Loss of The Phloem-Expressed Sugar Transporter VST1 Reduces Aphid Performance In Watermelon

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Research Article

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Abstract

Aphids can damage plants through sugar withdrawal by hijacking sugar metabolism and transport genes to increase their sugar sucking ability. Blocking plant diseases and pest access to nutrients has emerged as an exciting strategy for improving disease and insect resistance in plants. Our previous work identified a shift in the localization of the vacuolar sugar transporter (VST1) that contributes to sucrose (Suc) and glucose (Glc) unloading in the phloem of sweet watermelons. In this study, the potential role of VST1 in the response to aphid infestation was investigated. Loss of VST1 function adversely impacted aphid settling and honeydew production. The vst1 mutant displayed less aphid-induced hydrogen peroxide accumulation and cell death than wild-type (WT) plants. Additionally, the expression of the VST1 gene was induced by aphids. The mutation of VST1 reduced Suc and Glc accumulation in the phloem, indicating that Suc and Glc are important carbohydrate substrates in phloem sap that are transported by VST1 to support aphid propagation and infestation. Taken together, our results demonstrated that the mutation of VST1 by genome editing can decrease aphid performance in watermelon by blocking the sugar supply obtained from phloem sap.

Key Message

Vacuolar sugar transporter (VST1) contributes to sucrose and glucose unloading in the phloem of watermelon. We further revealed that the mutation of *VST1* restrains aphid infestation in watermelon by hindering its access to sucrose and glucose from phloem sap via genome editing, implying an alternative way to prevent aphid infestation.

Main Text

Aphids can damage plants through sugar withdrawal by hijacking sugar metabolism and transport genes to increase their sugar sucking ability (Sun et al., 2019). Plants block the access of aphids to sugars as an active self-protection mechanism (Åhman et al., 2019). This strategy can potentially be exploited to control aphids via the CRISPR/Cas9 editing of genes involved in phloem sugar transport.

Recently, we reported that a shift in the localization of a vacuolar sugar transporter (VST1) from the tonoplast to the plasma membrane in phloem can contribute to phloem sucrose and glucose unloading in sweet watermelons (*Citrullus lanatus*) (Ren et al., 2020). Herein, we tested whether the mutation of VST1 can increase tolerance to aphids after aphid feeding. Surprisingly, the emerging leaves of wild-type (WT) plants, but not those of *vst1* mutants (*vst1-1* harboring a 118-nt deletion and *vst1-2* harboring a C/T deletion generated with CRISPR/Cas9 technology) (Ren et al., 2020), showed leaf curling and a large amount of sticky honeydew following aphid infestation in the greenhouse under natural conditions (Figure 1a). To confirm the involvement of *VST1* in aphid tolerance, we examined aphid feeding performance on *ClVST1* overexpressing (OE) watermelon plants (Ren et al., 2020). Remarkably, the OE plants showed severe leaf curl phenotype than WT plants after aphid infestation (Figure S1a), suggesting that the sugar transporter VST1 plays a negative role in aphid tolerance. Plant defense response to aphid

infestation is often reflected by reduced offspring production (antibiosis) in a no-choice test with reduced feeding or by nonpreference (antixenosis) in a choice test (Lei et al., 2014). We performed these tests on different CRISPR and transgenic lines to assess their antibiotic and antixenotic resistance. Approximately twice as many aphids preferred WT versus *vst1* plants (Figure 1b); and *ClVST1* OE-1 and OE-2 plants showed the most susceptible to aphids in both choice and no-choice tests (Figure S1b). Honeydew production serves as an indicator of insect feeding activity, and honeydew stained by ninhydrin appears as purple spots (Lei et al., 2014). Consistent with the statistical results for the aphid population, the *vst1* mutant displayed lighter discoloration of filter paper following ninhydrin staining (Figure 1c), indicating that less honeydew secretion was induced by aphids on *vst1* plants than WT plants. This finding was later confirmed by data from quantification based on reading the optical density at 500 nm (Figure 1d). Insect infestation is often accompanied by the accumulation of reactive oxygen species (ROS) in host plants (Lei et al., 2014). We found that upon aphid infestation, *vst1* mutant leaves showed less H_2O_2 accumulation according to 3,3'-diaminobenzidine (DAB) staining (Figure 1e) and less cell death according to the trypan blue staining assay (Figure 1f). Collectively, these data indicate that *vst1* mutation can mitigate the damage caused by aphids in watermelon.

