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Silencing of the *SAMDC* gene decreases resistance of tomato to *Cladosporium fulvum*

Tingting Zhao, Huanhuan Yang, Jingbin Jiang, Guan Liu, He Zhang, Di. Xiao, Xiuling Chen, Jingfu Li, Xiangyang Xu*

College of Horticulture & Landscape Architecture, Northeast Agricultural University, Harbin 150030, PR China

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ABSTRACT

S-adenosylmethionine decarboxylase (SAMDC) is a key enzyme in polyamine (PA) biosynthesis, and overexpression of *SAMDC* in plants can increase the PA level and enhance tolerance to multiple abiotic and biotic stresses. In our previous study, this gene was significantly up-regulated during *Cladosporium fulvum* resistance gene (*Cf*)/*Avirulence* (*Avr*) interaction in tomato. In this study, to further understand the role that the *SAMDC* gene plays in *Cf*/*Avr* interaction, the virus-induced gene silencing (VIGS) method was applied to down-regulate *SAMDC* expression in CGN18423, a line carrying the *Cf-19* gene for resistance to *C. fulvum*. Then, these plants were infected with *C. fulvum* to analyze changes in resistance. The silencing of *SAMDC* was confirmed by quantitative real-time PCR (qRT-PCR) analysis and PA quantification. The resistance levels of *SAMDC*-silenced plants were lower than those of untreated CGN18423 plants. No visible signs of infection were observed on the leaves of untreated CGN18423 plants, while some small chlorotic spots were observed on parts of the leaf margins of *SAMDC*-silenced plants, and hyphae inside the chlorotic spots were observed late in the hypersensitive response (HR) process. These changes suggested that silencing of the *SAMDC* gene affected the process of *Cf*/*Avr* interaction and decreased the resistance of CGN18423 to *C. fulvum*.

1. Introduction

Spermidine (Spd), spermine (Spm) and their precursor, the diamine putrescine (Put), are present in all living organisms and are implicated in a wide range of cellular physiological processes. These basic, small molecules are thought to promote plant growth and development, such as cell division, growth and differentiation, induction of flowering, pollen development and fruit development, by activating the synthesis of nucleic acids and proteins [1–5]. Many studies have indicated that polyamines (PAs) also play an important role in the adaptation of plants to abiotic and biotic stresses [6–10]. PA accumulation has been widely described in plant tissues infected with various types of pathogens [11]. For example, PA accumulation has been observed in Arabidopsis inoculated with *Pseudomonas syringae*, in rice inoculated with *Magnaporthe grisea*, in tobacco inoculated with *Pseudomonas cichorii*, and in tobacco infected with the necrotrophic fungus *Sclerotinia sclerotiorum* and the biotrophic bacterium *Pseudomonas viridiflava* [11,12]. Accumulation of Put, Spd and Spm increased in barley leaves infected with powdery mildew fungus [13,14]. Exogenous application of Spm and Spd was shown to modulate resistance to *Tobacco mosaic virus* (TMV) in tobacco and Arabidopsis [11,15,16].

SAMDC is a key enzyme in PA biosynthesis (Fig. 1-a) [11] because it can catalyze decarboxylation of S-adenosylmethionine (SAM) into decarboxylated SAM (dSAM), the aminopropyl donor for the biosynthesis of Spd and Spm (SAMDC, EC 4.1.1.50) [2,5]. Overexpression of *SAMDC* in plants can increase polyamine levels and enhance tolerance to multiple abiotic stresses [17,18]. In contrast, repression of *SAMDC* changes enzyme activity and the putrescine-spermidine ratio and induces a reduced apical dominance phenotype with decreased plant length, reduced and delayed seed germination, and diminished tolerance to multiple abiotic stresses (drought, salinity, and chilling) [19]. Most studies of the *SAMDC* gene have focused on the function of this gene in plant growth, development and abiotic stress tolerance; studies of the relationship between *SAMDC* and biotic stress tolerance [20,21] have been relatively few.

* Corresponding author.
E-mail address: xxy709@126.com (X. Xu).

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VIGS offers an attractive and rapid alternative for knocking out the expression of a gene without the need to genetically transform the plant [22]. This method takes advantage of the plant RNA interference (RNAi)-mediated antiviral defense mechanism [23]. Using this method, a recombinant virus carrying a partial sequence of a host gene is used to infect the plant. When the virus spreads systemically, the endogenous gene transcripts, which are homologous to the insert in the viral vector (VIGS vector), are degraded by post-transcriptional gene silencing (PTGS) [24,25]. The VIGS method has been successfully applied in many functional studies in tomato and other plants [25–27].

Three genes in the tomato genome encode SAMDC. In our previous study, a transcript-derived fragment, TDF35 (accession number JZ717733.1), which was homologous to the *Solanum lycopersicum S-adenosylmethionine decarboxylase 1* (*SAMDC1*) gene (99%), was up-regulated significantly in tomato plants infected with *C. fulvum* [28]. This gene was first detected in the *Cf/Avr* interaction in our study, and the role it played in the resistance response process is unknown. To understand the function of the *SAMDC* gene and determine the relationship between *SAMDC* expression and *Cf/Avr* interaction, we applied VIGS technology to decrease the expression level of *SAMDC* in resistant tomato plants and analyzed changes in resistance ability in these plants.

