***eLife’s* transparent reporting form**

We encourage authors to provide detailed information *within their submission* to facilitate the interpretation and replication of experiments. If you have any questions, please contact us: [editorial@elifesciences.org](mailto:editorial@elifesciences.org).

**Sample-size estimation**

* You should state whether an appropriate sample size was computed when the study was being designed
* You should state the statistical method of sample size computation and any required assumptions
* If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., page numbers or figure legends), or explain why this information doesn’t apply to your submission:

We used a minimum sample size of n=5 plants (average = 9) for all VIGS experiments. As described below, each construct was tested at least twice across time-distributed replicates to ensure robustness of the results. Distributions were analyzed using Kolmogorov-Smirnov test to avoid assumption of normality, to account for the phenotypic variability between silenced lines, and to enable the visualization of the entire data distribution.

**Replicates**

* You should report how often each experiment was performed
* You should include a definition of biological versus technical replication
* The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
* If you encountered any outliers, you should describe how these were handled
* Criteria for exclusion/inclusion of data should be clearly stated
* High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., page numbers or figure legends), or explain why this information doesn’t apply to your submission:

Differential expression of candidates using RNA-seq was confirmed by results of two different biological replicates of trichome and non-trichome tissue per plant.

All enzyme assays have been repeated with at least 2 independent protein purifications (biological replicates).

All VIGS experiments have been repeated at least 2x per tested gene at different times (time-separated biological replicates), and the results of both replicates are described in the Main, Supplemental Figures and Supplemental Tables in the relevant sections.

For SsASAT2 VIGS, 1/12 and 1/7 Uninoculated and SsASAT2-1 plants, respectively, were excluded from the analysis because their normalized peak areas across the board for all acylsugars were >30-100X more than all other plants. This could occur due to improper measuring of dry weights or due to stochastic fluctuations in the acylsugar levels due to differential growth. All other variation across all experiments has been captured in the boxplots and/or barplots with standard deviation.

RNA-seq data has been uploaded to NCBI and is public. Project number is included in the Data Release section of the manuscript.

**Statistical reporting**

* Statistical analysis methods should be described and justified
* Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
* For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
* Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Raw data of VIGS experiments has been provided as Source Data.

For most VIGS analyses which required statistical estimations, we used the non-parametric Kolmogorov-Smirnov test to avoid assuming normal distribution of values. All sample sizes of the VIGS experiments are described in the figures.

Please outline where this information can be found within the submission (e.g., page numbers or figure legends), or explain why this information doesn’t apply to your submission:

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to page numbers in the manuscript.)

**Additional data files (“source data”)**

* We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
* Where provided, these should be in the most useful format, and they can be uploaded as “Source data” files linked to a main figure or table
* Include model definition files including the full list of parameters used
* Include code used for data analysis (e.g., R, MatLab)
* Avoid stating that data files are “available upon request”

Please indicate the figures or tables for which source data files have been provided:

Source Data has been included wherever possible. All RNA-seq data and sanger sequencing data has been uploaded to NCBI. Python code used for identifying VIGS fragments is attached as a File Supplement.