RESEARCH ARTICLE

A mutation in negative regulator of basal resistance _WRKY17_ of _Arabidopsis_ increases susceptibility to _Agrobacterium_-mediated genetic transformation [v1; ref status: indexed, http://f1000r.es/no]

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

Abstract

_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 _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 _Agrobacterium_ and its host plant, _Agrobacterium_ has evolved to take advantage of these plant defense pathways for its own purpose of advancement of the infection process. For example, _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 _Arabidopsis_, the VIP1 gene expression is repressed by WRKY17, a negative regulator of basal resistance to _Pseudomonas_. Thus, we examined whether WRKY17 is also involved in plant susceptibility to genetic transformation by _Agrobacterium_. Using reverse genetics, we showed that a _wrky17_ mutant displays higher expression of the VIP1 gene in roots, but not in shoots. In a root infection assay, the _wrky17_ mutant plants were hyper-susceptible to _Agrobacterium_ compared to wild type plants. WRKY17, therefore, may act as a positive regulator of _Arabidopsis_ resistance to _Agrobacterium_. This notion is important for understanding the complex regulation of _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.
Corresponding author: Benoît Lacroix (benoit.lacroix@stonybrook.edu)

How to cite this article: Lacroix B and Citovsky V. A mutation in negative regulator of basal resistance WRKY17 of Arabidopsis increases susceptibility to Agrobacterium-mediated genetic transformation [v1; ref status: indexed, http://f1000r.es/no] F1000Research 2013, 2:33 (doi: 10.12688/f1000research.2-33.v1)

Copyright: © 2013 Lacroix B and Citovsky V. This is an open access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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.

Competing interests: No competing interests were disclosed.

First published: 06 Feb 2013, 2:33 (doi: 10.12688/f1000research.2-33.v1)
First indexed: 08 Feb 2013, 2:33 (doi: 10.12688/f1000research.2-33.v1)
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. 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.

Among the target genes of wrky17/wrky11 is vip1, which is overexpressed in both wrky11 and wrky17 mutants. 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, including the PRI pathogenesis-related gene. VIP1 might also be involved in other stress-dependent regulation pathways, such as osmosensory signaling. 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.

VIP1, a host protein initially discovered as an interacting partner of the Agrobacterium T-DNA packaging protein VirE2, 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, their targeting to the host chromatin, and proteasomal uncoating of the T-DNA molecule from its associated proteins prior to integration. 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, one of which, VIP1, represents a major player in plant genetic transformation by Agrobacterium. However, microarray analyses of gene expression, although commonly used, often yield divergent data 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 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, after osmotic stress, and is differentially expressed in different tissues of Arabidopsis. WRKY17 functions as a transcription inhibitor of several genes involved in plant defense pathways. 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.
The wrky17 mutant is hypersusceptible to 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 Agrobacterium infection. To this end, we employed the classical 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 wrky17 plants were inoculated with 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 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 wrky17 roots to Agrobacterium infection correlates with elevated transcription levels of the VIP1 gene in this tissue. Considering the known role of VIP1 as an enhancer of Agrobacterium infectivity\textsuperscript{7–15}, it is likely that higher VIP1 expression in roots of the wrky17 mutant is responsible for the increased susceptibility to 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 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 wrky17 mutant displays elevated VIP1 expression in its roots as well as increased susceptibility to Agrobacterium-induced genetic transformation. This correlation allows a new insight into the interactions between 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 wrky17 mutant. Thus, WRKY17 may represent one of the host factors that elevate resistance to Agrobacterium\textsuperscript{20,21}. This is unlike the known role of WRKY17 as a negative regulator of plant resistance to Pseudomonas\textsuperscript{8}. 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**

Arabidopsis thaliana plants, wild-type (ecotype Col0) or 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, 13) were isolated from the original wrky17-1 stock.

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

| Line | Experiment 1 | Experiment 2 | Experiment 3 | Average |
|------|--------------|--------------|--------------|---------|
| WT   | 43.1% (53/123) | 44.7% (68/152) | 42.7% (56/131) | 43.5% |
| 7    | 69.2% (72/104) | 61.5% (88/143) | 70.2% (80/114) | 67.0% |
| 12   | 59.5% (97/163) | 63.5% (61/96) | 63.8% (81/127) | 62.3% |
| 13   | 60.8% (79/130) | 54.1% (72/133) | 59.4% (60/101) | 58.1% |

**Figure 2.** The effect of wrky17 mutation on susceptibility of Arabidopsis roots to 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 wrky17-1 mutant.
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’-ATGACCGTGTATTATGCGTTTAC3’/5’-TCAGCCTCTCTTGTGAATCC3’ that amplify the full length 1,026-bp VIP1 cDNA, and 5’-ATGGCTGAGGCGTG ATGATTT3’/5’TATAGAAACATTTTCTGTGAACGATTCC3’ that amplify the full length 1,134 bp ACTIN2 (At3g18780) cDNA.

Root transformation assay
All infection assays were performed as described by Gelvin (2006)14 with the Agrobacterium tumefaciens strain EHA105 (from S. Gelvin, Purdue University, USA), harboring a pBISN1 binary plasmid with an intron-containing GUS reporter gene that is not expressed in bacteria15. One-cm long root segments were excised 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 groups, each containing more than 100 root segments. Root bundles were overlaid with EHA105 harboring pBISN1 suspension culture at A600 = 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 timentine (BioWorld) to eliminate bacteria, and incubated for an additional three days on the MS medium supplemented with timentine. Root segments were then subjected to the GUS histochemical assay15, 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 wpky17 mutant seeds.