We further investigated the transcript levels of *VST1* upon aphid infestation by qRT-PCR. The expression level of *VST1* was increased at 12 hours post-infestation (hpi) by aphids (Figure 1g). Which further confirmed that VST1 plays an important role in aphid control. Sucrose serves as the optimal carbohydrate substrate for aphid growth and propagation (Hewer et al., 2010). To determine whether VST1-mediated sugar transport is necessary for aphid infestation, we detected the contents of sugars in phloem sap from WT and *vst1* mutants by using gas chromatography-mass spectrometry (GC-MS). The levels of both sucrose and glucose were higher in WT plants than in the *vst1* mutant (Figure 1h), indicating that sucrose and glucose are important carbohydrate substrates in phloem sap that are transported by VST1 for aphid propagation and infestation. Altogether, our results demonstrated that the mutation of *VST1* by genome editing can decrease aphid performance on watermelon by hindering the sugar supply obtained by aphids from phloem sap.

Watermelon is among the most commonly produced fruits around the world, and its production is limited by various insects, especially aphids, which survive on sugars in the phloem sap of their host plants (Clarke et al., 2020). In this context, considered together with the findings of a previous disease study, blocking insect access to nutrients has emerged as an exciting strategy for improving disease and insect resistance in plants (Oliva and Quibod, 2017). Here, we presented the first evidence demonstrating that sugar transporter mutants restrict aphid sucking on watermelon and revealed the possibility of improving crop resistance to aphids or other insects by gene editing. Sugar is also necessary for plant growth and development. It is worthwhile to consider how the constant tradeoff between blocking sugar starvation and defense can be modulated to avoid compromising plant yields under a long-term breeding strategy (Zaidi et al., 2018). Additionally, the fruit sink has the independent capacity for accumulating sugars without being influenced by leaf, stem, root or rootstock tissues in watermelon (Ren et al., 2021). A

potential solution to minimize the negative effect of VST1 on sugar content and yield is to perform tissue-specific editing via the precise CRISPR-mediated strategy in the future (Zhu et al., 2020).

Declarations

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Conflict of interest: The authors declare no conflict of interest.

Author Contributions

ML and YX designed the research. ML, YR and SG. did the experiments. GG, JZ, ST, JW, YY, HZ and HS analyzed the data. ML and YX wrote the manuscript. All authors read and approved the manuscript.

