

# Inter-organ defense networking: Leaf whitefly sucking elicits plant immunity to crown gall disease caused by *Agrobacterium tumefaciens*

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**Abbreviations:** ABA, abscisic acid; DAMP, damage-associated molecular pattern; ET, ethylene; ETI, effector-triggered immunity; GA, gibberellin; IAA, indole-3-acetic acid; JA, jasmonic acid; MAMP, microbe-associated molecular pattern; PAMP, pathogen-associated molecular pattern; PTI, PAMP-triggered immunity; PRR, pattern recognition receptors; SA, salicylic acid; VIGS, virus-induced gene silencing.

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Plants have elaborate defensive machinery to protect against numerous pathogens and insects. Plant hormones function as modulators of defensive mechanisms to maintain plant resistance to natural enemies. Recent our study suggests that salicylic acid (SA) is the primary phytohormone regulating plant responses to *Agrobacterium tumefaciens* infection. Tobacco (*Nicotiana benthamiana*) immune responses against *Agrobacterium*-mediated crown gall disease were activated by exposure to the sucking insect whitefly, which stimulated SA biosynthesis in aerial tissues; in turn, SA synthesized in aboveground tissues systemically modulated SA secretion in root tissues. Further investigation revealed that endogenous SA biosynthesis negatively modulated *Agrobacterium*-mediated plant genetic transformation. Our study provides novel evidence that activation of the SA-signaling pathway mediated by a sucking insect infestation has a pivotal role in subsequently attenuating *Agrobacterium* infection. These results demonstrate new insights into interspecies cross-talking among insects, plants, and soil bacteria.

Plants are routinely exposed to numerous pathogens and insect pests, and have developed elaborate chemical and physical defensive systems.<sup>1</sup> Plants perceive and recognize foreign molecules/signals of pathogens and insects from invaded plants, and mount complex innate immune systems against invaders.<sup>2,3</sup> Two plant immune systems have been widely studied. Plants express pattern recognition receptors (PRRs) that perceive microbe,

pathogen, and damage-associated molecular patterns (MAMPs, PAMPs, and DAMPs, respectively), and this recognition induces PAMP-triggered immunity (PTI).<sup>2,4-6</sup> Pathogens and insects suppress plant PTI through specific effectors that disrupt basal plant immune systems. Plants also recognize these specific effectors, which trigger disease-resistance responses called effector-triggered immunity (ETI).<sup>2,4-6</sup>

Two major phytohormones involved in plant defense responses are salicylic acid (SA) and jasmonic acid (JA), which are crucial components of downstream signaling pathways triggered by PTI and ETI.<sup>7,8</sup> These hormone pathways synergistically or antagonistically cross talk during responses to invading pathogens and insects.<sup>9-11</sup> In addition to SA and JA, other phytohormones involved in plant immune responses include ethylene (ET), abscisic acid (ABA), gibberellin (GA), auxin, cytokinin, and brassinosteroid.<sup>12</sup>

Cross-talk among plant hormone pathways is often observed in plants infested by the obligate phloem-feeding insect whitefly (*Bemisia tabaci*). In crop plants, whitefly infestation induces JA/ET signaling.<sup>13,14</sup> By contrast, whitefly infestation in *Arabidopsis* up-regulates marker gene expression for SA-response pathways but not for JA/ET-response pathways.<sup>15</sup> Recent work shows that whitefly infestation in pepper plants elicits a defense response to the leaf pathogen *Xanthomonas axonopodis* pv. *vesicatoria* and the soil-borne pathogen *Ralstonia solanacearum*.<sup>16</sup> The whitefly-induced defense responses in pepper plants include activation of defense-related SA and JA marker gene expression in leaves and roots, indicating

that SA- and JA-dependent pathways are activated in aboveground and belowground tissues in response to whitefly feeding.<sup>16</sup> Aphid infestation (another sucking insect) elicits pepper plant disease resistance to compatible and incompatible bacterial pathogens; both SA- and JA-signaling genes are up-regulated in leaves and roots.<sup>17</sup> Taken together, these results suggest that sucking insects such as whitefly and aphid can modulate plant defense responses to some pathogens *via* activation of SA- and/or JA/ET-signaling pathways.

Before our recent investigation, only a few studies reported that SA directly attenuated *Agrobacterium*-mediated crown gall formation and *vir* gene expression.<sup>18–20</sup> The observation that whitefly infestation modulated plant hormone-mediated defense responses inspired us to hypothesize that endogenously synthesized plant hormones may suppress or attenuate *Agrobacterium*-mediated gall formation. Therefore, we infested individual *Nicotiana benthamiana* plants with whitefly for 7 days, and subsequently infiltrated infested and control plants with the tumorigenic *Agrobacterium tumefaciens* strain C58 for 30 d. The levels of crown gall formation in aboveground and

belowground *N. benthamiana* tissues were significantly lower in whitefly-infested and BTH-treated plants than in control plant (Fig. 1).<sup>21</sup>

