A mutation in negative regulator of basal resistance \textit{WRKY17} of \textit{Arabidopsis} increases susceptibility to \textit{Agrobacterium}-mediated genetic transformation [version 1; referees: 2 approved]

Benoît Lacroix, Vitaly Citovsky
Department of Biochemistry and Cell Biology, State University of New York, New York, 11794-5215, USA

Abstract

\textit{Agrobacterium} is a phytopathogenic bacterium that induces crown gall disease in many plant species by transferring and integrating a segment of its own DNA (T-DNA) into its host genome. Whereas \textit{Agrobacterium} usually does not trigger an extensive defense response in its host plants, it induces the expression of several defense-related genes and activates plant stress reactions. In the complex interplay between \textit{Agrobacterium} and its host plant, \textit{Agrobacterium} has evolved to take advantage of these plant defense pathways for its own purpose of advancement of the infection process. For example, \textit{Agrobacterium} utilizes the host stress response transcriptional regulator VIP1 to facilitate nuclear import and proteasomal uncoating of its T-DNA during genetic transformation of the host cell. In \textit{Arabidopsis}, the VIP1 gene expression is repressed by WRKY17, a negative regulator of basal resistance to \textit{Pseudomonas}. Thus, we examined whether WRKY17 is also involved in plant susceptibility to genetic transformation by \textit{Agrobacterium}. Using reverse genetics, we showed that a \textit{wrky17} mutant displays higher expression of the VIP1 gene in roots, but not in shoots. In a root infection assay, the \textit{wrky17} mutant plants were hyper-susceptible to \textit{Agrobacterium} compared to wild type plants. WRKY17, therefore, may act as a positive regulator of \textit{Arabidopsis} resistance to \textit{Agrobacterium}. This notion is important for understanding the complex regulation of \textit{Agrobacterium}-mediated genetic transformation; thus, although this paper reports a relatively small set of data that we do not plan to pursue further in our lab, we believe it might be useful for the broad community of plant pathologists and plant biotechnologists.
Introduction
The WRKY protein family is composed of at least 74 members in Arabidopsis thaliana; they act as transcriptional regulators and participate mainly in the control of gene expression involved in the plant stress response, and, particularly, in the induction of gene expression by pathogen-derived elicitors. Arabidopsis WRKY17, together with another family member WRKY11, is a negative regulator of the basal defense response\(^1\). The wrky17 and wrky11 genes are usually induced during the defense response, and Arabidopsis loss-of-function mutants wrky17 and wrky11 display higher expression of numerous stress- or defense-related genes and show increased resistance to infection by Pseudomonas, but not by other pathogens. Thus, wrky17 and wrky11 have been suggested to play a role in the fine-tuning of the defense response, avoiding the effect of excessive reaction\(^2\).

Among the target genes of wrky17/wrky11 is vip1, which is overexpressed in both wrky11 and wrky17 mutants\(^2\). VIP1 is a multifunctional bZIP transcription factor that stimulates stress- and defense-related gene expression by binding to a specific DNA hexamer motif present in many promoters that respond to activation of the MPK3 pathway\(^3\), including the PRI-pathogenesis-related gene\(^1\). VIP1 might also be involved in other stress-dependent regulation pathways, such as osmosensory signaling\(^4\). Interestingly, the VIP1-related defense responses are activated during Agrobacterium-host plant interactions, and Agrobacterium has evolved to subvert them to facilitate the infection process\(^5\).

VIP1, a host protein initially discovered as an interacting partner of the Agrobacterium T-DNA packaging protein VirE2\(^6\), is involved in several critical aspects of plant genetic transformation by Agrobacterium. Specifically, VIP1 is thought to facilitate nuclear import of the T-DNA-protein complexes\(^7\), their targeting to the host chromatin\(^8\), and proteasomal uncoating of the T-DNA molecule from its associated proteins prior to integration\(^9\). Thus, we investigated one of the VIP1-controlling WRKY mutants, wrky17, in regard to vip1 expression and the potential effects on Agrobacterium infection.

Results and discussion
VIP1 represents one of the target genes of WRKY17
A previous microarray analysis of the wrky17 mutant identified a number of upregulated genes\(^7\), one of which, VIP1, represents a major player in plant genetic transformation by Agrobacterium\(^10,11\). However, microarrays analyses of gene expression, although commonly used, often yield divergent data\(^12, 13\) and, therefore, require direct confirmation by detection of the specific transcripts. Thus, we analyzed the wrky17 mutant for the levels of VIP1 expression.

First, we examined three different lines of Arabidopsis plants derived from the wrky17-1 mutant\(^1\) for the presence of the WRKY17 transcript using RT-PCR. Figure 1A shows that whereas the wild-type plants produced WRKY17 mRNA, neither of the mutant lines accumulated detectable levels of this transcript. Next, we investigated the effect of the wrky17 mutation on the expression of the VIP1 gene. Using RT-PCR, we analyzed the levels of the VIP1 transcript in plant roots (Figure 1B) and shoots (Figure 1C). The VIP1 transcription activity was substantially higher in the roots of all three wrky17 mutants than in those of wild-type plants (Figure 1B). Unexpectedly, we detected no changes in VIP1 expression in the shoots of the same plants, which accumulated VIP1 transcripts in amounts similar to those in the wild-type plants (Figure 1C). Analysis of ACTIN2-specific transcripts detected similar amounts of PCR products in all samples, indicating equal efficiencies of the RT-PCR reactions (Figure 1B, C). Collectively, these data suggest that WRKY17 represents one of the transcriptional regulators of the VIP1 gene, but that this regulation is tissue-specific.