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Figures

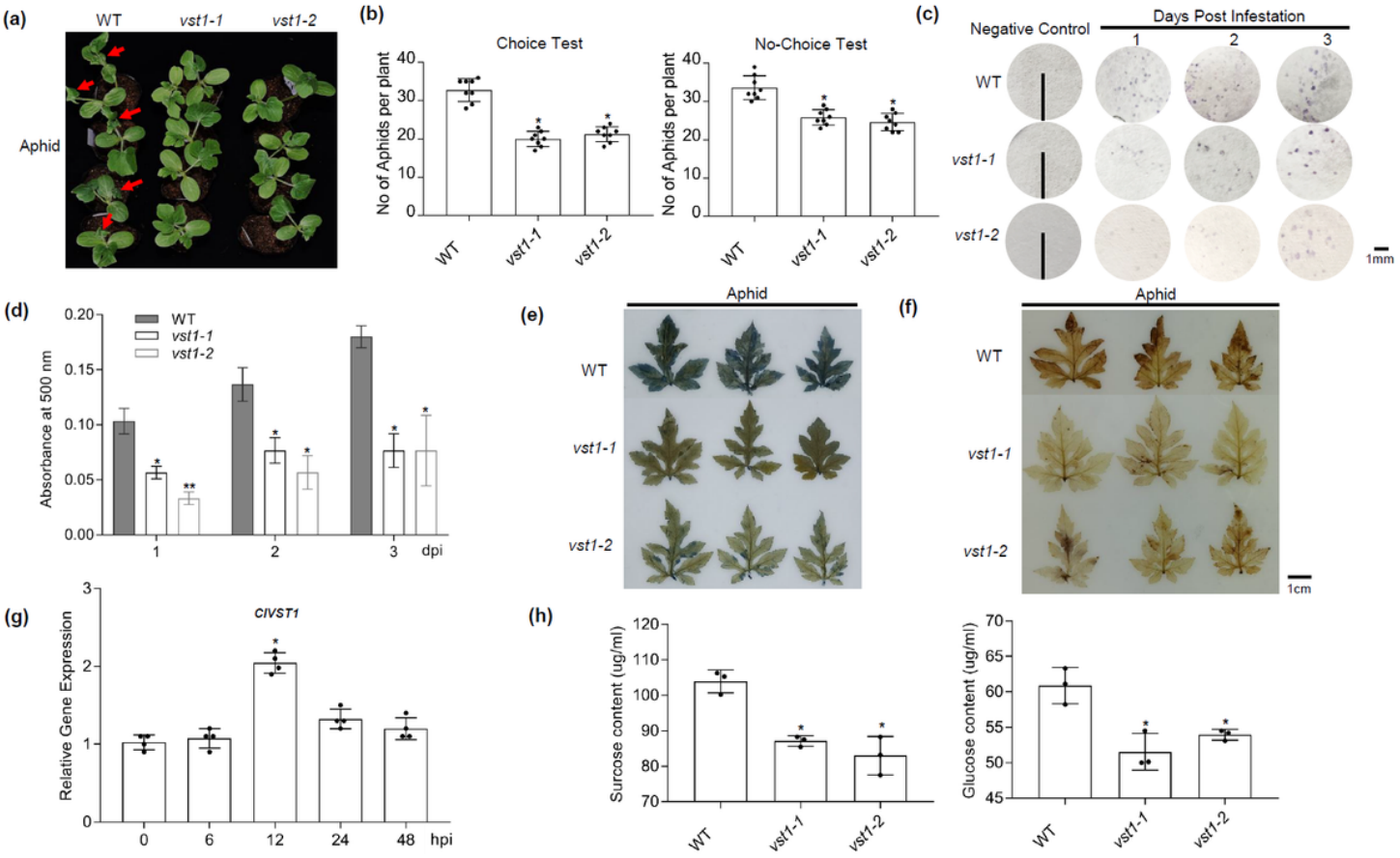

Figure 1

Loss of VST1 restricts sugar loading into the intercellular space for protection against aphid infestation in watermelon. (a) Leaf curl phenotypes of WT, vst1-1 and vst1-2 lines upon aphid infestation. Images of the leaf phenotypes were taken 7 days post aphid infestation (dpi). (b) Choice tests and no-choice tests on the WT, vst1-1 and vst1-2 lines. For choice tests, 21-day-old plants were used. Settled aphids were counted 6 hours after the release of 80 adults between two plants of the tested genotypes. For no-choice tests, 10 second-instar nymphs were inoculated onto each plant (21-day-old). Total aphid numbers were recorded seven days later. Each test was composed of eight replicates. (c) Ninhydrin staining and (d)

quantification of honeydew secretions. (e) DAB staining (H_2O_2 indicator) and (f) trypan blue staining (cell death indicator) after aphid infestation. Leaf samples from 21-day-old plants were collected after 7 dpi. (g) Relative expression of VST1 in WT plants in the presence of aphid infestation. Leaf samples from 21-day-old plants were collected for the indicated periods of time. (h) Quantification of sucrose and glucose contents in the phloem sap of the WT and vst1 mutant plants by GC-MS. Phloem sap samples were obtained from phloem exudates of the enclosed leaf petioles of 60-day-old watermelon plants by making a single shallow incision into the petiole. Statistical significance of treatment effects is indicated by *, $p < 0.05$. **, $p < 0.01$ (Student's t-test).

Supplementary Files

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- figS1.png