The Plant Cell, Vol. 26: 4991–5008, December 2014, www.plantcell.org

Virulence Factors of Geminivirus Interact with MYC2 to Subvert Plant Resistance and Promote Vector Performance[C][W]

Ran Li,[a] Berhane T. Weldegergis,[b] Jie Li,[c] Choonkyun Jung,[d] Jing Qu,[a] Yanwei Sun,[a,e] Hongmei Qian,[f] ChuanSia Tee,[a] Joop J.A. van Loon,[b] Marcel Dicke,[b] Nam-Hai Chua,[d] Shu-Sheng Liu,[c] and Jian Ye[a,e,1]

[a] Temasek Life Sciences Laboratory, National University of Singapore, Singapore 117604, Singapore
[b] Laboratory of Entomology, Wageningen University, Wageningen 6700 EH, The Netherlands
[c] Institute of Insect Sciences, Zhejiang University, Hangzhou 310058, China
[d] Laboratory of Plant Molecular Biology, The Rockefeller University, New York, New York 10065
[e] State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
[f] Department of Horticulture, Zhejiang University, Hangzhou 310058, China

ORCID ID: 0000-0002-7741-4354 (J.Y.)

A pathogen may cause infected plants to promote the performance of its transmitting vector, which accelerates the spread of the pathogen. This positive effect of a pathogen on its vector via their shared host plant is termed indirect mutualism. For example, terpene biosynthesis is suppressed in begomovirus-infected plants, leading to reduced plant resistance and enhanced performance of the whiteflies (*Bemisia tabaci*) that transmit these viruses. Although begomovirus-whitefly mutualism has been known, the underlying mechanism is still elusive. Here, we identified βC1 of *Tomato yellow leaf curl China virus*, a monopartite begomovirus, as the viral genetic factor that suppresses plant terpene biosynthesis. βC1 directly interacts with the basic helix-loop-helix transcription factor MYC2 to compromise the activation of MYC2-regulated terpene synthase genes, thereby reducing whitefly resistance. MYC2 associates with the bipartite begomoviral protein BV1, suggesting that MYC2 is an evolutionarily conserved target of begomoviruses for the suppression of terpene-based resistance and the promotion of vector performance. Our findings describe how this viral pathogen regulates host plant metabolism to establish mutualism with its insect vector.

INTRODUCTION

Vector-borne pathogens are virulent causal agents of diseases in humans, animals, and crop plants (Navas-Castillo et al., 2011; Rosenberg and Beard, 2011). Vector behavior has overwhelming ecological and evolutionary significance for the pathogens that they carry and transmit, as vector-borne pathogens rely on their vectors for transmission to and multiplication in new hosts. Therefore, the ability of a pathogen to alter the behavior of its vector in a manner that facilitates its own transmission would be highly adaptive. This is especially important for plant pathogens, as their hosts are sessile, thus limiting pathogen movement from one plant to another. Several animal pathogens can directly affect their vector to increase transmission rate (Hurd, 2003; Lefèvre et al., 2008). By contrast, plant pathogens have been shown mainly to modify vector behavior via their shared host plant to achieve an indirect mutualistic relationship between pathogen and vector (Casteel et al., 2014; Luan et al., 2014).

Indirect mutualistic relationships in pathogen-vector-plant interactions have two main aspects. First, the pathogen causes nutritional changes in infected plants, resulting in improved fitness of the vectors. Second, the pathogen increases plant attractiveness and suitability to the vectors by overcoming plant defenses against the vector species, thereby promoting vector performance and increasing pathogen spread (Beanland et al., 2000; Eigenbrode et al., 2002; Lacroix et al., 2005; Jiu et al., 2007; Wang et al., 2012; Luan et al., 2014). To date, only a few reports have examined the host plant genes that are involved in pathogen-vector-plant interactions. For instance, the phytoplasma effector protein SAP11 enhances the fitness of its vector by modulating plant defense-responsive TCP transcription factors (Sugio et al., 2011).

Being sessile, plants have evolved sophisticated mechanisms to integrate endogenous and exogenous signals to adapt to the changing environment, often by releasing a blend of ecologically important volatiles to the atmosphere (Hong et al., 2012). Plant volatiles are well known to mediate host location by herbivorous arthropods and their carnivorous enemies (Dicke and Baldwin, 2010). Pathogens may modulate plant volatile production to influence vector behavior. For instance, volatile terpenoids mediate direct defense against the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) (Bleeker et al., 2009; Luan et al., 2013). Infection of tobacco (*Nicotiana tabacum*) by *Tomato yellow leaf curl China virus* (TYLCCNV) and its betasatellite complex reduces the synthesis of the sesquiterpene cedrene. This reduction in turn benefits its vector, the whitefly *B. tabaci*, resulting in

[1] Address correspondence to jianye@im.ac.cn.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Jian Ye (jianye@im.ac.cn).
[C] Some figures in this article are displayed in color online but in black and white in the print edition.
[W] Online version contains Web-only data.
www.plantcell.org/cgi/doi/10.1105/tpc.114.133181

a vector-virus mutualism (Luan et al., 2013). Moreover, some pathogen-infected plants have an inferior nutritional profile not optimal for the vector. To attract more vectors, pathogens can modulate host-derived olfactory cues to deceive their vectors (Eigenbrode et al., 2002; Mauck et al., 2010; Mann et al., 2012; Luan et al., 2014). To date, the precise mechanism of how a pathogen regulates host-derived olfactory cues remains poorly understood.

The genus *Begomovirus* (family Geminiviridae) contains the largest number of plant viruses in tropical, subtropical, and temperate agroecosystems worldwide (Navas-Castillo et al., 2011). Begomoviruses can be categorized into two types according to their DNA genomes: bipartite and monopartite. Bipartite viruses, such as the *Cabbage leaf curl virus* (CaLCuV), contain two components: DNA-A and DNA-B. By contrast, monopartite viruses possess only the DNA-A component. DNA satellites (e.g., the betasatellite), however, are associated with some monopartite begomoviruses. Irrespective of the genome composition, begomoviruses are transmitted exclusively by members of the whitefly *B. tabaci* species complex. Increasing evidence reveals that the plant jasmonic acid (JA) signaling pathway plays an important role in whitefly resistance (Zarate et al., 2007; Zhang et al., 2012). Begomovirus infection or stable transgene-expressed viral proteins in plants lead to reduced transcription of some JA-responsive genes (Ascencio-Ibáñez et al., 2008; Yang et al., 2008; Lozano-Durán et al., 2011; Zhang et al., 2012), and the impairment of JA signaling enhances vector performance (Zhang et al., 2012; Luan et al., 2013). This is one of the strategies employed by begomoviruses for persistent transmission. However, the host protein targets of begomoviruses and the molecular processes in the suppression of JA signaling are still elusive. Yang et al. (2008) identified *Arabidopsis thaliana* ASYMMETRIC LEAVES1 (AS1) as one molecular target of the TYLCCNV pathogenicity factor βC1 to explain whitefly-geminivirus mutualism. βC1 directly binds to AS1 and depresses the AS1-mediated suppression of leaf development; by contrast, βC1 promotes the repressive role of AS1 in regulating JA signaling. Moreover, βC1 attenuates the expression of both *PDF1.2* and *VSP1*, which participate in two antagonistic branches of the JA downstream pathway. These effects on plant gene expression induced by βC1-AS1 interaction prompted us to search for additional target plant protein(s), which may be involved in whitefly-begomovirus mutualism.

Here, we show that begomoviruses suppress plant terpene-related defenses to achieve indirect vector-virus mutualism in two model plants: *Nicotiana benthamiana* and *Arabidopsis*. The βC1 protein, encoded by the betasatellite of the monopartite TYLCCNV, was identified as the key viral genetic factor for the suppression of terpene synthesis. Using the yeast two-hybrid system, we identified the transcription factor MYC2 as an additional interaction partner of βC1. As a key component in the JA pathway, MYC2 dimers directly regulate *TERPENE SYNTHASE* (*TPS*) genes. βC1 interferes with MYC2 dimerization, resulting in decreased *TPS* transcript levels and reduced terpene synthesis. Our results provide a molecular mechanism for how begomoviruses establish mutualistic relationships with their whitefly vectors by targeting the activity of the plant MYC2 protein.

RESULTS

TYLCCNV βC1 Protein Suppresses Plant Terpene Synthesis and Changes the Host Preference and Performance of Whitefly

Two model plants, *Arabidopsis* and *N. benthamiana*, were used in our work. *N. benthamiana* is closely related to tomato (*Solanum lycopersicum*), whose genome encodes 44 *TPS* genes with detailed functional characterization (Falara et al., 2011). Using the tomato TPS protein sequences as a reference, we identified 38 counterparts, and 13 of them encoded putative functional TPS (>300 amino acids) in the *N. benthamiana* genome (Supplemental Figure 1). Reverse transcription quantitative PCR (RT-qPCR) analysis showed that eight *TPS* genes were expressed in *N. benthamiana* leaves, where most vector-virus interactions take place. Two genes, Nb-*TPS1* and Nb-*TPS12*, encoding sesquiterpene synthases, as well as putative genes for the sesquiterpene modifier cytochrome P450 hydroxylase (Nb-*EAH*) (Ralston et al., 2001) were significantly downregulated in plants infected by TYLCCNV and its associated betasatellite (TYLCCNB) (hereafter referred to as TA+β) compared with plants infected with only TYLCCNV (hereafter referred to as TA) (Figure 1A). Nb-*TPS1* transcript levels in TA+β-infected plants were only 30% of that in control plants.

To understand the metabolic consequences of the reduced *TPS* expression levels upon TA+β infection, we investigated changes in the emission of volatile compounds after virus infection. Although basal *TPS* expression levels could be detected, no constitutive volatile from the headspace of *N. benthamiana* was detected with our experimental equipment and conditions. This might be because the expression of the majority of *TPS* genes in *N. benthamiana* plants is inducible, for example by whitefly infestation. This phenomenon was also found in *Arabidopsis* (Tholl and Lee, 2011). We noted that whitefly infestation increases the release of terpenes in both tobacco, a relative of *N. benthamiana*, and *Arabidopsis* (Luan et al., 2013; Zhang et al., 2013). Therefore, we applied methyl jasmonate (MeJA) to mimic whitefly infestation, since this plant hormone elicits the production of a variety of terpenes (Kessler and Baldwin, 2001). Figure 1B shows that five terpenes were reproducibly detected in the headspace of *N. benthamiana*. Among these, the emission of four volatile monoterpenes, namely α-pinene, β-pinene, D-limonene, and linalool, showed no significant difference between TA- and TA+β-infected plants (Figure 1B). By contrast, the presence of the betasatellite (TA+β) in infected plants decreased the emission of α-bergamotene, a sesquiterpene, by 75.4%. As expected, more whiteflies were attracted to TA+β-infected plants compared with TA-infected plants (Figure 1C).

Recent studies have shown that the TYLCCNB betasatellite encodes only one protein, βC1, which accounts for the attenuation of JA signaling in plants (Yang et al., 2008; Zhang et al., 2012). We examined whether the βC1 protein is responsible for the repression of plant terpene biosynthesis. To this end, we used a βC1 betasatellite mutant (mβ) in which the first start codon has been mutated (Cui et al., 2004). Relative to TA-infected plants, TA+mβ-infected plants showed no significant change in transcription levels of all *TPS* genes except Nb-*TPS3*, of which a mild

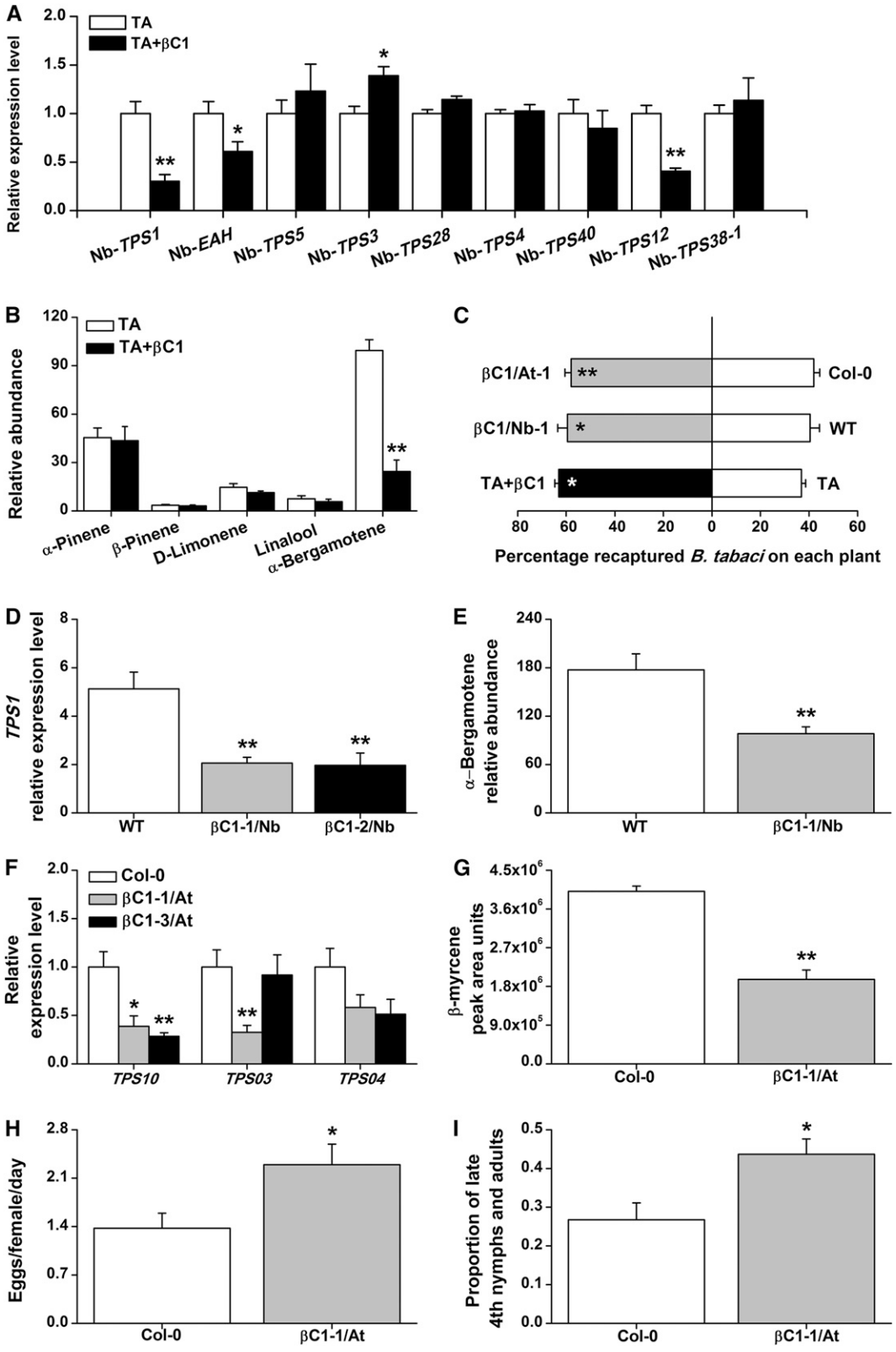

Figure 1. The βC1 Protein Encoded by the Betasatellite Increases Whitefly Attraction and Performance on Plants by Reducing Terpene Synthesis.

