ for some of the tested molecules on Agrobacterium virulence with their vir induction rates

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>3</th>
<th>1 (AS)</th>
<th>10</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol pKₐ</td>
<td>7.91</td>
<td>8.04</td>
<td>9.81</td>
<td>9.94</td>
</tr>
<tr>
<td>vir induction</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
</tbody>
</table>
transfer to the plants. Previously, we had shown that vir active molecules must have two methoxy groups in ortho position of phenolic function and one carbonyl group on the R1 chain [40].

The presence of strong active vir gene phenolic molecules such as AS1, benzalacetone 2 and chalcones 9, 10 and 11, increased the gene transfer in Tobacco and Petunia, and transformation rates 2–3 times higher than the control. Therefore, we confirmed that phenolic molecules are essential in the T-DNA transfer process. The presence of the double bonds in the phenolic compounds enhanced the molecular conjugation and enabled the electronic transfer from phenol to carbonyl and finally activated the protein receptor (Fig. 3).

According to the above hypothesis of Hess et al. [16], it is suggested that leaving-ability of the phenolic proton should be correlated with the vir induction power of molecules. In order to find out if the ability of ionisation of phenol function was the only induction factor on the VirA site, we calculated various pK_a of some tested molecules and compared them to their vir induction power. The values were predicted according to the method described by Perrin et al. [43]. We check this method experimentally by measurement and calculation pK_a of AS and 2. Tables 2 and 3 shows the pK_a and vir induction of these molecules. The less active compounds had the lowest pK_a, and the pK_a of molecules increased along with the vir induction power. Thus, it seems that effective induction of phenol binding (VirA) protein decreases with the phenol dissociation.

From the above suggested model (Fig. 3), a R radical can effect the induction power of phenols. This R radical might actively have the donor electronic effect, antagonist to the electronic flow from the phenol donor residue. Thus, we compared donor inductor and mesomeric effects of some R radicals with the vir induction powers of the corresponding molecules (Tables 2 and 3). For example, we noted that the compound 2, with a high vir induction, has a weak electron-donating group (–CH3) whereas the amide 7 with a high electron-donating effect (–N(CH3)2) has a low vir activity. Moreover, the literature showed [14] that three methyl esters of ferulic, sinapinic and syringic acids have a higher action on vir genes than the corresponding free acids, but in this case the acidity of the acid group was hidden by the esterification.

From the comparison of pK_a of phenol derivatives with the vir induction of some molecules (i), comparing the electron-donating effects with the vir induction (ii), and seeing that there is no possible conjugation between hydroxyl and carbonyl groups in compound 13 (iii), we believe that the model of phenol binding protein molecular activation proposed by Hess et al. [16] is highly significant. However, the physical distance between the phenol and carbonyl functions accounts for 20% with the two active compounds AS1 and 2 (5.9 and 7.5 Å respectively). In this case, we cannot easily imagine that there should be only one phenol receptor type. Recently an A. tumefaciens protein surface—Pbp39—which binds phenols was discovered [33]. We also know that the two proteins p10 and p21 have similar properties. Moreover, phenols of flavones class, such as kaempferol [22] showed good vir induction. These data suggest that VirA may be composed as a protein complex made of several phenol receptors, each of them specialized with one phenol class indirectly inducing VirA.

Finally, we report here that the three compounds with the most important conjugation fields (2, 9, and 10), also have the higher vir induction effect. Their great stability could result in an easier electron delocalization on the whole molecule, and therefore an easier (more efficient) VirA activation.

Acknowledgements

We thank B. Hohn and A. de Laat for generous gifts of A. tumefaciens strains; Y. Dessaux and S. Millam for critical reading and improving the English style of the manuscript.

References