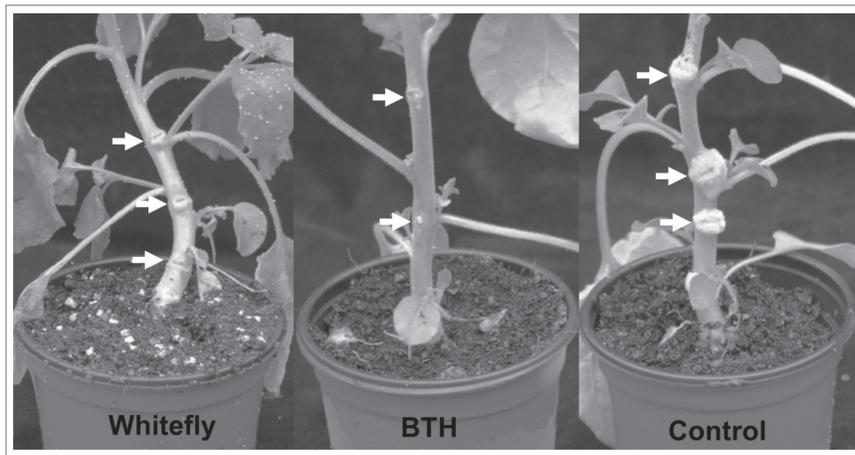
Marker genes of SA and JA pathways were differentially regulated in whitefly-infested plants after *Agrobacterium* application.<sup>21</sup> Genes involved in SA and JA biosynthesis were up- and down-regulated, respectively, in response to *Agrobacterium*, and *de novo* SA levels strongly accumulated in whitefly-infested plants exposed to *Agrobacterium*.<sup>21</sup> Virus-induced gene silencing (VIGS) knock-down of *isochorismate synthase 1 (ICS1)*, which is required for SA biosynthesis, resulted in high levels of crown gall formation in whitefly-infested plants.<sup>21,22</sup> These results suggest that SA is a major regulator of plant responses to *Agrobacterium* pathogenicity and virulence.<sup>21</sup> To understand these results and further investigate how whitefly infestation precisely modulates *Agrobacterium*-mediated crown gall disease, *in vitro* bioassays were performed.<sup>21</sup> Root exudates in the rhizosphere of whitefly-infested plants contained approximately 2-fold increases in SA levels.<sup>21</sup> Whitefly-infested *N. benthamiana* plants were inoculated with *A. tumefaciens* strain GV2260

carrying pBISN1, which is the T-DNA fused with the  $\beta$ -glucuronidase (*gusA*)-intron reporter gene, and GUS activity was lower than that observed in control plants.<sup>21</sup> Our data clearly indicate that whitefly-induced SA signaling can attenuate *Agrobacterium* transformation and gall formation.<sup>21</sup>

Indole-3-acetic acid (IAA) is another phytohormone with important roles in plant growth and development.<sup>23</sup> IAA levels were statistically higher in whitefly-infested plants 5 d after *Agrobacterium* inoculation compared with those in control plants.<sup>21</sup> IAA accumulated at sites of *Agrobacterium* inoculation and negatively affected *vir* gene expression.<sup>24</sup> These results suggest that IAA also suppresses *Agrobacterium*-mediated disease. Therefore, we speculate that the balance between plant defense responses to *Agrobacterium* and plant growth and development may be regulated by SA- and IAA-signaling pathways based on fitness costs.<sup>25</sup>

To extend our current understanding into other fields, further investigations into the following questions are needed: (1) what are the roles of whitefly-stimulated plant hormones SA, JA, and IAA in resistance responses on monocot such as rice and maize?; (2) what unique mechanisms orchestrate the global gene expression of plant-mediated modification of *Agrobacterium* and *Agrobacterium*-mediated modification of plant using a cutting-edge omics technology like dual-RNA-sequencing?; and (3) how do whitefly-activated plant defense responses modulate subsequent responses to abiotic stresses?

In conclusion, our study clearly demonstrates that whitefly-induced SA signaling attenuates subsequent *Agrobacterium*-mediated crown gall disease *via* its transformation ability. The molecular, biochemical, genetic, and phenotypic analyses performed in our study provide new insights into candidate molecular mechanisms that orchestrate interspecies interactions among insects, plants, and *Agrobacterium*.



**Figure 1.** Crown gall formation induced by *Agrobacterium tumefaciens* strain C58 is significantly attenuated in whitefly-infested tobacco plants. Three-week-old *Nicotiana benthamiana* plants were exposed to whitefly, treated with benzothiadiazole (BTH), or untreated (control) for 7 d. Plants were subsequently inoculated with a suspension (OD<sub>600</sub> = 2) of *A. tumefaciens* strain C58 by slightly injuring stems with a needle. Whitefly-infested and BTH-applied plants significantly attenuated subsequent crown gall formation compared with control. The arrows indicated the inoculation sites of *Agrobacterium* for each treatment. Representative plants were photographed at 30 d after *Agrobacterium* inoculation.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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