decrease in transcription level occurred (Supplemental Figure 2). These results indicate that a functional βC1 protein is indispensable for the repression of plant terpene synthesis by the TYLCCNV/TYLCCNB complex. To confirm this hypothesis, we determined Nb-*TPS1* expression levels in control and infected plants and transgenic *N. benthamiana* plants overexpressing βC1 (βC1/Nb). The expression level of Nb-*TPS1*, which was the most significantly downregulated gene in TA+β-infected plants, was greatly reduced in two βC1/Nb transgenic lines compared with wild-type plants (Figure 1D). Ectopic expression of βC1 in *N. benthamiana* or *Arabidopsis* altered plant development (Supplemental Figures 3A and 3B), which might in turn alter the interaction between whitefly and plant. To minimize the effect of plant development, we chose a weak phenotype to perform volatile extraction and whitefly experiments (βC1-1/Nb and βC1-1/At). As expected, the major changed component release from the headspace of βC1-1/Nb plants was α-bergamotene, only 55.4% of that of the wild-type plants (Figure 1E; Supplemental Figure 3B). The attenuated terpene synthesis in βC1-1/Nb lines was associated with the attraction of more whiteflies (Figure 1C). These results indicate that βC1 is the gene in the TYLCCNV/TYLCCNB complex responsible for the repression of terpene biosynthesis in *N. benthamiana*.

Next, we asked whether bipartite begomoviruses are also capable of repressing host plant terpene biosynthesis, like their monopartite counterparts. CaLCuV is a bipartite begomovirus that can infect *Arabidopsis*. We screened 15 leaf- or flower-specific *TPS* genes in *Arabidopsis* after CaLCuV infection (Tholl and Lee, 2011). Supplemental Figure 4A shows that six *TPS* genes were significantly repressed in plants infected with CaLCuV A+B (hereafter referred to as CA+CB) compared with plants infected with CaLCuV DNA-A alone (hereafter referred to as CA). Among them, the At-*TPS10* transcript level suffered the most severe reduction. Volatile metabolite analysis showed that production of the monoterpenes β-myrcene and limonene in CA+CB-infected plants was only 43 and 85.2%, respectively, as compared with those in control plants with CA infection alone (Supplemental Figure 4B). Moreover, compared with control plants, CA+CB-infected plants were more attractive to whiteflies (Supplemental Figure 4C), and more eggs were laid by whiteflies on the latter (Supplemental Figure 4D). Whitefly developed significantly faster on CA+CB-infected plants than on CA-infected plants, as indicated by the higher proportion of late fourth instar nymphs and adult whiteflies (Supplemental Figure 4E). These results show that suppression of terpene synthesis indeed indirectly benefits the whitefly vector in *Arabidopsis*. We note that At-*TPS10* transcript levels were also markedly more repressed in two *Arabidopsis* βC1/At transgenic plants than those of the wild type (Figure 1F), suggesting the conservation of a common host protein target through the evolution of both monopartite and bipartite begomoviruses. The expression level of At-*TPS10* and that of other key genes that differ significantly between *Arabidopsis* βC1/At transgenic plants and the wild type (Columbia-0 [Col-0] ecotype) (see below) showed no difference between transgenic empty vector plants and wild-type plants (Supplemental Figure 5). Therefore, we used wild-type plants as the control throughout our experiments. The reduced At-*TPS10* expression levels resulted in a 51.1% reduction in the release of β-myrcene (Figure 1G). Consequently, relative to *Arabidopsis* wild-type plants, βC1-1/At transgenic plants were more attractive to whiteflies, and more eggs were laid by whiteflies on the latter (Figures 1C and 1H). Moreover, whitefly development progressed significantly faster on βC1-1/At transgenic plants than on wild-type plants (Figure 1I). These data indicate that the TYLCCNV βC1 contributes to the suppression of host terpene biosynthesis and renders the host more attractive to the virus vector.

TPS10 Is an *Arabidopsis* Defense Gene against Whitefly

Because At-*TPS10* in *Arabidopsis* and Nb-*TPS1* in *N. benthamiana* were severely repressed after begomovirus infection, we investigated their roles in plant resistance to whiteflies. Quantitative PCR analysis revealed that the constitutive but low expression of At-*TPS10* increased rapidly when the plants were treated with

Figure 1. (continued).

(A) Relative expression levels of different *TPS* genes in *N. benthamiana*. Plants were infiltrated with the TYLCCNV DNA-A alone (TA) or along with an associated betasatellite DNA encoding only the protein βC1 (TA+β). Values are means + SE (n = 5).

(B) Terpenes emitted by *N. benthamiana* after TYLCCNV infection. Values are mean relative amounts (percentage of internal standard peak area) + SE (n = 6).

(C) Whitefly preference (as percentage recaptured whiteflies out of 200 released) on different plants. Values are means + SE (n = 8). All of the terpene extraction experiments and whitefly choice assays were performed at 6 h after MeJA treatment.

(D) Relative expression levels of Nb-*TPS1* in wild-type *N. benthamiana* and two *N. benthamiana* lines expressing βC1 (βC1-1/Nb and βC1-2/Nb). Values are means + SE (n = 5).

(E) α-Bergamotene emitted by the wild type or the transgenic βC1 expression line βC1-1/Nb. Values are means + SE (n = 6).

(F) Relative expression levels of At-*TPS* genes in the wild type and two *Arabidopsis* transgenic lines expressing βC1 (βC1-1/At and βC1-3/At). Values are means + SE (n = 5).

(G) Emission of β-myrcene in wild-type *Arabidopsis* and the transgenic βC1 expression line βC1-1/At. The results are expressed as peak area units per gram of plant fresh weight. Values are means + SE (n = 6).

(H) Daily number of eggs laid per female in wild-type *Arabidopsis* and the transgenic βC1 expression line βC1-1/At. Values are means + SE (n = 8).

(I) Effect of plant genotype on the proportion of progeny that had reached advanced stages of development (red eye yellow fourth nymph and open exuvia indicating emergence) by day 22. Values are means + SE (n = 8).

Asterisks indicate significant differences between different treatments or lines (*$P < 0.05$, **$P < 0.01$; Student's t test for all experiments except the whitefly choice experiments, which were analyzed by the Wilcoxon matched pairs test).

MeJA or mechanically wounded (Figures 2A and 2B). Consistent with these observations, whitefly infestation also increased At-*TPS10* expression (Supplemental Figure 6A). These results were further confirmed by At-*TPS10* promoter activity assays using an *Arabidopsis* transgenic line expressing an At-*TPS10* promoter:*GUS* (for β-glucuronidase) reporter gene. Figure 2C shows strong GUS expression after 6 h of mechanical wounding or MeJA treatment or after 8 h of feeding by whiteflies. The expression profile suggested that At-*TPS10*, similar to *VSP1* (Kazan and Manners, 2013), is a marker of the JA response in *Arabidopsis*.

To understand the role of At-*TPS10* in plant defense, we used a *tps10-2* (SALK_041114) mutant line, which carries a T-DNA insertion in the second exon of *TPS10* (Supplemental Figure 7A). There was no obvious difference in the growth phenotype between wild-type *Arabidopsis* and *tps10-2* mutants (Supplemental Figure 7B). Compared with wild-type plants, *tps10-2* mutant plants did not accumulate detectable levels of *TPS10* mRNA (Figure 2D), suggesting that *tps10-2* is a null mutant. At-TPS10 has been reported to convert geranyl diphosphate into multiple monoterpenes in *Escherichia coli*, and β-myrcene is the major monoterpene product (Bohlmann et al., 2000). To obtain direct genetic evidence for At-TPS10 enzyme activity in vivo, we trapped volatiles emitted from *tps10-2* mutant plants. Consistent with the above observations, the β-myrcene level was significantly decreased in *tps10-2* plants as compared with wild-type plants (Figure 2E). Moreover, *TPS10*-deficient *Arabidopsis* plants were more attractive to whiteflies than wild-type plants (Figure 2I). Whiteflies laid more eggs on *tps10-2* plants than on wild-type plants (Figure 2F). These results show that At-*TPS10* is involved in defense against whiteflies.

We used virus-induced gene silencing (VIGS) to downregulate *TPS1* transcript levels in *N. benthamiana*. In VIGS-silenced plants, Nb-*TPS1* transcript levels were reduced by 79% compared with control plants (Figure 2G). Phylogenetic tree analysis of TPS proteins from different species showed that Nb-*TPS1* belongs to the clade of sesquiterpene synthases (Supplemental Figure 1). Consistent with this prediction, the emission of α-bergamotene was reduced by 59% in Nb-*TPS1*-silenced plants compared with control plants (Figure 2H), indicating that Nb-*TPS1* encodes an α-bergamotene synthase. Moreover, Nb-*TPS1*-silenced plants attracted more whiteflies than control plants (Figure 2I). In conclusion, At-*TPS10* and Nb-*TPS1* are the major target genes in the terpene biosynthesis pathway of plants for host defense against whiteflies.

βC1 Interacts with the MYC2 Transcription Factor of Plant Hosts

To elucidate the molecular mechanism of how begomoviruses manipulate plant terpene biosynthesis pathways, we screened an *Arabidopsis* cDNA library by a yeast two-hybrid system to identify plant proteins that may interact with TYLCCNB βC1. In preliminary experiments, we obtained a few putative positive interactors, including At-MYC2, which is a key downstream component of the JA signaling pathway (Kazan and Manners, 2013). The importance of JA signaling in plant-insect interactions (Wu and Baldwin, 2010) prompted us to investigate MYC2 further.

First, we confirmed the interaction between *Arabidopsis* MYC2 and βC1 by a cotransformation assay in yeast. BD-βC1 and AD-At-MYC2 yeast transformants were able to grow on SD-Leu-Trp-His selection plates with 2 mM 3-amino-1,2,4-triazole, whereas yeast transformants carrying two control constructs were unable to do so. These observations confirmed the At-MYC2/βC1 interaction in yeast (Figure 3A). Next, we performed protein pull-down assays to determine whether the two proteins can indeed interact directly in vitro. Figure 3B shows that MBP-At-MYC2 was pulled down by GST-βC1, whereas no signal was observed when GST-βC1 was replaced by GST in the assay, indicating specific At-MYC2 interaction with βC1 in vitro. We then extended these observations to the *N. benthamiana* system. Nb-*bHLH1* is a homolog of At-*MYC2* in *N. benthamiana* (De Boer et al., 2011), and this gene is referred to as Nb-*MYC2* here. As expected, MBP-Nb-MYC2 was also pulled down by GST-βC1 (Figure 3B), indicating the interaction between βC1 and Nb-MYC2 as well.

We used the *N. benthamiana* transient expression system to determine the intracellular localization of βC1 and plant MYC2 proteins. Supplemental Figure 8 shows that βC1 localized in both the nucleus and the cytosol, whereas At-MYC2 and Nb-MYC2 specifically localized in the nucleus. Coexpression of βC1-CFP and At-MYC2 or Nb-MYC2-YFP showed colocalization of βC1 with MYC2 proteins in the nuclear compartment (Supplemental Figure 8, third and fifth panels); however, these results did not provide direct evidence that they indeed interact in vivo. To further examine the interaction between βC1 and MYC2 proteins, we performed a bimolecular fluorescence complementation (BiFC) assay. *Agrobacterium tumefaciens* strains containing expression vectors for βC1-cEYFP and At-MYC2 (or Nb-MYC2)-nEYFP were coinfiltrated into *N. benthamiana* leaf cells. Figure 3D (third and fourth panels) shows strong fluorescence in nuclei, as confirmed by 4′,6-diamidino-2-phenylindole (DAPI) staining. As a negative control, no fluorescence was observed when βC1-cEYFP was coexpressed with an empty nEYFP vector (Figure 3D, first panel).

