

Tomato responds to green peach aphid infestation with the activation of trehalose metabolism and starch accumulation

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The disaccharide trehalose and trehalose-6-phosphate that are present in trace amounts are suggested to have a signaling function in plants. Recently, it was demonstrated that trehalose metabolism contributes to *Arabidopsis thaliana* defense against the green peach aphid (GPA; *Myzus persicae* Sülzer), an important insect pest of a large variety of plants. *TPS11* (*TREHALOSE PHOSPHATE SYNTHASE11*)-dependent trehalose metabolism was shown to curtail GPA infestation by promoting starch accumulation and expression of the *PAD4* (*PHYTOALEXIN-DEFICIENT4*) gene, which has important roles in regulating antibiosis and antixenosis against GPA. Here we show that trehalose metabolism is similarly activated in leaves of GPA-infested tomato (*Solanum lycopersicum*) plants and likely contributes to tomato defense against GPA. GPA-infested leaves of tomato accumulated trehalose, which was accompanied by the transient upregulation of *SITPS11*, a homolog of the *Arabidopsis* *TPS11*. GPA-infestation was also accompanied by starch accumulation and the upregulation of *SIPAD4*, the tomato homolog of *Arabidopsis* *PAD4*. Furthermore, trehalose application induced *SIPAD4* expression and starch accumulation, and curtailed GPA infestation, suggesting that like in *Arabidopsis* trehalose contributes to tomato defense against GPA.

Sugars have important functions in carbon and energy metabolism of plants.¹ In addition, sugars also have critical roles as signaling molecules in plant growth, development and response to environmental stress.^{1–3} The role of sucrose as a signaling molecule has been well documented for some time.¹ In recent years, the α,α -1,1-linked glucose disaccharide trehalose and its metabolic intermediate trehalose-6-phosphate (T6P), which are present in trace amounts in most plants, were also shown to function as signaling molecules in plant growth, development and stress response.^{4,5} We recently demonstrated the involvement of trehalose metabolism in *Arabidopsis thaliana* defense against the green peach aphid (*Myzus persicae* Sülzer),⁶ which is an important pest of more than 50 families of plants.⁷ Trehalose levels and expression of the *TREHALOSE PHOSPHATE SYNTHASE 11* (*TPS11*) gene, which encodes a trehalose synthesizing enzyme, transiently increased in GPA-infested *Arabidopsis* leaves.⁶ In addition to trehalose, sucrose levels also increased in GPA-infested *Arabidopsis* leaves.⁶ The upregulation of *TPS11* promoted the flux of C into starch at the expense of sucrose accumulation. Starch accumulation in GPA-infested leaves was required for the full extent of basal resistance against GPA.⁶ *TPS11* promoted the timely upregulation of *PAD4* (*PHYTOALEXIN-DEFICIENT4*),⁶ which is an important modulator of antibiotic and antixenotic defenses against GPA. Here we present evidence that like in *Arabidopsis*,

tomato leaves also respond to GPA-infestation with the activation of trehalose metabolism.

Trehalose Content Transiently Increases in GPA-infested Tomato Leaves

Tomato is a host for GPA. To determine if tomato, like *Arabidopsis* responds to GPA infestation with an increase in trehalose content, GPA was released on tomato variety Motelle plants and leaves from GPA-infested and un-infested plants harvested over a 24 h period. Trehalose content in these leaves was monitored as previously described in reference 6. As shown in **Figure 1A**, although trehalose levels were undetectable in leaves of un-infested plants, trehalose content gradually increased in the GPA-infested leaves, reaching peak levels by 12 hpi and then declining at 24 hpi.

Sucrose and Starch Content Increase in GPA-infested Tomato Leaves

In *Arabidopsis*, GPA infestation results in an increase in sucrose and starch content.⁶ *TPS11* promoted the accumulation of starch at the expense of sucrose in GPA-infested *Arabidopsis* leaves. No-choice assays with the starch-deficient *Arabidopsis* *pgm1* mutant indicated that starch accumulation was required for controlling GPA infestation in *Arabidopsis*. Hence, it was suggested

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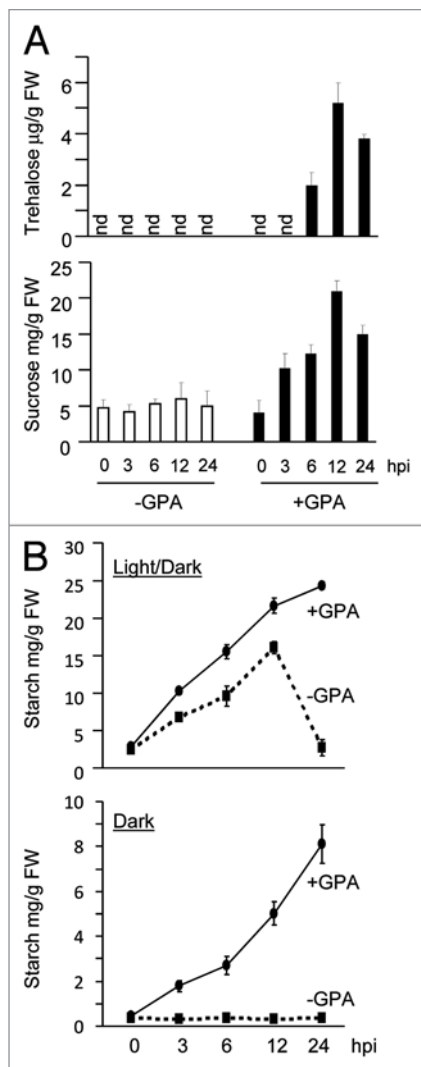