References
1. Eulgem T, Somssich IE: Networks of WRKY transcription factors in defense signaling. Curr Opin Plant Biol. 2007, 10(4): 366–371. PubMed Abstract | Publisher Full Text
2. Journot-Catalino N, Somssich IE, Roby D, et al.: The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in Arabidopsis thaliana. Plant cell. 2006, 18(1): 3289–3302. PubMed Abstract | Publisher Full Text | Free Full Text
3. Pitzschke A, Djamai A, Teige M, et al.: VIP1 response elements mediate mitogen-activated protein kinase 3-induced stress gene expression. Proc Natl Acad Sci U S A. 2009, 106(43): 18414–18419. PubMed Abstract | Publisher Full Text | Free Full Text
4. Djamai A, Pitzschke A, Nakagami H, et al.: Trojan horse strategy in Agrobacterium transformation: abusing MAPK defense signaling. Science. 2007, 318(5849): 452–458. PubMed Abstract | Publisher Full Text
5. Tsugama D, Liu S, Takano T: A BZIP protein, VIP1, is a regulator of osmosensory signaling in Arabidopsis. Plant Physiol. 2012, 159(1): 144–155. PubMed Abstract | Publisher Full Text | Free Full Text
6. Zaltsman A, Kirchovsky A, Kozlovsky SV, et al.: Plant defense pathways subverted by Agrobacterium for genetic transformation. Plant Signal Behav. 2010, 5(10): 1245–1248. PubMed Abstract | Publisher Full Text | Free Full Text
7. Tzfira T, Vaidya M, Citovsky V: VIP1, an Arabidopsis protein that interacts with Agrobacterium VirE2, is involved in VirE2 nuclear import and Agrobacterium infectivity. EMBO J. 2001, 20(13): 3596–3607. PubMed Abstract | Publisher Full Text | Free Full Text
8. Tzfira T, Vaidya M, Citovsky V: Increasing plant susceptibility to Agrobacterium infection by overexpression of the Arabidopsis nuclear protein VIP1. Proc Natl Acad Sci U S A. 2002, 99(16): 10435–10440. PubMed Abstract | Publisher Full Text | Free Full Text
9. Citovsky V, Kapetnov A, Otel S, et al.: Protein interactions involved in nuclear import of the Agrobacterium VirE2 protein in vivo and in vitro. J Biol Chem. 2004, 279(28): 29528–29533. PubMed Abstract | Publisher Full Text | Free Full Text
10. Lacroc B, Loyer A, Citovsky V: Association of the Agrobacterium T-DNA-protein complex with plant nucleosomes. Proc Natl Acad Sci U S A. 2008, 105(40): 15429–15434. PubMed Abstract | Publisher Full Text | Free Full Text
11. Li J, Kirchovsky A, Vaidya M, et al.: Uncoupling of the functions of the Arabidopsis VIP1 protein in transient and stable plant genetic transformation by Agrobacterium. Proc Natl Acad Sci U S A. 2005, 102(16): 5733–5738. PubMed Abstract | Publisher Full Text | Free Full Text
12. Loyer A, Rosenbluh J, Zakai N, et al.: The plant VirE2 interacting protein 1. A molecular link between the Agrobacterium T-complex and the host cell chromatin?. Plant Physiol. 2005, 138(3): 1318–1321. PubMed Abstract | Publisher Full Text | Free Full Text
13. Tzfira T, Vaidya M, Citovsky V: Involvement of targeted proteolysis in plant genetic transformation by Agrobacterium. Nature. 2004, 431(7004): 87–92. PubMed Abstract | Publisher Full Text | Free Full Text
14. Zaltsman A, Kirchovsky A, Loyer A, et al.: Agrobacterium induces expression of a plant host F-box protein required for tumorogenicity. Cell Host Microbe. 2010, 7(3): 197–209. PubMed Abstract | Publisher Full Text | Free Full Text
15. Magori S, Citovsky V: Agrobacterium counteracts host-induced degradation of its F-box protein effectors. Sci Signal. 2011, 4(195): ra69. PubMed Abstract | Publisher Full Text
16. van der Spek PJ, Kremer A, Murry L, et al.: Are gene expression microarray analyses reliable? A review of studies of retinoic acid responsive genes. Genomics Proteomics Bioinformatics. 2003; 1(1): 9–14. PubMed Abstract

17. Shi L, Campbell G, Jones WD, et al.: The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models. Nat Biotechnol. 2010; 28(8): 827–838. PubMed Abstract | Publisher Full Text | Free Full Text

18. Avivi Y, Morad V, Ben-Meir H, et al.: Reorganization of specific chromosomal domains and activation of silent genes in plant cells acquiring pluripotentiality. Dev Dyn. 2004; 230(1): 12–22. PubMed Abstract | Publisher Full Text

19. Gelvin SB: Agrobacterium transformation of Arabidopsis thaliana roots: a quantitative assay. Methods Mol Biol. 2006; 343: 105–113. PubMed Abstract | Publisher Full Text

20. Gelvin SB: Agrobacterium-mediated plant transformation: the biology behind the “gene-jockeying” tool. Microbiol Mol Biol Rev. 2003; 67(1): 16–37. PubMed Abstract | Publisher Full Text | Free Full Text

21. Lacroix B, Tzfira T, Vainstein A, et al.: A case of promiscuity: Agrobacterium’s endless hunt for new partners. Trends Genet. 2006; 22(1): 29–37. PubMed Abstract | Publisher Full Text

22. Liu CN, Li XQ, Gelvin SB: Multiple copies of virG enhance the transient transformation of celery, carrot, and rice tissues by Agrobacterium tumefaciens. Plant Mol Biol. 1992; 20(6): 1071–1087. PubMed Abstract | Publisher Full Text

23. Jefferson RA, Kavanagh TA, Bevan MW: GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. 1987; 6(13): 3901–3907. PubMed Abstract | Free 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.