Because the bipartite begomovirus CaLCuV also suppressed terpene biosynthesis, we asked if any of the CaLCuV-encoded proteins can interact with MYC2. *BV1* in the DNA-B component of CaLCuV is located in a similar genomic locus as the *βC1* gene of TYLCCNV/TYLCCNB (Cui et al., 2004; Fontes et al., 2004). Confocal microscopy experiments using BV1-CFP and At-MYC2-YFP showed that the two proteins colocalized in the nucleus (Supplemental Figure 8, fourth panel). Pull-down and BiFC assays provided direct evidence that BV1 interacts with At-MYC2 in vitro and in vivo, respectively (Figures 3C and 3D, fifth panels). Considered together, these results demonstrate that MYC2 is a conserved interaction partner shared by TYLCCNV/TYLCCNB βC1 and CaLCuV BV1 proteins.

MYC2 Directly Regulates Transcript Levels of *TPS* Genes

Our results so far confirmed and extended previous data (Luan et al., 2013) that TYLCCNV/TYLCCNB infection suppresses plant terpene synthesis and that βC1 modulates this process (Figure 1; Supplemental Figure 2). The interaction between βC1 and MYC2 raises the question of whether the host protein is directly involved in terpene-mediated whitefly resistance. MYC2, which belongs to the basic helix-loop-helix (bHLH) transcription

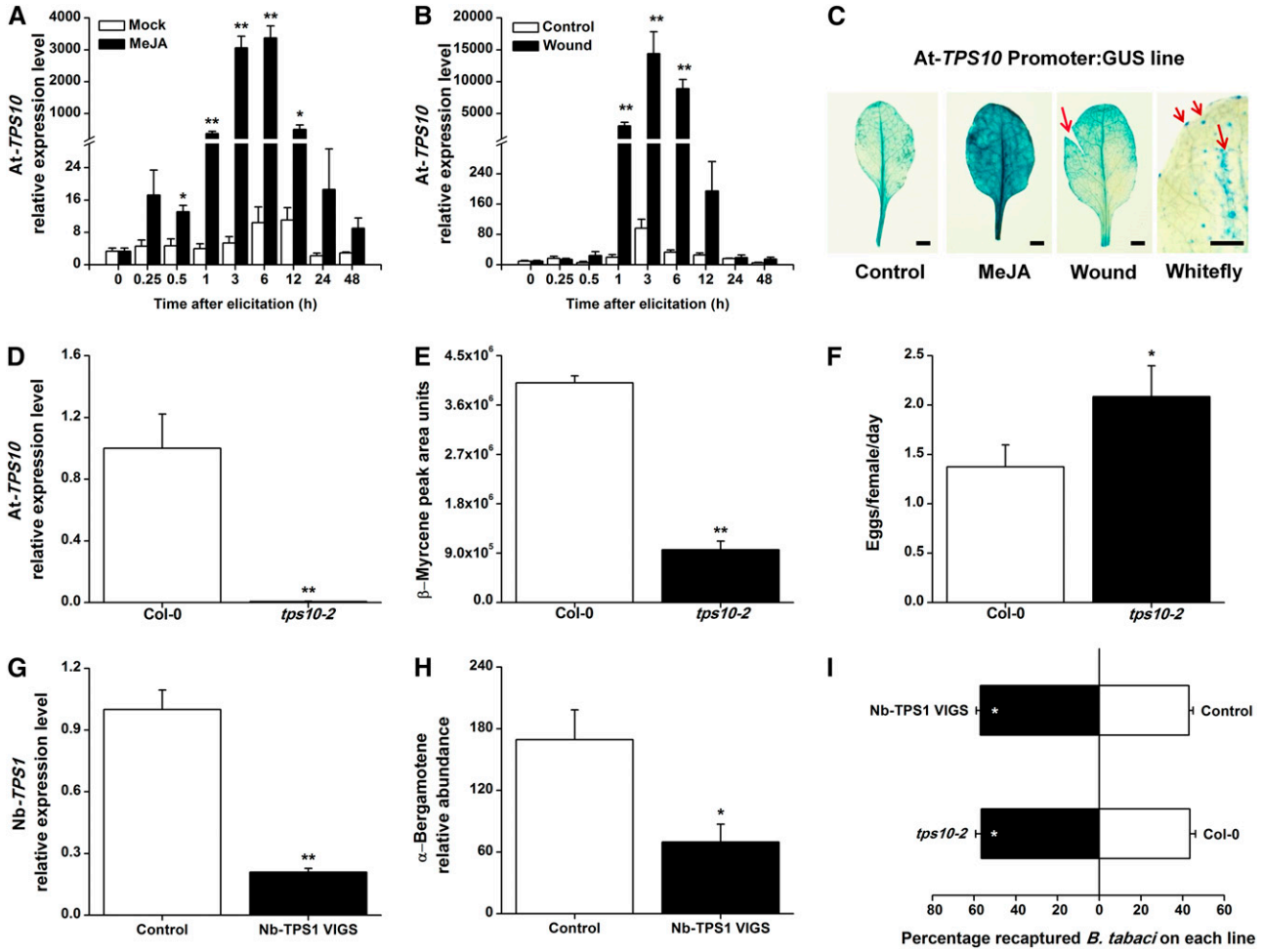

Figure 2. Silencing of *TPS* Decreases Terpenoid Synthesis and Compromises Plant Defense against Whitefly.

(A) Relative At-*TPS10* expression levels (means + SE; $n = 5$) in *Arabidopsis* treated with MeJA or 0.01% Tween 20 (Mock).
(B) Relative At-*TPS10* expression levels (means + SE; $n = 5$) in control and wounded *Arabidopsis* plants.
(C) GUS activity of the At-*TPS10* promoter:*GUS* reporter line after MeJA treatment and mechanical wounding for 6 h or whitefly feeding for 8 h. An untreated line was used as a control. Arrows indicate mechanical wounding or whitefly feeding sites. Bars = 2 mm.
(D) Relative expression level of At-*TPS10* in *Arabidopsis* wild type and *tps10-2*. Values are means + SE ($n = 5$).
(E) Emission of β-myrcene in *Arabidopsis* wild type and *tps10-2*. Values are means + SE ($n = 5$).
(F) Daily number of eggs laid per female in *Arabidopsis* wild type and *tps10-2*. Values are means + SE ($n = 8$).
(G) Relative expression level of Nb-*TPS1* in control and Nb-*TPS1*-silenced *N. benthamiana* plants. Values are means + SE ($n = 5$).
(H) α-Bergamotene emitted by control and Nb-*TPS1*-silenced *N. benthamiana* plants. Values are means + SE ($n = 6$).
(I) Whitefly preference (as percentage recaptured whiteflies out of 200 released) on each plant. Values are means + SE ($n = 8$).
All terpene extraction experiments and whitefly choice assays were performed at 6 h after MeJA treatment. Asterisks indicate significant differences between different treatments or different lines (*$P < 0.05$, **$P < 0.01$; Student's *t* test for all experiments except the whitefly choice experiments, which were analyzed by the Wilcoxon matched pairs test).
[See online article for color version of this figure.]

factor family, binds to the G-box or G-box-like (CANNTG) *cis*-element (Dombrecht et al., 2007; Godoy et al., 2011). Analysis of a 1.8-kb upstream region of At-*TPS10* by PLACE (http://www.dna.affrc.go.jp/htdocs/PLACE/) uncovered five G-box-like elements distributed in three regions (Figure 4A). Three additional G-box-like elements were also found in the At-*TPS10* 5′ terminal non-coding region. To check whether MYC2 can bind directly to these elements in vivo, we performed a chromatin immunoprecipitation (ChIP) assay using transgenic lines expressing a MYC2-GFP fusion protein under the control of the cauliflower mosaic virus *35S* promoter in the *myc2-2* mutant background of *Arabidopsis*. Quantitative PCR analysis showed that region III was significantly enriched in *35S:MYC2-GFP/myc2-2* lines relative to wild-type plants (Figure 4B). This region is 200 to 400 bp upstream of the At-*TPS10* transcription start site and contains two G-box-like elements, G-box-like 1 (CAAGTG, −449 to −454 bp from the

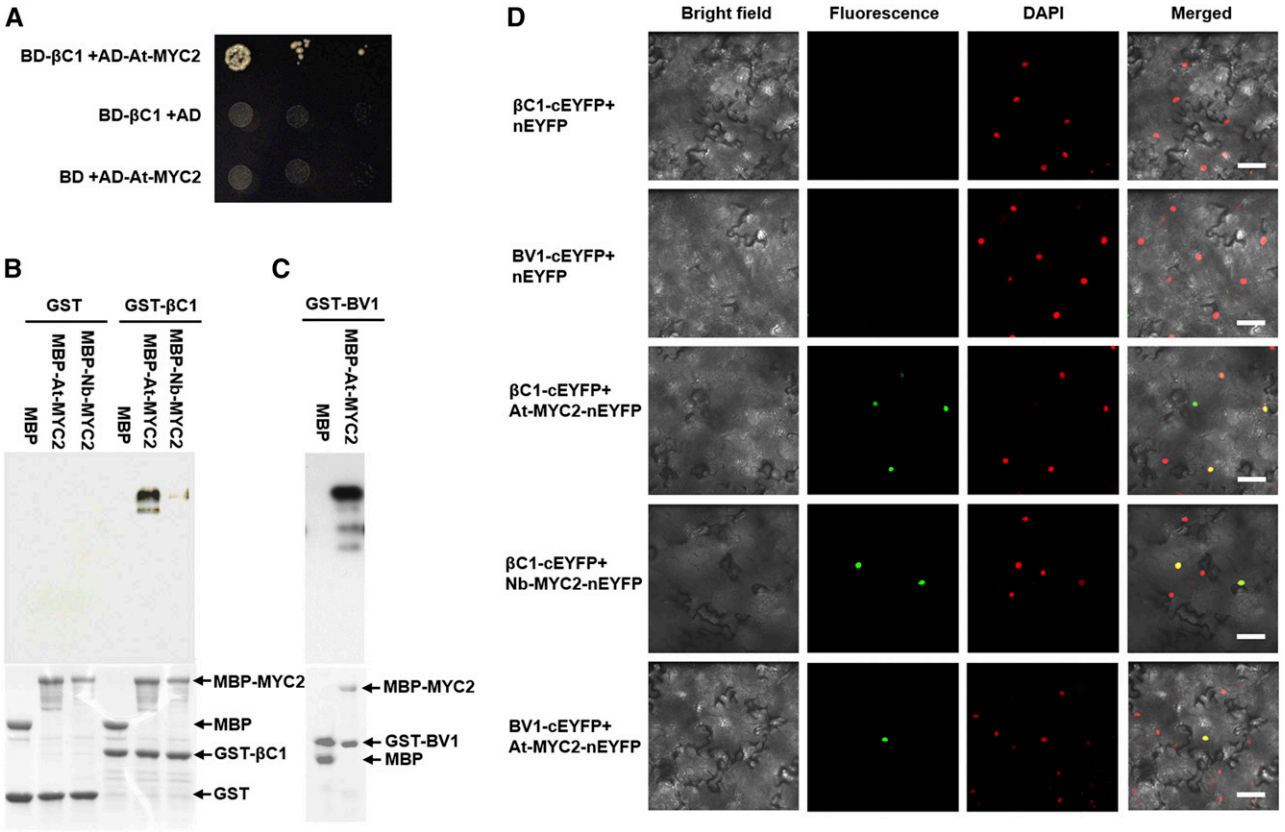

Figure 3. Protein-Protein Interaction between βC1 and Plant MYC2 Proteins.

(A) Interaction between βC1 and At-MYC2 in the yeast two-hybrid system. Yeast strain Y2HGold cotransformed with the indicated plasmids was spotted on synthetic medium SD-Leu-Trp-His with 2 mM 3-amino-1,2,4-triazole. The empty vectors pGBKT7 and pGADT7 were used as negative controls.

(B) and **(C)** In vitro pull-down assays. Two micrograms of GST or GST fusion proteins was used to pull down 2 μg of MBP or MBP fusion proteins. Immunoblots were performed using anti-MBP antibody to detect the associated proteins. Membranes were stained with Coomassie Brilliant Blue to monitor input protein amount.

(D) In vivo BiFC analysis of βC1 or BV1 interaction with MYC2. Fluorescence was observed owing to complementation of the BV1 or βC1 fused with the C-terminal part of EYFP with At-MYC2 or Nb-MYC2 fused with the N-terminal part of EYFP. Nuclei of tobacco leaf epidermal cells were stained with DAPI. Unfused nEYFP was used as a negative control. Bars = 50 μm.

transcription start site) and G-box-like 2 (CACATG, −188 to −183 bp from the transcription start site). To further confirm the binding of MYC2 to these two G-box-like elements, we performed an electrophoretic mobility shift assay using the purified recombinant His-MYC2 protein. His-MYC2 can bind to G-box-like 2 (CACATG) but not to G-box-like 1 (CAAGTG) nor to a mutant of G-box-like 2 (TTCAAA) (Supplemental Figure 9; Godoy et al., 2011). DNA binding specificity was further confirmed in a competition experiment using excess unlabeled G-box-like 2 probe as a cold competitor, by which reduced DNA/protein was detected (Supplemental Figure 9). To test if G-box-like 2 in region III was indeed responsible for the JA-induced response, we generated At-*TPS10* mutant promoter:*GUS* transgenic lines, in which 5′-CACATG-3′ was substituted by 5′-TTCAAA-3′. At-*TPS10* promoter:*GUS* lines showed strong GUS activity 6 h after MeJA treatment, whereas little GUS activity was observed in At-*TPS10* mutant promoter:*GUS* lines (Figures 4C and 4D). These data indicate that MYC2 binding to the G-box-like 2 element is essential for At-*TPS10* transcriptional activity.