Figure 1. Trehalose, sucrose and starch content in GPA-infested tomato. (A) Trehalose and sucrose levels were measured at the indicated hours post infestation (hpi) in un-infested (-GPA) and GPA-infested (+GPA) leaves of the tomato variety Motelle ($n = 3$). (B) Starch content was measured in un-infested (-GPA) and GPA-infested (+GPA) tomato leaves of Motelle kept for 24 h in a 14/10 h light/dark cycle (Top part) or for 24 h in complete darkness (Lower part) ($n = 3$). For the light/dark cycle, insects were released on plants (0 h) immediately after start of the light period (8:00 AM). In (A and B), each plant received 25 adult aphids. Error bars represent SE. nd, non-detectable.

that starch accumulation is a mechanism that is utilized by the host plant to generate a secondary sink that counter's the ability of the insect to manipulate host metabolism to increase sucrose content.⁶ We therefore examined if GPA-infestation also results in starch accumulation in tomato leaves. As shown in **Figure 1B**, a rapid increase in starch content was observed in the GPA-infested leaves of tomato. Starch accumulation paralleled sucrose accumulation in these GPA-infested leaves (**Fig. 1A**). Starch accumulation was also observed in experiments where the GPA-infested tomato plants were kept in darkness for the duration of the experiment (**Fig. 1B**). These results confirm that like

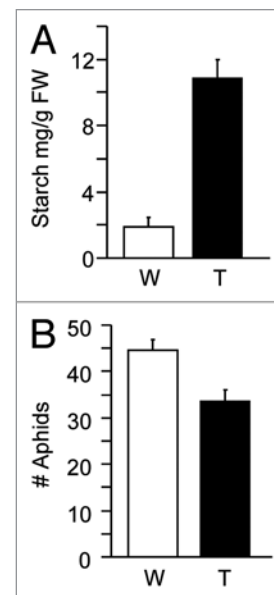


Figure 2. Trehalose application promotes tomato defense against GPA. (A) Starch content in trehalose-treated tomato leaves. Starch content was measured in leaves of tomato variety Motelle 12 h after infiltration of 10 mM Trehalose (T) and as control with water (W). (B) Trehalose application enhances resistance against GPA. No-choice assay: GPA numbers (adults + nymphs) on plants of the tomato variety Motelle infiltrated with 10 µM Trehalose (T) and as control with water (W), 12 h prior to release of 25 adult aphids per plant. Aphid numbers were taken at 2 dpi ($n = 5$). In (A and B), error bars represent SE.

Arabidopsis, tomato also responds to GPA-infestation by promoting sucrose and starch accumulation.

Trehalose Promotes Resistance against GPA in Tomato Leaves

Trehalose applied to tomato leaves promoted starch accumulation (**Fig. 2A**) and curtailed GPA infestation (**Fig. 2B**), suggesting that similar to Arabidopsis trehalose metabolism may have a role in tomato defense against GPA. In Arabidopsis, expression of *TPS11*, which is required for trehalose accumulation, parallels the transient increase in trehalose in GPA-infested leaves.⁶ A survey of the nucleotide sequence databases revealed that the tomato genome contains a *TPS11* homolog, which we have designated *SITPS11* (GenBank: AB368491). *SITPS11* expression was transiently induced in the GPA-infested leaves of tomato (**Fig. 3A**). The time-frame of *SITPS11* upregulation parallels the time-frame of trehalose accumulation in GPA-infested tomato leaves (**Fig. 1A**), suggesting that *SITPS11* expression is likely associated with the increase in trehalose in GPA-infested tomato leaves. By comparison to the transient upregulation of *SITPS11*, expression of tomato *SIPAD4* (GenBank: AI781175), which is homologous to *PAD4* that is involved in Arabidopsis defense against GPA,⁸⁻¹⁰ gradually increased over the duration of the experiment (**Fig. 3A**). Similar to the Arabidopsis *PAD4*, *SIPAD4* expression in tomato was upregulated in response to trehalose application (**Fig. 3B**). These results suggest that the expression of *SIPAD4* in response

to GPA infestation is similar to the expression of *PAD4* in GPA-infested Arabidopsis. Furthermore, similar to our observations in Arabidopsis,⁶ GPA population was smaller on tomato plants that were pre-treated with trehalose as opposed to the water treated control plants (Fig. 2B).