2. Materials and methods

2.1. Plant material

Tomato (*Solanum lycopersicum*) line CGN18423 (obtained from the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences), which carries the *Cf*-19 gene, is resistant to leaf mold disease caused by *C. fulvum*. All plants were grown at the Horticultural Experimental Station of Northeast Agricultural University. At the three-to four-leaf stage, the seedlings were used for VIGS study. The susceptible tomato line Moneymaker was used as an inoculation control. Root, stem, leaf, and other samples were collected from CGN18423 tomato plants at the flowering stage for gene expression pattern analysis.

2.2. Target fragment amplification and vector construction

Total RNA was extracted from CGN18423 seedling leaves using TRIzol (Invitrogen) according to the manufacturer's handbook. First-strand cDNA was synthesized using an M-MLV RTase cDNA synthesis kit (Takara) according to the manufacturer's instructions. Primers for target fragment TF1 were designed based on the sequence of TDF35, and primers for target fragment TF2 were designed based on the partial coding sequence (CDS) of the tomato *SAMDC1* gene (accession number NM_001247770.2, homologous gene of TDF35) (Table 1). The positions of TA1 and TA2 and the structures of the *SAMDC1* gene and protein are shown in Fig. 1-b. All primers were designed using Primer 5.0 software. The resulting PCR products were analyzed by agarose gel electrophoresis, and bands with the correct size were excised from the gel and purified with a PCR purification kit (Takara). The purified products were cloned into pMD18-T vector (Takara) and sequenced (Sangon Biotech Co., Ltd., Shanghai, China).

After the target fragments were identified, reamplification was performed using primers with added restriction site sequences (*Eco*RI and *Bam*HI). Target products were excised from the gel, purified and cloned into *Tobacco rattle virus* (TRV) RNA2 (TRV2), which was digested with restriction endonucleases *Eco*RI and *Bam*HI (Takara). The cloned TRV2 vectors were transformed into competent cells of *Escherichia coli* DH5α and incubated at 37 °C overnight on lysogeny broth (LB) medium containing kanamycin/X-gal/IPTG. The white clones were picked and cultured in liquid LB with 50 μg/mL of kanamycin, and the plasmids were extracted and verified by sequencing. The identified TRV2-TA1 and TRV2-TA2 strains were cultured in liquid LB with 50 μg/mL of kanamycin and used for plasmid extraction. The TRV constructs were transformed

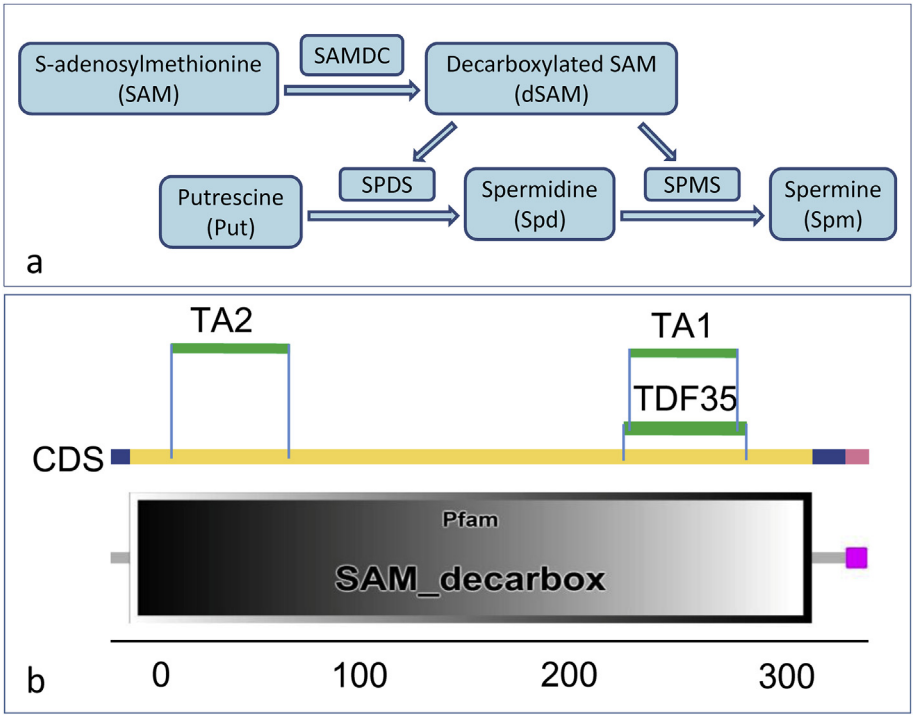

Fig. 1. Biochemical function and gene map of the *SAMDC* gene. a: Put, Spd and Spm biosynthetic pathway; b: relative positions of TA1 and TA2 with respect to the TDF35 fragment. SAMDC: S-adenosylmethionine decarboxylase; SPDS: spermidine synthase; SPMS: spermine synthase.