Next, we compared *TPS* gene expression levels in the wild type and *myc2* mutants. Constitutive and MeJA-induced transcript levels of At-*TPS10* were significantly decreased in *myc2-1* and *myc2-2* mutants compared with the wild type. Another herbivore-inducible gene, At-*TPS04*, was also downregulated (Figure 4E; Supplemental Figure 10). The emission level of β-myrcene, a product of At-TPS10, was only 45.8% in *myc2-2* as compared with wild-type plants (Figure 4F). Consistent with the reduced *TPS* expression and volatile emission, insect choice assays showed that *myc2-2* lines were more attractive to whiteflies than wild-type plants (Figure 4J). Whiteflies laid more eggs on *myc2-2* plants than on wild-type plants (Figure 4G). We used VIGS to silence Nb-*MYC2*, and in the silenced plants Nb-*MYC2* transcript levels were reduced by 62.0% (Figure 4H). Nb-*TPS1* expression was also greatly repressed in Nb-*MYC2*-silenced

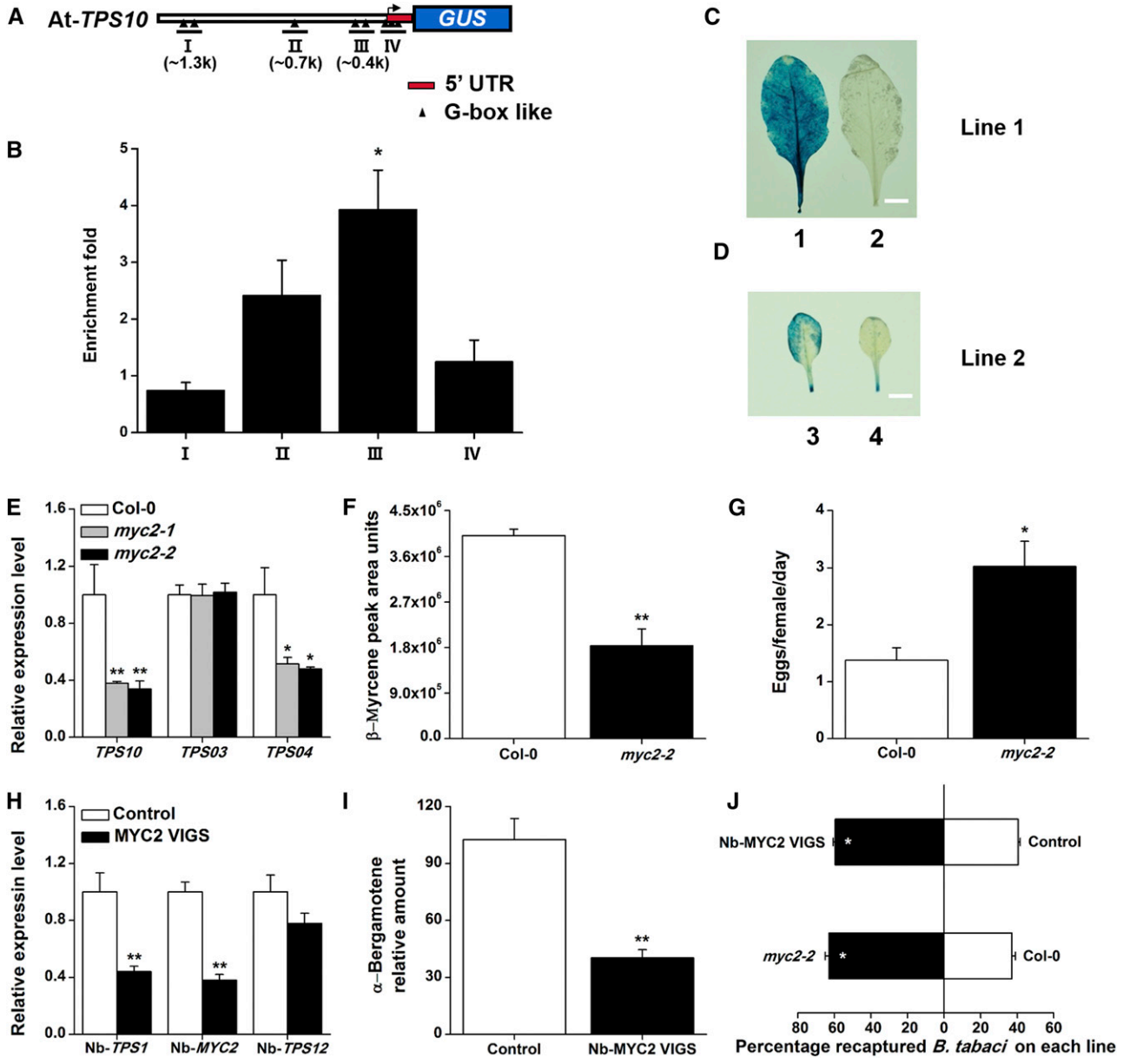

Figure 4. MYC2 Directly Regulates *TPS* Genes and Modulates Plant Defense against Whitefly.

(A) Schematic diagram of the At-*TPS10* promoter-*GUS* reporter construct. The bent arrow indicates the transcription start site (+1). The small triangles represent the G-box-like motif. The four lines (I, II, III, and IV) under the triangles indicate fragments amplified in ChIP assays. The end positions of each fragment (kb) relative to the transcription start site are indicated below. UTR, untranslated region.

(B) Fold enrichment of GFP-MYC2 associated with each of the four promoter fragments. The enrichments are for *35S:MYC2-GFP/myc2-2* lines relative to wild-type plants. Error bars indicate SE of three separate samples.

(C) and **(D)** GUS activity in *GUS* reporter lines after being treated with MeJA for 6 h. Two independent lines of each constructs were used (plants 1 and 3, At-*TPS10* promoter:*GUS* lines; plants 2 and 4, At-*TPS10* G-box-like III mutant promoter:*GUS* lines). Bars = 3 mm.

(E) Relative expression levels of *Arabidopsis TPS* genes in the wild type and *myc2* mutants. Values are means + SE (n = 5).

(F) Emission of β-myrcene in the wild type and *myc2-2*. Values are means + SE (n = 5).

(G) Daily number of eggs laid per female in wild-type *Arabidopsis* and *myc2-2*. Values are means + SE (n = 8).

(H) Relative expression levels of Nb-*TPS1* and Nb-*TPS12* in control and Nb-*MYC2*-silenced *N. benthamiana* plants. Values are means + SE (n = 5).

(I) α-Bergamotene emitted by control and Nb-*MYC2* VIGS *N. benthamiana* plants. Values are means + SE (percentage of internal standard peak area) (n = 6).

(J) Whitefly preference (as percentage recaptured whiteflies out of 200 released) on each plant. Values are means + SE (n = 8).

plants compared with control plants, resulting in diminished emission of α-bergamotene in the former (Figures 4H and 4I). Consistent with the results with *Arabidopsis*, more whiteflies chose to settle on Nb-*MYC2*-silenced plants than on control plants (Figure 4J). Taken together, our results show that MYC2 modulates whitefly resistance by direct transcriptional regulation of *TPS* genes.

TYLCCNV βC1 Protein Suppresses the MYC2-Regulated Synthesis of Indole and Aliphatic Glucosinolates

In *Arabidopsis*, MYC2 can interact with indole and aliphatic glucosinolate (GS)-related MYB transcription factors, which play a crucial role in the regulation of herbivore-induced plant production of GSs. Moreover, MYC2 can bind directly to promoter regions of multiple indole and aliphatic GS pathway genes (Schweizer et al., 2013). Indole and aliphatic GSs in *Arabidopsis* have been implicated in the performance of the whitefly *B. tabaci* (Elbaz et al., 2012; Markovich et al., 2013). Thus, we further asked if indole and aliphatic GSs were also affected by TYLCCNV βC1. To investigate this issue, the expression level of several GS pathway genes in βC1 overexpression *Arabidopsis* lines (βC1/At) were examined. Interestingly, all tested MYC2-regulated indole and aliphatic GS-related genes were downregulated in βC1/At lines compared with wild-type plants (Figures 5A to 5E). The transcript level of a MYC2 interaction partner, MYB34, which controls the synthesis of indole GSs, also decreased in βC1/At lines (Figure 5F); by contrast, another indole GS regulator, MYB51, was not transcriptionally affected by βC1 expression (Figure 5G). Consistent with the GS-related gene expression levels, three aliphatic GSs (3-methylsulfinylpropyl glucosinolate, 4-methylthiobutyl glucosinolate, and 8-methylsulfinyloctyl glucosinolate) and two indolic GSs (indolyl-3-methyl glucosinolate and 4-methoxy-indolyl-3-methyl glucosinolate) were decreased significantly in the *Arabidopsis* transgenic line expressing βC1 (βC1-1/At) compared with wild-type *Arabidopsis* (Figure 5H). These results suggest that the interaction between βC1 and MYC2 likewise reduces whitefly resistance-related indole and aliphatic GS levels in *Arabidopsis*.

βC1 Suppresses MYC2 Activity by Interfering with Its Dimerization

Because MYC2 positively regulates *TPS* genes whereas βC1 attenuates their expression, we asked if βC1 interacts with MYC2 protein and, therefore, affects the activity of the latter. At-MYC2 contains a typical bHLH domain in its C terminus, and a JAZ interaction domain and a MED25 interaction domain have been identified in its N-terminal region (Fernández-Calvo et al., 2011; Chen et al., 2012). To localize βC1-interacting domains, we constructed different At-MYC2 deletion derivatives fused with nEYFP and performed BiFC assays with βC1-cEYFP. As indicated by yellow fluorescent protein (YFP) signal, At-MYC2 fragments that contained the nuclear localization signal (NLS) and/or the bHLH domain were sufficient to interact with βC1 in vivo (Figure 6A, right panel). These interactions were observed in the nucleus, with the exception of the NLS-deleted At-MYC2, which was observed in the cytosol (Figure 6A). The loss of the NLS resulted in aberrant localization of the interaction partners (Figure 6A, fourth panel). The NLS is essential for protein translocation into the nucleus, whereas the bHLH region plays a vital role in protein dimerization. In addition to homodimers, MYC2 forms heterodimers with other MYC-related bHLH transcription factors (Supplemental Figure 11; Fernández-Calvo et al., 2011). These dimerizations may contribute to the binding to the G-boxes and the regulation of multiple signaling events (Kazan and Manners, 2013). We used a protein competition pull-down assay to test whether βC1 may affect the dimerization of MYC2. The amount of GST-At-MYC2 pulled down by MBP-At-MYC2 was reduced by increasing the amount of SUMO-βC1 (ΔN8) in the mix. By contrast, increasing the amount of SUMO protein alone did not affect MYC2 self-association (Figure 6B). These results provide preliminary evidence that βC1 blocked MYC2 dimerization by competing with the MYC2 binding bHLH domain.

Next, we asked whether βC1 could affect the DNA binding activity of At-MYC2. Using At-*TPS10* promoter:*GUS* as a reporter and At-MYC2-YFP and βC1-CFP as effectors, *N. benthamiana* leaf cells were transiently expressed with the indicated effector and reporter constructs and GUS activities were subsequently quantified. YFP and cyan fluorescent protein (CFP) were used as negative control effectors. Figure 6C shows that At-MYC2 significantly increased the GUS activity, whereas βC1 decreased constitutive and MYC2-induced GUS activity. These results indicate that βC1 attenuated the activity of At-MYC2 in promoting At-*TPS10* transcription.

The βC1 Interaction Partners AS1 and MYC2 Mediate Different Signaling Pathways against Whitefly

The interaction between βC1 and *Arabidopsis* AS1 also attenuates a subset of JA-responsive genes (Yang et al., 2008). To clarify the biological difference between the roles of AS1 and MYC2 in whitefly resistance, we checked the expression level of MYC2-dependent whitefly resistance genes in the *as1-1* mutant. The constitutive expression of most MYC2-dependent genes in *as1-1* was the same as in the wild type; only the transcript of one aliphatic GS-related gene, *BCAT4*, was slightly higher in *as1-1* (Figure 7). Compared with the wild-type plant, MeJA-induced expression of At-*TPS10* was not changed in *as1-1* plants; however, At-*TPS10* expression decreased strongly in *myc2* mutants (Figure 7A; Supplemental Figure 10). The transcripts of *CYP79B3* and *SUR1* were weakly suppressed in *as1-1* mutants 6 h after MeJA treatment (Figures 7B and 7D). These results

Figure 4. (continued).

All terpene extraction experiments and whitefly choice assays were performed at 6 h after MeJA treatment. Asterisks indicate significant differences between different lines (*P < 0.05, **P < 0.01; Student's *t* test for all experiments except the whitefly choice experiments, which were analyzed by the Wilcoxon matched pairs test).

[See online article for color version of this figure.]

