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Article

Integrated transcriptome and metabolome reveal that SlSYTA modulates ROS responses driving resistance defense in *Solanum lycopersicum*

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Abstract

Synaptotagmin A (SYTA), renowned for its indispensable role in mammalian vesicle trafficking, has recently captured attention in plant biology owing to its potential regulatory functions. This study meticulously delves into the involvement of *Solanum lycopersicum* SlSYTA in plant immunity, focusing on its response to an array of pathogens affecting tomatoes. Our comprehensive inquiry uncovers that SlSYTA overexpression heightens susceptibility to tobacco mosaic virus (TMV), *Phytophthora capsici*, *Botrytis cinerea*, and *Pseudomonas syringae* pv. *tomato* DC3000, whereas RNA interference (RNAi) plants show a robust and encompassing resistance to these pathogens. Remarkably, our findings shed light on SlSYTA's negative regulation of pivotal aspects of pattern-triggered immunity (PTI) defense, notably hindering the reactive oxygen species (ROS) burst, impeding stomatal closure, and curtailing callose deposition. Through meticulous scrutiny via transcriptome and metabolome analyses, our studies reveal SlSYTA's profound impact on diverse plant defense pathways, specifically influencing phenylpropanoid metabolism, hormone signaling, and oxidative phosphorylation, primarily via NADPH synthesis modulation in the pentose phosphate pathway, and ultimately interplay within ROS signaling. Collectively, our research presents groundbreaking insights into the intricate molecular mechanisms governing plant immunity, emphasizing the significant role of SlSYTA in orchestrating plant responses to biotic stress.

Introduction

Solanum lycopersicum (tomato) stands as one of the foremost globally consumed vegetables and fruits. As consumer preferences evolve, considerations for sustainable production, palatability, and potential health benefits have emerged as paramount priorities, reflecting essential facets of a long-term sustainable economy [1]. Concurrently, biotic stresses posed by fungi, bacteria, and viruses exert substantial influence on the yield potential of current crop varieties. Notably, tomatoes, a pivotal vegetable for human health and nutrition, are particularly susceptible to diseases exacerbated by climate change. This necessitates a proactive approach in harnessing resistance-related resources to foster the cultivation of robust and disease-resistant tomatoes, aligning with the imperative of ensuring the sustained health and nutritional well-being of global populations [1]. In our preceding study, we successfully cloned *S. lycopersicum* synaptotagmin A (SlSYTA) in tomatoes and elucidated its function in negatively regulating the anti-tobacco mosaic virus (TMV) processes in tobacco [2]. However, a more in-depth investigation is warranted to comprehensively understand the role of SlSYTA involvement in disease resistance in tomatoes.

In the context of vesicle dynamics, SYT plays a crucial role in mammalian neurons, mediating vesicle fusion for neurotransmitter release and participating in endocytosis and vesicle activation [3–5]. This function has prompted investigations into its homologs in plants, with a family of SYT1–7 being identified in *Arabidopsis* [6]. Notably, SYT1 and SYT5 exhibit the highest expression levels, SYT1 being particularly prominent [7]. Moreover, SYT1 has been implicated in membrane fusion, contributing to plant responses to abiotic stress; its mutant *syt1-2* displays heightened sensitivity in high NaCl concentration, showing the relevance of SYT1 in salt stress environments [8]. Additionally, loss of SYT function in *Arabidopsis* leads to compromised plasma membrane integrity and reduced cell stability, with these effects exacerbated under salt stress conditions [8]. Besides, studies by Kawamura and Uemura observed an upregulation in *SYT1* expression during cold acclimation in *Arabidopsis*, correlating with enhanced tolerance to cold stress [9], while Yamazaki *et al*. demonstrated that *Arabidopsis* SYT1 plays a vital role in Ca^{2+}-dependent antifreeze processes, maintaining plasma membrane integrity and cell viability and participating in membrane transport [10]. Overexpressing plants maintained lower membrane lipid peroxidation levels, contributing to enhanced survival rates [10]. While the role of the SYT family in abiotic stress responses is well documented [11, 12], its response to biotic stress, particularly in tomatoes, remains largely unexplored. Existing research indicates that *Arabidopsis* SYT1,

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peak area extraction. Metabolites were identified by matching accurate mass and MS/MS data against established databases and a self-built metabolite standard library, focusing on features with significant measurement values across the samples. The raw metabolome data have been uploaded to Metabolights, and the project ID is MTBLS9722. Metabolites with P values <0.05 in univariate statistical analysis were considered significantly different. Data visualization was done using ChiPlot (https://www.chiplot.online).