This is consistent with the previous observations of differential regulation of VIP1 expression during plant development as well as in response to various stimuli. For example, VIP1 transcription is activated upon induction of cell division\(^1\), after osmotic stress, and is differentially expressed in different tissues of Arabidopsis\(^1\). WRKY17 functions as a transcription inhibitor of several genes involved in plant defense pathways\(^1\). Our results suggest that VIP1 is one of the target genes down-regulated, directly or indirectly, by WRKY17 in tissue-specific fashion. Alternatively, VIP1 expression in the shoot tissue could be regulated by additional factors which mask the effect of the WRKY17 knock-out mutation.

Figure 1. RT-PCR analysis of ACTIN2, WRKY17 and VIP1 gene expression in wild-type and wrky17 mutant Arabidopsis plants. (A) WRKY17 expression in whole plants. (B, C) VIP1 expression in roots and shoots, respectively. WT, wild-type plants; 7, 12, and 13 are the three different lines of the homozygous wrky17-1 mutant.
The \textit{wrky17} mutant is hypersusceptible to \textit{Agrobacterium}-mediated genetic transformation

Once we had identified plant tissue showing a clear effect of WRKY17 on VIP1 expression, we investigated whether this effect altered susceptibility to \textit{Agrobacterium} infection. To this end, we employed the classical \textit{Arabidopsis} root infection assay\textsuperscript{19}, in which the efficiency of infection is monitored and quantified by measuring the level of transient T-DNA expression, that is early expression of the invading T-DNA molecules before their stable integration in the host genome. Root segments from the wild-type and \textit{wrky17} plants were inoculated with \textit{Agrobacterium} strain EHA105 harboring the binary plasmid pBISN1 with the β-glucuronidase (GUS) gene expression reporter in its T-DNA region. T-DNA expression was quantified based on the percentage of root segments exhibiting GUS histochemical staining. These experiments revealed that T-DNA expression frequencies in roots of all three \textit{wrky17} mutant lines were 30–50% higher than those measured in roots of the wild-type plants (Table 1 and Figure 2).

The increased susceptibility of the \textit{wrky17} roots to \textit{Agrobacterium} infection correlates with elevated transcription levels of the VIP1 gene in this tissue. Considering the known role of VIP1 as an enhancer of \textit{Agrobacterium} infectivity\textsuperscript{7–15}, it is likely that higher VIP1 expression in roots of the \textit{wrky17} mutant is responsible for the increased susceptibility to \textit{Agrobacterium}. This notion is consistent with our earlier observations that overexpression of VIP1 in tobacco further elevates transformation efficiency\textsuperscript{8}. That we detected this effect of the \textit{wrky17} mutation using a transient T-DNA expression assay indicates that increased VIP1 expression affects the early steps of the infection process, i.e., those that occur prior to T-DNA integration and stable expression.

Conclusion
We show here that the \textit{wrky17} mutant displays elevated VIP1 expression in its roots as well as increased susceptibility to \textit{Agrobacterium}-induced genetic transformation. This correlation allows a new insight into the interactions between \textit{Agrobacterium} and its host plants. Specifically, this interaction appears to be affected negatively by WRKY17 such that the infection process is enhanced in the loss-of-function \textit{wrky17} mutant. Thus, WRKY17 may represent one of the host factors that elevate resistance to \textit{Agrobacterium}\textsuperscript{20,21}. This is unlike the known role of WRKY17 as a negative regulator of plant resistance to \textit{Pseudomonas}\textsuperscript{5}. Although this paper reports a relatively small set of data that we do not plan to pursue further in our lab, we believe its publication will be useful for the broad community of plant pathologists and plant biotechnologists.

Materials and methods
Transgenic plants
\textit{Arabidopsis thaliana} plants, wild-type (ecotype Col0) or \textit{wrky17-1} T-DNA insertion mutants (obtained from D. Roby, CNRS Montpellier, France), were grown either in soil or on Gamborg’s B5 medium (20 g L\textsuperscript{-1} sucrose, 8 g L\textsuperscript{-1} agar), after seed surface sterilization. All plants were grown in an environment-controlled growth chamber at 22°C under long day (16h light/8h dark) conditions. Three lanes of homozygous plants (lanes 7, 12, and 13) were isolated from the original \textit{wrky17-1} stock.

![Figure 2. The effect of \textit{wrky17} mutation on susceptibility of \textit{Arabidopsis} roots to \textit{Agrobacterium} infection. Transformation efficiency is expressed as the percent of GUS-stained roots from the total number of roots tested. All data represent average values of three independent experiments with indicated standard deviations. WT, wild-type plants; 7, 12, and 13 are the three different lines of the homozygous \textit{wrky17-1} mutant.]