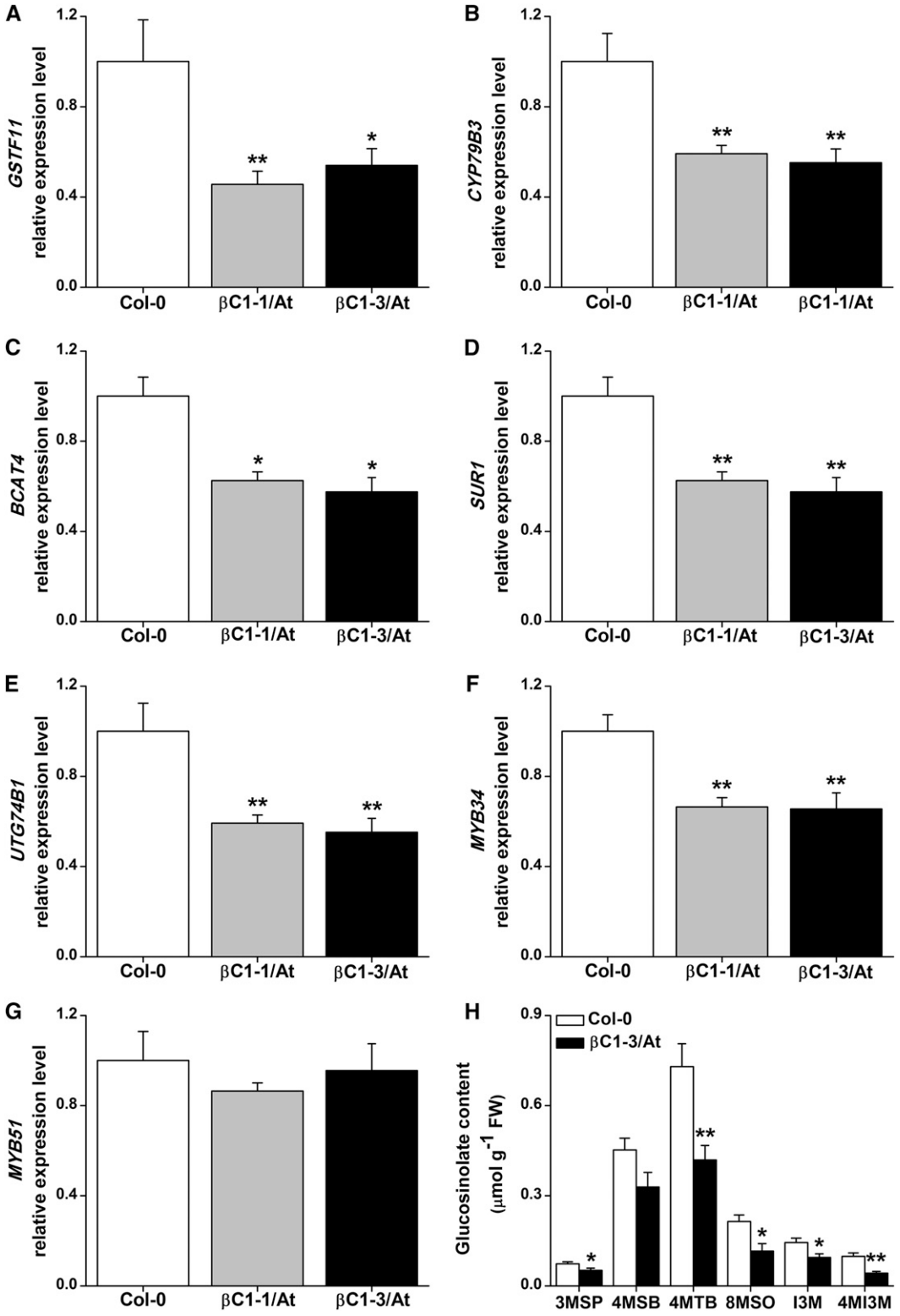

Figure 5. GS Biosynthesis in *Arabidopsis* Transgenic Lines Expressing βC1.

(A) to **(G)** Relative expression levels of the GS biosynthesis genes *GSTF11* **(A)**, *CYP79B3* **(B)**, *BCAT4* **(C)**, *SUR1* **(D)**, *UTG74B1* **(E)**, *MYB34* **(F)**, and *MYB51* **(G)** in the wild type and two *Arabidopsis* transgenic lines expressing βC1 (βC1-1/At and βC1-3/At). Values are means + SE (n = 6).

reveal that MYC2 and AS1 regulate different JA signaling branches.

DISCUSSION

Begomoviruses Suppress Host Terpene Synthesis to Promote the Performance of Their Vectors

Begomoviruses share host plants with their whitefly vectors, and these viruses manipulate host defense to indirectly influence whitefly behavior and performance. Here, we demonstrate that the emission of several terpenes was greatly decreased in TYLCCNV/TYLCCNB-infected *N. benthamiana* and CaLCuV-infected *Arabidopsis* plants, and reduction in the terpenes promoted the performance of the whitefly vector (Figure 1; Supplemental Figure 4). Meanwhile, changes of terpenes in the plants did not affect the multiplication of begomoviruses (Supplemental Figure 12), implying that whitefly feeding may exert little direct effect on the multiplication of these viruses. However, since begomoviruses depend on the whitefly *B. tabaci* for transmission, the enhanced performance of whitefly on virus-infected plants will be favorable to population increase of the vectors and, in turn, further the transmission and spread of the viruses, thereby establishing an indirect mutualistic relationship between the viruses and the vector. Tobacco and tomato plants displayed a similar reduction of terpene emission after begomovirus infection (Fang et al., 2013; Luan et al., 2013).

Plant terpenes play three main roles in resistance to whiteflies. First, some sesquiterpenes function as toxins that inhibit whitefly feeding and affect insect oviposition behavior (Luan et al., 2013); second, specific terpenes confer repellence to whiteflies and influence their host selection behavior (Bleeker et al., 2009); and third, some terpenes, for instance β-myrcene, are involved in indirect defense against whiteflies by attracting natural enemies (Zhang et al., 2013). Poor terpene release in viruliferous plants results in attenuated resistance to whitefly (Figure 1; Supplemental Figure 4; Luan et al., 2013). In *Arabidopsis*, CaLCuV infection significantly decreased the emission of β-myrcene (Supplemental Figure 4B). When β-myrcene was exogenously applied to *Arabidopsis* plants, direct repellence to whiteflies was observed (Supplemental Figure 13). By contrast, β-myrcene-treated tomato plants showed no effect on whitefly behavior (Bleeker et al., 2009). This discrepancy could be due to different β-myrcene concentrations used in the experiments (Terry et al., 2007). Another possibility is that β-myrcene may function by changing volatile profiles, and the resulting alterations may differ in different plant species, leading to variable effects on whiteflies. In *N. benthamiana*, TYLCCNV/TYLCCNB infection also increased the transcription level of *NbTPS3*, which is predicted to be a monoterpene synthase (Supplemental Figure 1). However, no increase of monoterpene was detected in TYLCCNV/TYLCCNB-infected plants compared with that in control plants (Figure 1B). We speculated that the products of Nb-TPS3 may change to other forms (e.g., hydroxylated or acylated) of metabolites that are involved in the disease resistance against pathogens, as the diterpene WAF-1 does in tobacco mosaic virus-induced defense in tobacco (Seo et al., 2003). Manipulation of plant volatiles by pathogens in favor of their vectors has been reported in many other pathogen-vector-plant systems (Eigenbrode et al., 2002; Mauck et al., 2010, 2012; Mann et al., 2012). We hypothesize that "odor manipulation" could be a general strategy employed by plant pathogens, especially vector-borne pathogens, to promote the performance of their vectors and, in turn, facilitate their transmission and spread.

The Plant Transcription Factor MYC2 Is a Target of Begomoviruses

In this study, we present several lines of evidence to support the claim that MYC2 is a target of βC1. First, βC1 and MYC2 co-localize in the nucleus (Supplemental Figure 8). Pull-down and BiFC assays showed that βC1 can interact specifically with plant MYC2 in vivo and in vitro (Figure 3). Second, MYC2 directly regulates transcript levels of the whitefly defense-related genes At-*TPS10* and Nb-*TPS1*, but these two *TPS* genes are repressed by begomoviral infection (Figure 4; Supplemental Figure 10). Third, βC1 reduces At-*TPS10* transcript levels by impairing the DNA binding activity of MYC2 (Figure 6C). Fourth, MYC2 is a key component downstream of JA biosynthesis. The JA-responsive *VSP1* gene is positively regulated by MYC2 (Kazan and Manners, 2013), and *VSP1* transcript levels decreased in βC1-overexpressing *Arabidopsis* lines (Yang et al., 2008). Compared with wild-type plants, the JA biosynthesis-related *LOX2* gene is down-regulated in *myc2* null mutant plants, suggesting that a positive feedback loop regulates JA levels via MYC2 (Zhai et al., 2013). Suppression of JA synthesis by TYLCCNV/TYLCCNB infection is probably caused by the interaction between βC1 and MYC2. Fifth, in βC1 overexpression plants, several MYC2-regulated indole and aliphatic GS biosynthesis genes such as *GSTF11* were highly repressed. Indole and aliphatic GSs function as defensive chemicals against whitefly (Elbaz et al., 2012). The biosynthesis of indole and aliphatic GSs is directly transcriptionally regulated by MYC2 (Schweizer et al., 2013). Most likely, both terpenes and GSs are involved in βC1-suppressed plant defense against whitefly. However, the deficiency of a key indole GS biosynthesis regulator, MYB34, did not affect the host location of whitefly like TPS10 (Figure 2I; Supplemental Figure 14). One possibility could be that, because of their nonvolatility, indole GSs are not as effective as terpenes as a host location signal for whiteflies. Another possibility is that the reduced amount of indole GSs in *myb34* mutants is not

Figure 5. (continued).

(H) Individual aliphatic (3MSP, 4MSB, 4MTB, and 8MSO) and indolic (I3M and 4MI3M) GS contents in wild-type *Arabidopsis* (Col-0) and the transgenic βC1 expression line βC1-1/At. 3MSP, 3-methylsulfinylpropyl glucosinolate; 4MSB, 4-methylsulfinylbutyl glucosinolate; 4MTB, 4-methylthiobutyl glucosinolate; 8MSO, 8-methylsulfinyloctyl glucosinolate; I3M, indolyl-3-methyl glucosinolate; 4MI3M, 4-methoxy-indolyl-3-methyl glucosinolate; FW, fresh weight.

Asterisks indicate significant differences between different lines (*$P < 0.05$, **$P < 0.01$; Student's *t* test).

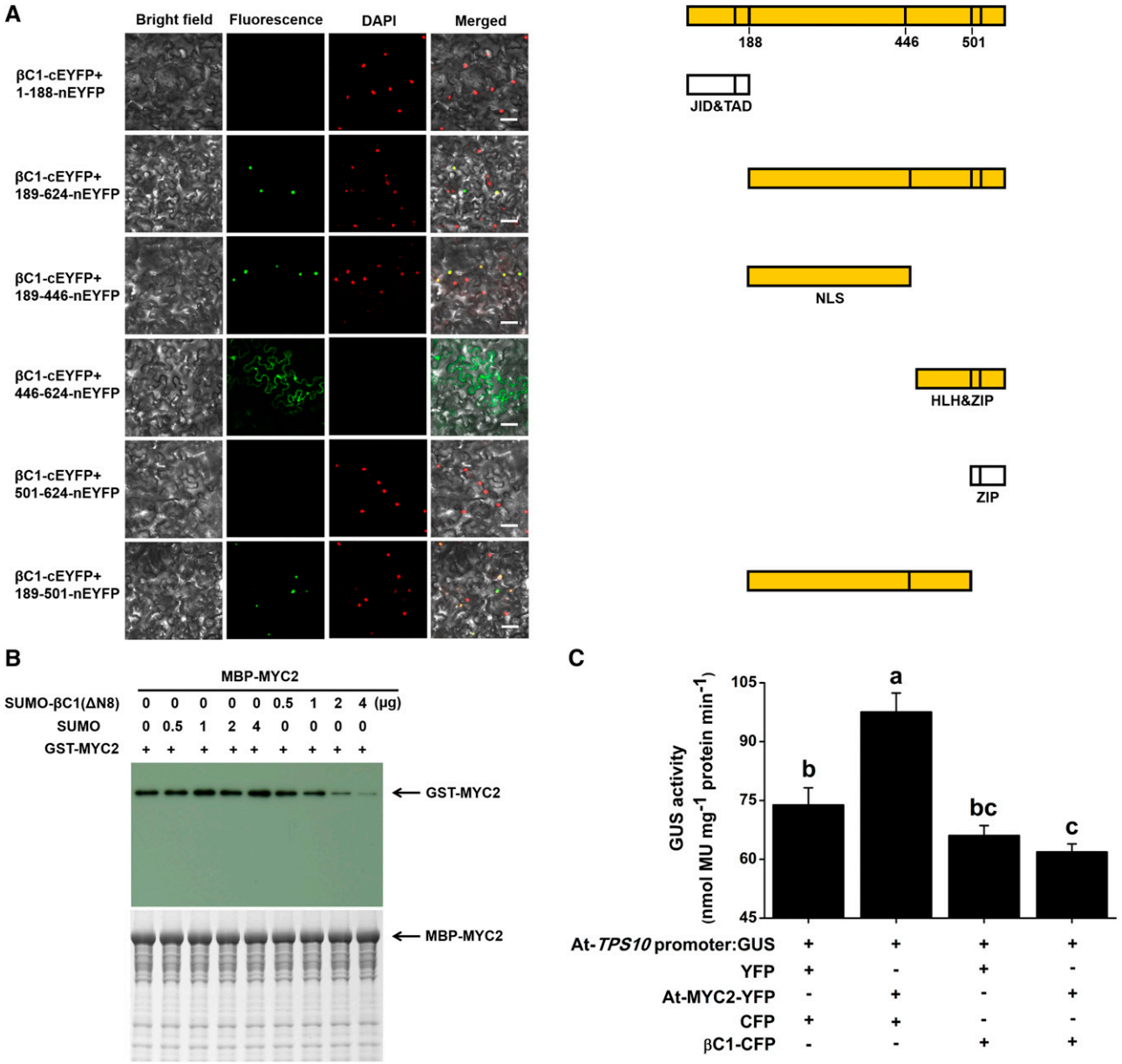

Figure 6. βC1 Suppresses MYC2 DNA Binding Activity by Interacting with the bHLH Domain of MYC2.

(A) Mapping of βC1-interacting domains of MYC2 using BiFC assays. The right panel shows a schematic protein structure of MYC2. JID, JAZ interaction domain; TAD, MED25 interaction domain; ZIP, leucine zipper. MYC2 and its deletion derivatives were fused with the N-terminal part of EYFP. βC1 was fused with the C-terminal part of EYFP. Nuclei in leaf epidermal cells were stained with DAPI. Bars = 50 μm.