Detection of ROS accumulation

Luminol assays were used to detect ROS bursts in #63, #19, RNAi, and WT plants in the presence of flg22 and chitin. Briefly, 3-mm tomato leaf discs were placed in a 96-well plate, three per well. Each well was filled with 100 μl of ddH_2O and left overnight at room temperature in the dark to clear residual ROS. After removing the ddH_2O, reaction mixture was added, and luminescence was measured immediately after adding flg22 or chitin, with readings taken every 90 s for 1 h.

DAB staining was used to observe ROS accumulation in PAMP-treated tomato leaf tissues. A uniform spray of 10 μmol/l flg22 and 25 mg/ml chitin was applied on tomato leaves. Staining and destaining procedures followed previous reports [57].

Antioxidant enzyme activities were measured in WT and transgenic plants using kits from Solarbio (BC0200, BC0090) for CAT and POD, Sinobestbio (YXC6PG, YXCA102) for D-glucose 6-phosphate (6PG) and glucose-6-phosphate (6PGH), and Geruisi-bio (G0138F) for NADPH oxidase (NAO). Briefly, 0.1 g of tomato leaf sample was homogenized in 1 ml of extraction buffer, centrifuged, and the supernatant used for activity assays. Absorbance was measured at 240 and 470 nm for CAT and POD activity determination, 450 nm for NAO activity determination and 450 and 340 nm for 6PG and 6PGH content determination.

Callose deposition assay

To assess the impact of SlSYTA on callose deposition in tomatoes, leaves were stained with aniline blue following a 15-min chitin infiltration. The leaves were decolorized overnight in chloral hydrate solution. Transparent leaf sections were then stained with 0.01% aniline blue in a 150-mmol/l K_2HPO_4 solution (pH 8.5) for 2 h under dark conditions. Stained leaves were stored in sterile 50% glycerol for fluorescence observation. Image J software was used for analysis of mean fluorescence intensity.

Scanning electron microscopic observation

To observe how SlSYTA affects stomatal immunity, leaves from WT and transgenic plants were fixed overnight in 2.5% glutaraldehyde. After removing glutaraldehyde, fungal hyphae were dehydrated in an ethanol gradient (30–100%) and then completely dried before sputter-coating with gold. Finally, the stomatal apertures of the leaves were examined using an SU3500 (Hitachi) microscope at 400× and 2000× magnification.

Statistical analysis

All experiments presented in this study were conducted with a minimum of three independent replicates to ensure robustness and reliability. Data are reported as means accompanied by standard deviations. Statistical analyses were carried out utilizing SPSS software (version 23.0). Student's t-test was employed for comparisons and one-way analysis of variance (ANOVA) was applied, followed by the least significant difference (LSD, $P < 0.05$) test for *post hoc* comparisons when necessary.

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Author contributions

S.T. performed the experiments and wrote the original draft. C.L. performed the formal analysis. F.L. and X.W. leveraged software to analyze raw data. G.Q. and J.D. verified data validation. Q.W., Y.W. and Q.P. graphed the data. X.M. guided the experiment and provided protocol. X.S. performed writing, review, and editing, supervision, project administration, and funding acquisition. All authors critically revised and approved the final manuscript.

Data availability statement

Sequence reads of transcriptome have been deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1086573. The raw metabolome data has been uploaded to Metabolights (https://www.ebi.ac.uk/metabolights/) and the National Genomics Data Center (https://www.cncb.ac.cn/), and the project IDs are MTBLS9722 (In Curation) and OMIX006287, respectively. Other data generated or analyzed during this study are included in this article.

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary information

Supplementary data are available at *Horticulture Research* online.

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