Taken together, the results presented here indicate similarities in the molecular and physiological alterations in tomato and Arabidopsis leaves in response to GPA-infestation. We suggest that similar to Arabidopsis, tomato engages trehalose metabolism to promote the activation of defenses that target GPA. This study also bolsters the idea that plants have evolved mechanisms that invoke their primary metabolic pathways to counter insect herbivory. Additional genetic studies are required to validate the contribution of trehalose and starch metabolism, and the *SITPS11* and *SIPAD4* genes in tomato defense against GPA.

Note

GenBank IDs: *SIPAD4* (AI781175); *SITPS11* (AB368491); *SIEF1a* (X53043.1).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Rolland F, Baena-Gonzalez E, Sheen J. Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annu Rev Plant Biol* 2006; 57:675-709; PMID:16669778; <http://dx.doi.org/10.1146/annurev.arplant.57.032905.105441>.
2. Koch KE. Carbohydrate modulated gene expression in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1996; 47:509-40; PMID:15012299; <http://dx.doi.org/10.1146/annurev.arplant.47.1.509>.
3. Roitsch T. Source-sink regulation by sugar and stress. *Curr Opin Plant Biol* 1999; 2:198-206; PMID:10375568; [http://dx.doi.org/10.1016/S1369-5266\(99\)80036-3](http://dx.doi.org/10.1016/S1369-5266(99)80036-3).
4. Paul MJ, Primavesi LF, Jhurreea D, Zhang Y. Trehalose metabolism and signaling. *Annu Rev Plant Biol* 2008; 59:417-41; PMID:18257709; <http://dx.doi.org/10.1146/annurev.arplant.59.032607.092945>.
5. Fernandez O, Béthencourt L, Quero A, Sangwan RS, Clément C. Trehalose and plant stress responses: friend or foe? *Trends Plant Sci* 2010; 15:409-17; PMID:20494608; <http://dx.doi.org/10.1016/j.tplants.2010.04.004>.
6. Singh V, Louis J, Ayre BG, Reese JC, Pegadaraju V, Shah J. TREHALOSE PHOSPHATE SYNTHASE11-dependent trehalose metabolism promotes *Arabidopsis thaliana* defense against the phloem-feeding insect *Myzus persicae*. *Plant J* 2011; 67:94-104; PMID:21426427; <http://dx.doi.org/10.1111/j.1365-313X.2011.04583.x>.
7. Blackman RL, Eastop VF. Aphids on the World's Crops: An Identification and Information Guide, Chichester, UK: John Wiley 2002; 2.
8. Pegadaraju V, Knepper C, Reese J, Shah J. Premature leaf senescence modulated by the Arabidopsis *PHYTOALEXIN DEFICIENT4* gene is associated with defense against the phloem-feeding green peach aphid. *Plant Physiol* 2005; 139:1927-34; PMID:16299172; <http://dx.doi.org/10.1104/pp.105.070433>.
9. Pegadaraju V, Louis J, Singh V, Reese JC, Bautor J, Feys BJ, et al. Phloem-based resistance to green peach aphid is controlled by Arabidopsis *PHYTOALEXIN DEFICIENT4* without its signaling partner *ENHANCED DISEASE SUSCEPTIBILITY1*. *Plant J* 2007; 52:332-41; PMID:17725549; <http://dx.doi.org/10.1111/j.1365-313X.2007.03241.x>.
10. Louis J, Lorenc-Kukula K, Singh V, Reese J, Jander G, Shah J. Antibiosis against the green peach aphid requires the Arabidopsis *thaliana MYZUS PERSICAE-INDUCED LIPASE1* gene. *Plant J* 2010; 64:800-11; PMID:21105927; <http://dx.doi.org/10.1111/j.1365-313X.2010.04378.x>.

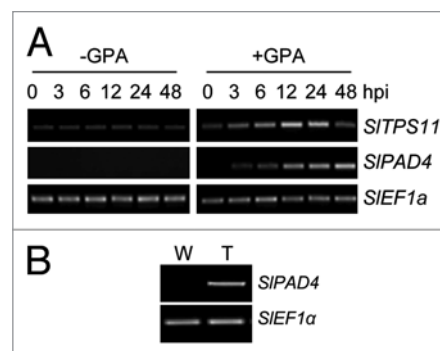


Figure 3. Expression of defense-related genes in tomato leaves. (A) RT-PCR analysis of tomato *SITPS11* (GenBank: AB368491) and *SIPAD4* (GenBank: AI781175) gene expression in un-infested (-GPA) and GPA-infested (+GPA) leaves of tomato variety Motelle at the indicated hours post infestation (hpi). (B) RT-PCR analysis of tomato *SIPAD4* expression in Motelle leaves 12 h post infiltration with a 10 μ M solution of trehalose (T). Water (W) treated leaves provided negative controls. In (A and B), expression of the *SIEF1a* gene (GenBank: X53043.1) provided the control for RT-PCR.