(B) Pull-down protein competition assays. The indicated protein amount of SUMO-βC1 (ΔN8) or SUMO was mixed with 2 μg of GST-MYC2 and pulled down by 2 μg of MBP-MYC2. Immunoblots were performed using anti-GST antibody to detect the associated proteins. Membranes were stained with Coomassie Brilliant Blue to monitor input protein amount.

(C) Effects of βC1 on the activation of the At-*TPS10* promoter by At-MYC2. *AtTPS10* promoter:*GUS* was used as a reporter construct. CFP, YFP, At-MYC2-YFP, and βC1-CFP were used as effector constructs. The reporter and the respective effector constructs were coinfiltrated into tobacco leaf cells. Quantitative GUS activity of infiltrated leaf cells was measured after 2 d. Letters indicate significant differences among different treatments ($P < 0.05$; Duncan's multiple range test).

strong enough to affect whitefly preference, as two other MYB family regulators, MYB51 and MYB122, are also involved in indole GS biosynthesis (Frerigmann and Gigolashvili, 2014). Further work with mutants depleted in GSs is required to confirm the role of GSs in the host location and performance of whitefly.

MYC2 is a master regulator and has several structural domains (Kazan and Manners, 2013). Here, we found that βC1 binds to the bHLH domain of the MYC2 protein (Figure 6A), which is essential for its dimerization. In general, MYC2 forms homodimers with itself or heterodimers with other bHLH (e.g.,

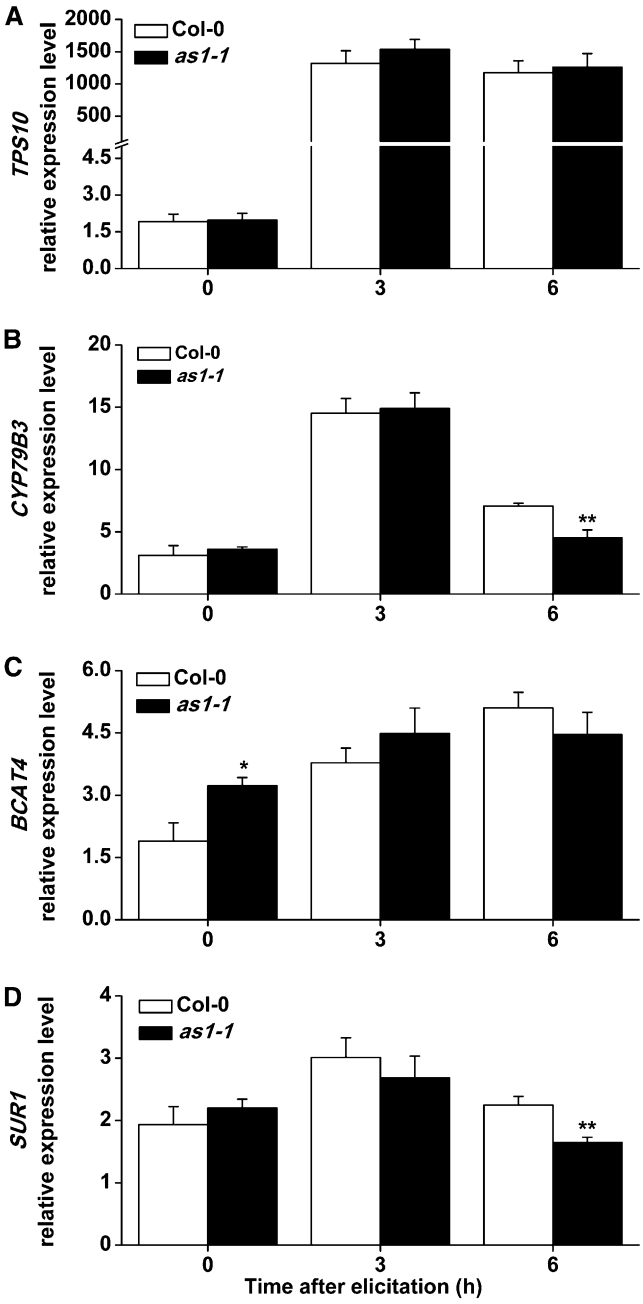

Figure 7. Expression of MYC2-Dependent Genes in the *Arabidopsis as1-1* Mutant.

Relative expression levels are shown for At-*TPS10* **(A)**, *CYP79B3* **(B)**, *BCAT4* **(C)**, and *SUR1* **(D)** in the wild type and *as1-1*. Values are means + SE (n = 6). Asterisks indicate significant differences between different lines (*P < 0.05, **P < 0.01; Student's *t* test).

MYC3 and MYC4) and MYB (e.g., MYB51 and MYB34) transcription factors to bind to *cis*-regulating elements of target promoters (Kazan and Manners, 2013; Schweizer et al., 2013). In vitro competitive pull-down assays showed that βC1 interferes with MYC2 dimerization (Figure 6B). The two α-helix structures of βC1 contribute to the heterodimerization with MYC2 (Supplemental Figure 15). Animal viruses use molecular mimicry to reprogram their host signaling pathways (e.g., the Notch and Wnt pathways) to enhance viral gene expression and downregulate host defensive gene expression (Hayward et al., 2006). Here, we show that begomoviruses use a structure-mimicking (Supplemental Figure 15) strategy to manipulate the MYC2-mediated defensive JA signaling pathway to benefit their insect vector. It has been reported that the phytoplasma effector SAP11 protein interacts with and destabilizes *Arabidopsis* TCP transcription factors to impair plant defense against its vector. The HLH domain of the TCP protein was predicted to be the putative binding site of SAP11 (Sugio et al., 2011). We speculate that the formation of abnormal heterodimers may be one way for a pathogen to suppress plant defense and enhance vector performance.

MYC2-TPS Is a Conserved and Efficient Defense Pathway against Whitefly

Terpenoids confer effective resistance to whiteflies (Bleeker et al., 2009, 2012; Luan et al., 2013), and some terpene biosynthesis genes have been identified to be involved in this process. 7-Epizingiberene synthase, a key enzyme in the biosynthesis of the sesquiterpene 7-epizingiberene in wild tomato, is a major factor in the resistance of wild tomatoes (*Solanum habrochaites* LA1777) to whiteflies (Bleeker et al., 2012). *TPS*-silenced *Arabidopsis* or tobacco plants were more susceptible to whiteflies (Figure 2F; Luan et al., 2013). The terpenoids may be constitutively emitted by plants such as tomato, and their emission levels become elevated after whitefly infestation (Bleeker et al., 2009; Luan et al., 2013). However, the emission of terpenes in *Arabidopsis* leaves requires induction by some chemicals such as JA or biotic stress (Supplemental Figure 4B; Tholl and Lee, 2011). *Arabidopsis* plants exposed to whitefly feeding upregulate the transcription of both *TPS10* and *MYC2* (Figure 2C; Supplemental Figure 6), suggesting that the *Arabidopsis* MYC2-TPS10 pathway is part of an induced defense against these insects. Furthermore, tomato Sl-MYC1, the homolog of At-MYC2, can transiently transactivate some potato (*Solanum tuberosum*) terpene synthase promoters in *N. benthamiana* leaves (Spyropoulou et al., 2014). We hypothesize that MYC2-TPS is a conserved element of plant resistance against whitefly infestation. Hong et al. (2012) showed that MYC2 positively regulates genes for sesquiterpene synthase (At-*TPS11* and At-*TPS21*) in the inflorescence, although they did not show roles of the sesquiterpenes in plant-insect interaction. The discovery that three *TPS* genes are directly regulated by MYC2 suggests that both *TPS* and *MYC2* are promising targets in improving the resistance of plants to whitefly.

βC1 Represses the JA Signaling Pathway to Achieve Begomovirus-Whitefly Mutualism by Targeting at Least Two Distinct Molecular Targets

βC1 has been identified to interact with AS1, which is well known for its function in developmental regulation. The *Arabidopsis* AS complex represses homeobox genes such as *KNAT2* to stably silence these stem cell regulators in differentiating leaves (Lodha et al., 2013). βC1 mimics AS2 to compete with AS1 to disable the

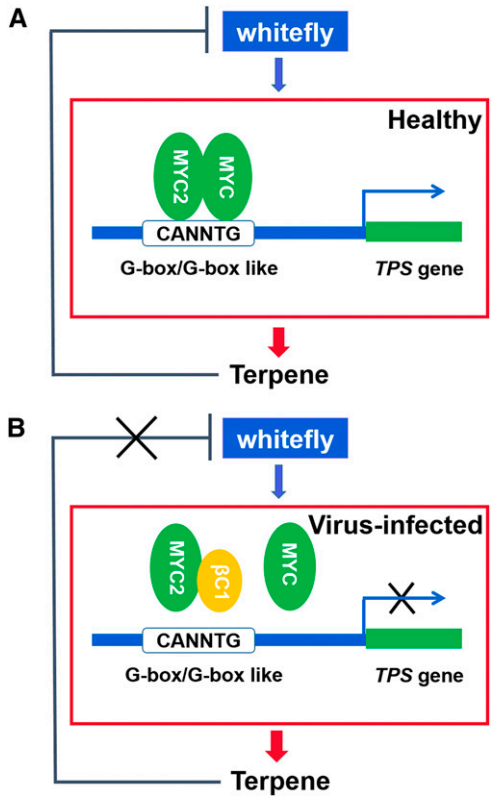

Figure 8. A Working Model of Plant MYC2 and MYC2-Like Transcription Factors in Begomovirus-Whitefly-Plant Tripartite Interactions.

(A) Plant MYC2 mediates the transcription activation of *TPS* genes by direct binding to G-box/G-box-like elements of the promoter region. Whitefly feeding activates the transcription of *MYC2* and *TPS* genes. Monoterpenes or sesquiterpenes are released from plants to defend against whitefly.

(B) In begomovirus-infected plants, however, βC1 interacts with MYC2, interfering with MYC2 dimerization, which is necessary for the activation of JA-mediated plant resistance. This interaction decreases the DNA binding activity of MYC2 and suppresses transcript levels of *TPS*, leading to reduced release of terpenes. Therefore, begomovirus-infected plants become more susceptible to whiteflies.

[See online article for color version of this figure.]

repression complex and to upregulate several stem cell genes, including the homeobox genes *KNAT1*, *KNAT2*, and *KNAT6* and *ARF2*, *ARF3*, and *ARF4*, whose expression levels are well correlated with the phenotype severity (Supplemental Figures 3A and 3C). However, the fact that the expression levels of the MYC2-regulated genes *TPS* and *GS* do not differ in two phenotypically distinct plant lines (Figures 1C, 1E, and 5) strongly suggests that the targets of βC1 on the developmental process and JA defensive pathway are distinct.

Plant viral pathogens have a compact genome in which the encoded proteins, especially the pathogenicity factors, are frequently multifunctional. βC1 has evolved to target at least two different transcription factors in the JA pathway, and the majority of the genes regulated by AS1 and MYC2 are different (Supplemental Figure 16). The first subset of genes repressed by βC1 is via AS1; AS1 is a negative regulator of genes such as *PLANT DEFENSIN1.2* and *PATHOGENESIS-RELATED4*, which in turn are induced by infection with necrotrophic fungi (Nurmberg et al., 2007). βC1 enhances the repressive activity of AS1 for these two genes (Yang et al., 2008). The second subset of genes is repressed via a MYC2-dependent pathway (e.g., At-*TPS10* and *GS* genes) (Figure 6). The expression of At-*TPS10* in *as1-1* mutants is the same as in wild-type plants after MeJA treatment (Figure 7A), suggesting that At-*TPS10* is an AS1-independent gene. The third subset of genes is genetically controlled by both MYC2 and AS1 (e.g., *CYP79B3* and *SUR1*), whose expression is decreased in either the *as1-1* or *myc2-1* mutant. Nevertheless, further work is required to distinguish the epistasis relationship between AS1 and MYC2 in controlling this subset of genes. In addition, transcriptome analysis in combination with metabolic analysis of βC1-expressing plants, *myc2-1*, *as1-1*, and *as2*, also will be very helpful to ascertain the exact roles of MYC2 and AS1 in βC1-mediated whitefly-begomovirus mutualism.

In conclusion, whitefly feeding activates the MYC2 transcription factor in plants that are not infected with the begomovirus. Dimerized MYC2 binds to the G-box/G-box-like elements of *TPS* gene promoter regions, resulting in elevated *TPS* transcript levels and increased TPS, which contribute to direct and indirect resistance to whiteflies (Figure 8A). However, in begomovirus-infected plants, MYC2 protein activity is repressed by βC1 due to heterodimerization, resulting in reduced *TPS* transcript levels. The decreased terpene synthesis in begomovirus-infected plants renders them more susceptible to whiteflies (Figure 8B). Begomoviruses benefit from the virus-vector indirect mutualism through an increased probability of transmission to new hosts via whiteflies. Here, we have provided evidence that the plant transcription factor MYC2 is involved in begomovirus-whitefly-plant interactions. Our mechanistic analysis of begomovirus-vector mutualism will help to understand virus epidemiology and may provide novel strategies for pest/viral disease management.

METHODS

Plant Materials and Growth Conditions

Arabidopsis thaliana (Col-0) was used as the wild type. The following mutants and transgenic plants, all in the Col-0 background, were used: *myc2-1*, *myc2-2*, *myb34*, *as1-1*, *35S:HA-βC1*/Col-0 (Yang et al., 2008), and *tps10-2* (SALK_041114). Sterilized seeds were incubated on Murashige and Skoog medium at 4°C for 3 d before being transferred to a growth chamber (22°C with 10 h of light/14 h of dark). Transgenic *Nicotiana benthamiana* plants carrying *35S:βC1* have been reported previously (Yang et al., 2008). *N. benthamiana* plants were grown in an insect-free growth chamber at 25°C with 12 h of light/12 h of dark.