Table 1. Number of root segments staining positive for β-glucuronidase (GUS). Percentage (number of GUS positive root segments/total number of root segments).

<table>
<thead>
<tr>
<th>Line</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>43.1% (53/123)</td>
<td>44.7% (68/152)</td>
<td>42.7% (56/131)</td>
<td>43.5%</td>
</tr>
<tr>
<td>7</td>
<td>69.2% (72/104)</td>
<td>61.5% (88/143)</td>
<td>70.2% (80/114)</td>
<td>67.0%</td>
</tr>
<tr>
<td>12</td>
<td>59.5% (97/163)</td>
<td>63.5% (61/96)</td>
<td>63.8% (81/127)</td>
<td>62.3%</td>
</tr>
<tr>
<td>13</td>
<td>60.8% (79/130)</td>
<td>54.1% (72/133)</td>
<td>59.4% (60/101)</td>
<td>58.1%</td>
</tr>
</tbody>
</table>
RT-PCR
Total RNA was extracted from plant tissues using Trizol (Invitrogen), and cDNA synthesis was performed with a RevertAid cDNA synthesis Kit (Fermentas) according to the manufacturer’s instructions. Transcript levels were then estimated by PCR, with 30 cycles of amplification. The resulting cDNA was PCR-amplified for 30 cycles using primers specific for the tested gene or for ACTIN2 as an internal control of a constitutively expressed gene. The following primer pairs were used: 5′-ATGACCGTGTGATATATGCGTTTAC-3′ and 5′-TCAGCCTCTCTTGGTGAAATCC-3′ that amplify the full length 1,134 bp ACTIN2 (At3g18780) cDNA.

Root transformation assay
All infection assays were performed as described by Gelvin (2006)\(^5\) with the Agrobacterium tumefaciens strain EHA105 (from S. Gelvin, Purdue University, USA), harboring a pBISN1 binary plasmid containing 100 mg.L\(^{-1}\) of kanamycin, and bundles of root segments were placed on the MS (Murashige and Skoog) medium. For each experiment, roots were pooled from 3–4 week-old Arabidopsis plants grown on Gamborg’s B5 medium, and bundles of root segments were placed on the MS (Murashige and Skoog) medium. For each experiment, roots were pooled from more than 20 plants and divided into three bundles, each containing more than 100 root segments. Root bundles were overlaid with EHA105 harboring pBISN1 suspension culture at A\(_{600}\) = 0.25 in NaCl 0.9%, and excess liquid was removed by pipette aspiration after 15 min of incubation. Root segments were then incubated for two days at 22°C under the long day conditions, rinsed in water containing 100 mg.L\(^{-1}\) of timetine (BioWorld) to eliminate bacteria, and incubated for an additional three days on the MS medium supplemented with timetine. Root segments were then subjected to the GUS histochemical assay\(^6\), with overnight incubation at 37°C, and the number of root segments displaying GUS staining was recorded.

Author contributions
BL designed experiments, performed experiments, analyzed data and wrote manuscript; VC designed experiments, analyzed data and wrote manuscript. Both authors approved the final manuscript for publication.

Competing interests
No competing interests were disclosed.

Grant information
The work in our laboratory is supported by grants from United States Department of Agriculture/National Institute of Food and Agriculture (USDA/NIFA) 2008-01012, National Institutes of Health (NIH) R01 GM50224, National Science Foundation (NSF) MCB 1118491, United States-Israel Binational Science Foundation (BSF) 2011070 and United States-Israel Binational Agricultural Research and Development Fund (BARD) IS-4237-09C, to Vitaly Citovsky.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments
We thank Dr Dominique Roby (INRA, Toulouse, France) for the gift of Arabidopsis wky17 mutant seeds.

References
Published Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text | Free Full Text
PubMed Abstract | Publisher Full Text


Open Peer Review

Current Referee Status:  ✔  ✔

Version 1

Referee Report 08 February 2013
doi:10.5256/f1000research.852.r759

Kiran Mysore
Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA

This is a very short report regarding a finding that may be important for some researchers. Even though the science is acceptable several things need to be changed. Genetic transformation normally refers to stable transformation. The authors have only looked at transient transformation which may or may not be stable (they could have tested this). Therefore, please change the title and the abstract to indicate transient transformation rather than just saying genetic transformation.

All wild-type gene names should be in capital (e.g., WRKY17, WRKY 11, VIP1 etc.,). Only the mutants should be in small letters (e.g., wrky17).

The authors mention that the VIP1 gene is induced substantially in the wrky17 mutant. However, to my eyes the induction is subtle (probably 2-3 fold). They could have done a better quantification using real-time RT-PCR. Please remove the word “substantial”.

Please give the concentration of the Agrobacterium used for infection in CFU.

I believe the antibiotic used should be “timetin” and not “timentine”

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 07 February 2013
doi:10.5256/f1000research.852.r754

Herman Scholthof
Department of Plant Pathology and Microbiology, Bioenvironmental Sciences, The College of Agriculture and Life Sciences, Texas A&M University, Texas, USA
I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

**Competing Interests:** No competing interests were disclosed.