Whitefly Culture

Whiteflies were captured in fields in Singapore and were identified as *Bemisia tabaci* MEAM1 (mtCOI GenBank accession number GQ332577). The whitefly population was maintained on cotton (*Gossypium hirsutum*) grown in a growth chamber (25°C, 65% RH) with a 14-h-light/10-h-dark light cycle.

Virus Inoculation

For *Tomato yellow leaf curl China virus* infection, *N. benthamiana* plants with four to six true leaves were infiltrated with *Agrobacterium tumefaciens*

carrying TYLCCNV and betasatellite DNAβ (isolate Y10) as described previously (Cui et al., 2004; Yang et al., 2008). Infiltration with TYLCCNV alone or TYLCCNV plus a mutant betasatellite carrying a βC1 mutation was used as a control (Cui et al., 2004). For *Cabbage leaf curl virus* experiments, infectious clones (DNA-A and DNA-B) suitable for agroinfiltration were generated and used to infect *Arabidopsis* plants with six to eight true leaves. Infiltration of *Agrobacterium* containing DNA-A alone was used as a control.

Plant Treatments

Four week-old *Arabidopsis* and *N. benthamiana* plants were sprayed with 100 μM MeJA (Sigma-Aldrich) containing 0.01% (v/v) Tween 20. Plants used for volatile analysis and whitefly choice experiments were treated with MeJA and were used in experiments 6 h after treatment. Control plants were treated with 0.01% (v/v) Tween 20 in parallel for the same time period. For wounding experiments, rosette leaves of 3- to 4-week-old *Arabidopsis* plants were crushed twice across the apical lamina with a forceps. Nontreated plants were used as controls. Samples were collected at the indicated time points. For whitefly feeding experiments, 4-week old *Arabidopsis* plants were placed, one plant per cup, inside transparent cups (diameter, 118 mm; height, 112 mm) with a nylon-screen donut lid. One hundred adult whiteflies were captured and released into each cup. Samples were harvested after whitefly feeding at the indicated time points. Five plants were used for each treatment at each time interval. For terpene treatment, 100 μg of β-myrcene dissolved in 20 μL of lanolin paste was applied to *Arabidopsis* rosette leaves. Control plants were treated with 20 μL of pure lanolin.

Whitefly Bioassay

The choice experiments were performed as described previously (Bleeker et al., 2009). Two plants of similar size and leaf numbers were pretreated with MeJA and placed in a cage (30 × 30 × 30 cm). Two hundred adult whiteflies were captured, placed on ice for 1 min, and then released in between the two plants. At 15 min after insect release, the settled whiteflies were recaptured, and the number on each of the two plants was recorded. For whitefly oviposition experiments, three female and three male whitefly adults were released to a single plant placed in a transparent cylinder (diameter, 118 mm; height, 112 mm) with a nylon-screen donut lid. All the eggs on the *Arabidopsis* leaves were counted with a microscope after 10 d, and the number of eggs per emerged female was determined. For the whitefly developmental progression experiment, 16 female adults were inoculated to a single plant placed in a transparent cylinder with a nylon-screen donut lid. After 2 d of oviposition, all adults were removed, and the eggs were allowed to develop. The total number of progeny and their development were recorded after 22 d. Developmental progression was estimated by calculating the proportion of late fourth instar nymphs (red-eye yellowish) and empty exuvia on each plant. Eight plants of each line were used in each experiment.

Volatile Analysis

The collection, isolation, and identification of volatiles from *N. benthamiana* plants were performed using the method of Li et al. (2013). Volatiles emitted from individual plants of each line treated with MeJA were collected. The compounds were expressed as percentages of peak areas relative to the internal standard (camphor) per 18 h of trapping using one plant. The volatiles of *Arabidopsis* were collected and analyzed as described previously (Pineda et al., 2013), with minor modification. Volatile trapping was done for 3 h at a flow of 300 mL/min, and the gas chromatography oven temperature program was as follows: 40°C for 2 min, raised at 10°C/min to 280°C, and then held for 4 min. Six plants of each line were used.

In Vitro Pull-Down and Protein-Protein Competitive Binding Assays

The recombinant GST and MBP tag proteins were purified using GST- and MBP-Trap (GE Healthcare) according to the manufacturer's instructions. The in vitro pull-down assay was performed with 2 μg of GST fusion proteins and 2 μg of MBP fusion proteins as described (Yang et al., 2008). For competitive pull-down assay, the pET29a-βC1 (△N8) construct was prepared as described (Yang et al., 2008). His fusion proteins were purified using His-Trap (GE Healthcare) according to the manufacturer's instructions. Indicated amounts of βC1 (△N8) were mixed with 2 μg of MBP-At-MYC2 and 30 μL of Dextrin Sepharose High Performance (GE Healthcare) overnight. After centrifugation two times and two washes with binding buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.25% Triton X-100, and 35 mM β-mercaptoethanol), 2 μg of GST-At-MYC2 was added and the mixture was incubated for 2 h at 4°C. After washing six times with binding buffer, pulled-down proteins were separated on 12% SDS-polyacrylamide gels and detected by immunoblot using anti-GST antibody.

BiFC

Leaves of 3-week-old *N. benthamiana* plants were infiltrated with agrobacterial cells containing the indicated constructs. Two days after incubation, fluorescence and DAPI staining were analyzed by confocal microscopy (Liu et al., 2009).

VIGS

Leaves of 3-week-old *N. benthamiana* plants were agroinfiltrated with *psTRV1* and *psTRV2-NbTPS1* or *psTRV2-NbMYC2*. Plants coinfiltrated with *psTRV1* and *psTRV2* were used as controls (Qu et al., 2012).

Yeast Two-Hybrid Analysis

The *Arabidopsis* Mate and Plate Library was kindly provided by Yuehui He (Temasek Life Sciences Laboratory). Full-length βC1 was inserted into the pGBKT7 vector. The library was screened using yeast mating according to the Matchmaker Gold Yeast Two-Hybrid System manufacturer's protocol (Clontech). Confirmation of the interaction between βC1 and At-MYC2 was performed according to the manufacturer's protocol (Clontech). The yeast strain Y2HGold was cotransformed with pGBKT7-βC1 and pGADT7-At-MYC2 constructs and plated on SD-Leu-Trp selective dropout medium. Colonies were transferred onto SD-Leu-Trp-His plates with 2 mM 3-amino-1,2,4-triazole.

Quantitative RT-PCR

Total RNA was isolated using the RNeasy Plant Mini Kit (Qiagen), and 800 ng of total RNA for each sample was reverse transcribed using the PrimeScript RT-qPCR Kit (TaKaRa). Four to six independent biological samples were collected and analyzed. RT-qPCR was performed on the ABI 7900HT fast real-time system (Life Technologies) using SYBR Green Real-Time PCR Master Mixes (Life Technologies). The primers used for mRNA detection of target genes by real-time PCR are listed in Supplemental Table 1. The *Arabidopsis* α-*TUBULIN2* (At5g62690) mRNA and *N. benthamiana EF1*α mRNA were used as internal controls.

GUS Activity Assay

Leaves of *N. benthamiana* were agroinfiltrated with the indicated constructs. Two days after infiltration, leaves were harvested and frozen in liquid nitrogen. Each treatment was repeated eight times. GUS quantitative assay and histochemistry were performed as described (Jefferson, 1987).

ChIP Assay

Transgenic *Arabidopsis* plants expressing *35S:MYC2-GFP/myc2-2* were used for ChIP assay. About 3 g of seedlings was harvested and fixed in 1% formaldehyde solution under vacuum for 10 min. Glycine was added to a final concentration of 0.125 M, and the sample was treated with vacuum for an additional 5 min. After three washes with distilled water, samples were frozen in liquid nitrogen. ChIP experiments were performed as described (Jang et al., 2011) using anti-GFP agarose beads (GFPtrack) for immunoprecipitation. The resulting DNA samples were purified with the QIAquick PCR purification kit (Qiagen). The experiments were repeated with three independent biological samples. The relative abundance of the indicated DNA fragments was normalized using the *Arabidopsis ACTIN2* promoter as a control.

GS Assay

GSs were extracted and analyzed as described previously (Guo et al., 2013). Leaf samples (200 mg) from the wild type (Col-0) or the transgenic βC1 expression line (βC1-1/At) were used. Each line was replicated eight times.

Data Analysis

Differences in gene expression levels and volatile organic compound levels in different lines or in response to different treatments or times of treatment were determined by Student's *t* tests. Differences in quantitative GUS activity between different treatments were analyzed by one-way ANOVA; if the ANOVA result was significant ($P < 0.05$), Duncan's multiple range tests were used to detect significant differences between groups. Differences in whitefly choice between different lines or treatments were analyzed by nonparametric Wilcoxon matched pairs tests (with two dependent samples). All tests were performed with Statistica (SAS Institute; http://www.sas.com/).

Accession Numbers

Sequence data from this work can be found in GenBank/EMBL or TAIR (www.Arabidopsis.org) under the following accession numbers: At-MYC2 (At1g32640), At-TPS10 (At2g24210), AS1 (At2g37630), Nb-MYC2 (GQ859152), Nb-TPS1 (KF990999), TYLCCNV βC1 (AJ421621), and CaLCuV BV1 (NP_624352).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. Phylogenetic Relationships of Terpene Synthases from Plants.

Supplemental Figure 2. Effect of βC1 on *terpene synthase* Transcript Levels in *N. benthamiana*.

Supplemental Figure 3. Ectopic Expression of *βC1* Affects *Arabidopsis* and *N. benthamiana* Leaf Development.

Supplemental Figure 4. Bipartite Begomovirus Increases Whitefly Attraction and Performance on *Arabidopsis* by Decreasing Terpene Synthesis.

Supplemental Figure 5. Transcript Levels of Different Genes in Wild-Type and Empty Vector Transgenic *Arabidopsis*.

Supplemental Figure 6. Expression Profiles of At-*TPS10* and At-*MYC2* Induced by Whitefly Feeding.

Supplemental Figure 7. Phenotype of *TPS10* T-DNA Insertion Mutant in *Arabidopsis* (SALK_041114).

Supplemental Figure 8. Colocalization of TYLCCNB βC1, CaLCuV BV1 Protein, and Plant MYC2 Transcription Factors.

Supplemental Figure 9. EMSA Analysis of MYC2 Binding to the G-Box-Like Elements.

Supplemental Figure 10. Transcript Level of *TPS10* in Wild-Type and *myc2* Knockout *Arabidopsis*.

Supplemental Figure 11. Self-Interaction of At-MYC2 Assayed by BiFC Assay.

Supplemental Figure 12. The Role of MYC2 and TPS in Begomoviruses Viral Titer.

Supplemental Figure 13. The Role of β-Myrcene in Whitefly Feeding Behavior.

Supplemental Figure 14. The Role of GS Biosynthesis-Related Gene *MYB34* in Whitefly Feeding Behavior.

Supplemental Figure 15. Helix Domain of βC1 Is Sufficient to Bind with MYC2 Protein.

Supplemental Figure 16. AS1 and MYC2 Regulate Different JA Pathways against Whitefly.

Supplemental Table 1. DNA Primers Used in This Study.

Supplemental Methods.

Supplemental References.

Supplemental Data Set 1. ClustalW2 Alignment Corresponding to Supplemental Figure 1, in PIR Format.

ACKNOWLEDGMENTS

We thank Gang Wang, Xiyuan Jiang, Yunlin Su, KharMeng Ng, Yuehui He, Junyi Yang (National Chung Hsing University) and Xueping Zhou (Zhejiang University) for their invaluable assistance with experiments. This study was supported by the Chinese Academy of Sciences (Strategic Priority Research Program Grant XDB11040300 to J.Y.), the Singapore National Research Foundation (CRP Award NRF-CRP8-2011-02 to J.Y.), and in part by grants from the Singapore Millennium Foundation, the Cooperative Research Program for Agricultural Science and Technology Development (Grant PJ906910), the Rural Development Administration, Republic of Korea (to N.-H.C.), and the National Natural Science Foundation of China (Grant 31390421 to S.-S.L.).

AUTHOR CONTRIBUTIONS

J.Y. and R.L. designed experiments. R.L. performed gene expression analysis, *N. benthamiana* volatile analysis, whitefly bioassays, protein interaction assays, GUS activity assays, and VIGS assays. J.L., Y.W.S., and H.M.Q. participated in these experiments. B.T.W. performed *Arabidopsis* volatile analysis. C.J. discovered the BV1/At-MYC2 interaction and performed the assay. J.Q. performed ChIP experiments. R.L., N.-H.C., S.-S.L., and J.Y. wrote the article, which was reviewed and approved by all authors.

Received October 14, 2014; revised November 11, 2014; accepted November 24, 2014; published December 9, 2014.

REFERENCES

Ascencio-Ibáñez, J.T., Sozzani, R., Lee, T.J., Chu, T.M., Wolfinger, R.D., Cella, R., and Hanley-Bowdoin, L. (2008). Global analysis of Arabidopsis gene expression uncovers a complex array of changes

impacting pathogen response and cell cycle during geminivirus infection. Plant Physiol. **148:** 436–454.

Beanland, L., Hoy, C., Miller, S., and Nault, L. (2000). Influence of aster yellows phytoplasma on the fitness of aster leafhopper (Homoptera: Cicadellidae). Ann. Entomol. Soc. Am. **93:** 271–276.

Bleeker, P.M., Diergaarde, P.J., Ament, K., Guerra, J., Weidner, M., Schütz, S., de Both, M.T., Haring, M.A., and Schuurink, R.C. (2009). The role of specific tomato volatiles in tomato-whitefly interaction. Plant Physiol. **151:** 925–935.

Bleeker, P.M., Mirabella, R., Diergaarde, P.J., VanDoorn, A., Tissier, A., Kant, M.R., Prins, M., de Vos, M., Haring, M.A., and Schuurink, R.C. (2012). Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. Proc. Natl. Acad. Sci. USA **109:** 20124–20129.

Bohlmann, J., Martin, D., Oldham, N.J., and Gershenzon, J. (2000). Terpenoid secondary metabolism in *Arabidopsis thaliana*: cDNA cloning, characterization, and functional expression of a myrcene/(*E*)-β-ocimene synthase. Arch. Biochem. Biophys. **375:** 261–269.

Casteel, C.L., Yang, C., Nanduri, A.C., De Jong, H.N., Whitham, S.A., and Jander, G. (2014). The NIa-Pro protein of *Turnip mosaic virus* improves growth and reproduction of the aphid vector, *Myzus persicae* (green peach aphid). Plant J. **77:** 653–663.

Chen, R., Jiang, H., Li, L., Zhai, Q., Qi, L., Zhou, W., Liu, X., Li, H., Zheng, W., Sun, J., and Li, C. (2012). The *Arabidopsis* mediator subunit MED25 differentially regulates jasmonate and abscisic acid signaling through interacting with the MYC2 and ABI5 transcription factors. Plant Cell **24:** 2898–2916.

Cui, X., Tao, X., Xie, Y., Fauquet, C.M., and Zhou, X. (2004). A DNAβ associated with *Tomato yellow leaf curl China virus* is required for symptom induction. J. Virol. **78:** 13966–13974.

De Boer, K., Tilleman, S., Pauwels, L., Vanden Bossche, R., De Sutter, V., Vanderhaeghen, R., Hilson, P., Hamill, J.D., and Goossens, A. (2011). APETALA2/ETHYLENE RESPONSE FACTOR and basic helix-loop-helix tobacco transcription factors cooperatively mediate jasmonate-elicited nicotine biosynthesis. Plant J. **66:** 1053–1065.

Dicke, M., and Baldwin, I.T. (2010). The evolutionary context for herbivore-induced plant volatiles: Beyond the 'cry for help.' Trends Plant Sci. **15:** 167–175.

Dombrecht, B., Xue, G.P., Sprague, S.J., Kirkegaard, J.A., Ross, J.J., Reid, J.B., Fitt, G.P., Sewelam, N., Schenk, P.M., Manners, J.M., and Kazan, K. (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*. Plant Cell **19:** 2225–2245.

Eigenbrode, S.D., Ding, H., Shiel, P., and Berger, P.H. (2002). Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proc. Biol. Sci. **269:** 455–460.

Elbaz, M., Halon, E., Malka, O., Malitsky, S., Blum, E., Aharoni, A., and Morin, S. (2012). Asymmetric adaptation to indolic and aliphatic glucosinolates in the B and Q sibling species of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Mol. Ecol. **21:** 4533–4546.

Falara, V., Akhtar, T.A., Nguyen, T.T., Spyropoulou, E.A., Bleeker, P.M., Schauvinhold, I., Matsuba, Y., Bonini, M.E., Schilmiller, A.L., Last, R.L., Schuurink, R.C., and Pichersky, E. (2011). The tomato terpene synthase gene family. Plant Physiol. **157:** 770–789.

Fang, Y., Jiao, X., Xie, W., Wang, S., Wu, Q., Shi, X., Chen, G., Su, Q., Yang, X., Pan, H., and Zhang, Y. (2013). *Tomato yellow leaf curl virus* alters the host preferences of its vector *Bemisia tabaci*. Sci. Rep. **3:** 2876.

Fernández-Calvo, P., et al. (2011). The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant Cell **23:** 701–715.

Fontes, E.P., Santos, A.A., Luz, D.F., Waclawovsky, A.J., and Chory, J. (2004). The geminivirus nuclear shuttle protein is a virulence factor that suppresses transmembrane receptor kinase activity. Genes Dev. **18:** 2545–2556.

Frerigmann, H., and Gigolashvili, T. (2014). MYB34, MYB51, and MYB122 distinctly regulate indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. Mol. Plant **7:** 814–828.

Godoy, M., Franco-Zorrilla, J.M., Pérez-Pérez, J., Oliveros, J.C., Lorenzo, O., and Solano, R. (2011). Improved protein-binding microarrays for the identification of DNA-binding specificities of transcription factors. Plant J. **66:** 700–711.

Guo, R., Qian, H., Shen, W., Liu, L., Zhang, M., Cai, C., Zhao, Y., Qiao, J., and Wang, Q. (2013). BZR1 and BES1 participate in regulation of glucosinolate biosynthesis by brassinosteroids in Arabidopsis. J. Exp. Bot. **64:** 2401–2412.

Hayward, S.D., Liu, J., and Fujimuro, M. (2006). Notch and Wnt signaling: Mimicry and manipulation by gamma herpesviruses. Sci. STKE **2006:** re4.

Hong, G.J., Xue, X.Y., Mao, Y.B., Wang, L.J., and Chen, X.Y. (2012). *Arabidopsis* MYC2 interacts with DELLA proteins in regulating sesquiterpene synthase gene expression. Plant Cell **24:** 2635–2648.

Hurd, H. (2003). Manipulation of medically important insect vectors by their parasites. Annu. Rev. Entomol. **48:** 141–161.

Jang, I.C., Chung, P.J., Hemmes, H., Jung, C., and Chua, N.H. (2011). Rapid and reversible light-mediated chromatin modifications of *Arabidopsis phytochrome A* locus. Plant Cell **23:** 459–470.

Jefferson, R.A. (1987). Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol. Biol. Rep. **5:** 387–405.

Jiu, M., Zhou, X.P., Tong, L., Xu, J., Yang, X., Wan, F.H., and Liu, S.S. (2007). Vector-virus mutualism accelerates population increase of an invasive whitefly. PLoS ONE **2:** e182.

Kazan, K., and Manners, J.M. (2013). MYC2: The master in action. Mol. Plant **6:** 686–703.

Kessler, A., and Baldwin, I.T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. Science **291:** 2141–2144.

Lacroix, R., Mukabana, W.R., Gouagna, L.C., and Koella, J.C. (2005). Malaria infection increases attractiveness of humans to mosquitoes. PLoS Biol. **3:** e298.

Lefèvre, T., Roche, B., Poulin, R., Hurd, H., Renaud, F., and Thomas, F. (2008). Exploiting host compensatory responses: The 'must' of manipulation? Trends Parasitol. **24:** 435–439.

Li, R., Afsheen, S., Xin, Z., Han, X., and Lou, Y. (2013). *OsNPR1* negatively regulates herbivore-induced JA and ethylene signaling and plant resistance to a chewing herbivore in rice. Physiol. Plant. **147:** 340–351.

Liu, C., Xi, W., Shen, L., Tan, C., and Yu, H. (2009). Regulation of floral patterning by flowering time genes. Dev. Cell **16:** 711–722.

Lodha, M., Marco, C.F., and Timmermans, M.C. (2013). The ASYMMETRIC LEAVES complex maintains repression of KNOX homeobox genes via direct recruitment of Polycomb-repressive complex2. Genes Dev. **27:** 596–601.

Lozano-Durán, R., Rosas-Díaz, T., Gusmaroli, G., Luna, A.P., Taconnat, L., Deng, X.W., and Bejarano, E.R. (2011). Geminiviruses subvert ubiquitination by altering CSN-mediated derubylation of SCF E3 ligase complexes and inhibit jasmonate signaling in *Arabidopsis thaliana*. Plant Cell **23:** 1014–1032.

Luan, J.B., Wang, X.W., Colvin, J., and Liu, S.S. (2014). Plant-mediated whitefly-begomovirus interactions: Research progress and future prospects. Bull. Entomol. Res. **104:** 267–276.

Luan, J.B., Yao, D.M., Zhang, T., Walling, L.L., Yang, M., Wang, Y.J., and Liu, S.S. (2013). Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. Ecol. Lett. **16:** 390–398.

Mann, R.S., Ali, J.G., Hermann, S.L., Tiwari, S., Pelz-Stelinski, K.S., Alborn, H.T., and Stelinski, L.L. (2012). Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. PLoS Pathog. **8:** e1002610.

Markovich, O., Kafle, D., Elbaz, M., Malitsky, S., Aharoni, A., Schwarzkopf, A., Gershenzon, J., and Morin, S. (2013). *Arabidopsis thaliana* plants with different levels of aliphatic- and indolylglucosinolates affect host selection and performance of *Bemisia tabaci*. J. Chem. Ecol. **39:** 1361–1372.

Mauck, K., Bosque-Pérez, N.A., Eigenbrode, S.D., De Moraes, C.M., and Mescher, M.C. (2012). Transmission mechanisms shape pathogen effects on host-vector interactions: Evidence from plant viruses. Funct. Ecol. **26:** 1162–1175.

Mauck, K.E., De Moraes, C.M., and Mescher, M.C. (2010). Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proc. Natl. Acad. Sci. USA **107:** 3600–3605.

Navas-Castillo, J., Fiallo-Olivé, E., and Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. Annu. Rev. Phytopathol. **49:** 219–248.

Nurmberg, P.L., Knox, K.A., Yun, B.W., Morris, P.C., Shafiei, R., Hudson, A., and Loake, G.J. (2007). The developmental selector AS1 is an evolutionarily conserved regulator of the plant immune response. Proc. Natl. Acad. Sci. USA **104:** 18795–18800.

Pineda, A., Soler, R., Weldegergis, B.T., Shimwela, M.M., van Loon, J.J., and Dicke, M. (2013). Non-pathogenic rhizobacteria interfere with the attraction of parasitoids to aphid-induced plant volatiles via jasmonic acid signalling. Plant Cell Environ. **36:** 393–404.

Qu, J., Ye, J., Geng, Y.F., Sun, Y.W., Gao, S.Q., Zhang, B.P., Chen, W., and Chua, N.H. (2012). Dissecting functions of *KATANIN* and *WRINKLED1* in cotton fiber development by virus-induced gene silencing. Plant Physiol. **160:** 738–748.

Ralston, L., Kwon, S.T., Schoenbeck, M., Ralston, J., Schenk, D.J., Coates, R.M., and Chappell, J. (2001). Cloning, heterologous expression, and functional characterization of 5-epi-aristolochene-1,3-dihydroxylase from tobacco (*Nicotiana tabacum*). Arch. Biochem. Biophys. **393:** 222–235.

Rosenberg, R., and Beard, C.B. (2011). Vector-borne infections. Emerg. Infect. Dis. **17:** 769–770.

Schweizer, F., Fernández-Calvo, P., Zander, M., Diez-Diaz, M., Fonseca, S., Glauser, G., Lewsey, M.G., Ecker, J.R., Solano, R., and Reymond, P. (2013). *Arabidopsis* basic helix-loop-helix transcription factors MYC2, MYC3, and MYC4 regulate glucosinolate biosynthesis, insect performance, and feeding behavior. Plant Cell **25:** 3117–3132.

Seo, S., Seto, H., Koshino, H., Yoshida, S., and Ohashi, Y. (2003). A diterpene as an endogenous signal for the activation of defense responses to infection with tobacco mosaic virus and wounding in tobacco. Plant Cell **15:** 863–873.

Spyropoulou, E.A., Haring, M.A., and Schuurink, R.C. (2014). RNA sequencing on *Solanum lycopersicum* trichomes identifies transcription factors that activate terpene synthase promoters. BMC Genomics **15:** 402.

Sugio, A., Kingdom, H.N., MacLean, A.M., Grieve, V.M., and Hogenhout, S.A. (2011). Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. Proc. Natl. Acad. Sci. USA **108:** E1254–E1263.

Terry, I., Walter, G.H., Moore, C., Roemer, R., and Hull, C. (2007). Odor-mediated push-pull pollination in cycads. Science **318:** 70.

Tholl, D., and Lee, S. (2011). Terpene specialized metabolism in *Arabidopsis thaliana*. The Arabidopsis Book **9:** e0143, doi/10.1199/tab.0143.

Wang, J., Bing, X.L., Li, M., Ye, G.Y., and Liu, S.S. (2012). Infection of tobacco plants by a begomovirus improves nutritional assimilation by a whitefly. Entomol. Exp. Appl. **144:** 191–201.

Wu, J., and Baldwin, I.T. (2010). New insights into plant responses to the attack from insect herbivores. Annu. Rev. Genet. **44:** 1–24.

Yang, J.Y., Iwasaki, M., Machida, C., Machida, Y., Zhou, X., and Chua, N.H. (2008). betaC1, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. Genes Dev. **22:** 2564–2577.

Zarate, S.I., Kempema, L.A., and Walling, L.L. (2007). Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol. **143:** 866–875.

Zhai, Q., Yan, L., Tan, D., Chen, R., Sun, J., Gao, L., Dong, M.Q., Wang, Y., and Li, C. (2013). Phosphorylation-coupled proteolysis of the transcription factor MYC2 is important for jasmonate-signaled plant immunity. PLoS Genet. **9:** e1003422.

Zhang, P.J., Xu, C.X., Zhang, J.M., Lu, Y.B., Wei, J.N., Liu, Y.Q., David, A., Boland, W., and Turlings, T.C. (2013). Phloem-feeding whiteflies can fool their host plants, but not their parasitoids. Funct. Ecol. **27:** 1304–1312.

Zhang, T., Luan, J.B., Qi, J.F., Huang, C.J., Li, M., Zhou, X.P., and Liu, S.S. (2012). Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. Mol. Ecol. **21:** 1294–1304.