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Functional Analysis of *SmMYB39* in Salt Stress Tolerance of Eggplant (*Solanum melongena* L.)

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Abstract: Eggplant (*Solanum melongena* L.), a widely cultivated vegetable of the Solanaceae family, faces significant challenges in growth and yield due to soil salinization. This study aimed to investigate the functional role of the transcription factor *SmMYB39* in salt stress tolerance in eggplant. This investigation was conducted through the utilization of bioinformatics analysis, quantitative real-time polymerase chain reaction (qRT-PCR), subcellular localization, validation of transcriptional activation activity, Virus-Induced Gene Silencing (VIGS), and protein interactome analysis. Bioinformatics analysis revealed that *SmMYB39* has the closest relationship with *SlMYB41*, and its promoter contains multiple stress-responsive elements. qRT-PCR results demonstrated that *SmMYB39* was significantly upregulated after 12 h of salt stress. Subcellular localization results indicated that the SmMYB39 protein is localized in the nucleus and exhibits transcriptional activation activity. Using VIGS, we observed that silencing of *SmMYB39* led to reduced salt stress tolerance in eggplant. In addition, we have conducted research on the protein interactome of SmMYB39. In conclusion, our study demonstrates that *SmMYB39* is a crucial transcription factor involved in salt stress response and has the potential to enhance salt tolerance in eggplant.

Keywords: eggplant; salt stress; MYB transcription factor; functional analysis; VIGS

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1. Introduction

Eggplant (*Solanum melongena* L.), a vegetable belonging to the Solanaceae family, is cultivated worldwide. In recent years, improper crop rotation and excessive fertilizer use have exacerbated soil salinization and alkalinization [1]. This has resulted in severe impacts on the growth and development of plants, becoming a significant limiting factor for greenhouse crop production [2]. Specifically for eggplant, soil salinization has a detrimental effect on both vegetative and reproductive growth stages. During the vegetative growth phase, it manifests as reduced leaf expansion, plant height, and stem diameter, leading to weakened growth vigor. Additionally, it also affects the fruit setting rate and individual fruit quality during the reproductive growth phase, ultimately resulting in decreased yield and quality [3]. As a consequence, soil salinization has become a bottleneck factor in restricting the development of eggplant production. Therefore, it is of utmost importance to conduct research on the salt tolerance mechanisms of eggplant and breed new salt-tolerant varieties.

MYB (Myeloblastosis) transcription factors are one of the most widely distributed transcription factors in eukaryotes, playing crucial roles in plant responses to various abiotic stresses, including high salinity, drought, and extreme temperatures [4]. When plants are subjected to stress, including nutrient deficiency and biotic stress, it can induce the specific binding of MYB proteins to cis-elements in the promoter regions of downstream target genes. This binding activates or inhibits the transcriptional expression of these genes, thereby regulating plant stress tolerance. MYB transcription factors consist of 1 to 4 repeated units forming the MYB domain, with each repeat composed of 50 to 53 amino acids [5]. The MYB domain adopts a helix–turn–helix structure, which contains a highly conserved DNA-binding domain [6]. Based on the number of imperfect

repeats (R) within this domain, MYB transcription factors are classified into four classes: those containing 1R domain (1R-MYB/MYB-related) [7], those containing 2R domains (R2R3-MYB) [8], those containing 3R domains (R1R2R3-MYB) [5], and those containing 4R domains (4R-MYB). Among which, 1R-MYB is translated as a telomere-binding protein, primarily involved in regulating chromosomal stability and cellular morphogenesis [9]. R2R3-MYB is a widely distributed class of transcription factors that regulate the biosynthesis of secondary metabolites, participate in various stress responses, and respond to hormone signaling [10]. R1R2R3-MYB is relatively less abundant in plant cells but is widely distributed in animal and fungal cells, mainly involved in cell differentiation processes and responses to certain stresses [11]. 4R-MYB has been found only in a few plant species, and its function remains unclear.

MYB transcription factors exert their functions by binding to cis-regulatory elements in the promoter regions of genes. The cis-regulatory elements recognized by MYB transcription factors are referred to as "MBS" (MYB-binding sites). *SbMYB44* could bind to the "TAACTG" sequence present in the promoters of many stress-responsive genes and activate their expression [12]. A MYB binding site with the sequence "TAACTG" was discovered in the promoter region of the stress-responsive gene *OsIMP* in rice (*Oryza sativa*) [13]. Research findings revealed that the *MYB3R1* protein in mulberry (*Morus alba*) can bind to the "CAACGG" sequence within the promoter region of polyphenol oxidase 1 (*MnPPO1*), thereby facilitating its expression [14]. In addition to directly regulating target gene expression by binding to cis-regulatory elements, some MYB transcription factors can interact with bHLH transcription factors, regulating the expression of downstream target genes [15]. MYB transcription factors can also form ternary complexes, known as "MBW," with bHLH and WD40 transcription factors, regulating certain anthocyanin biosynthesis pathways [16]. MYB transcription factors play important roles in plant responses to both biotic and abiotic stresses. Previous studies have found that some MYB transcription factors regulate salt tolerance by the abscisic acid (ABA) signaling pathway and modulating ABA biosynthesis. Yang et al. found that under salt stress, the seed germination of high-expression lines of *OsMYB2* in rice is more sensitive to ABA compared to the wild type, indicating the involvement of ABA in the response mechanism of rice *OsMYB2* to salt stress. The upregulation of *OsMYB2* expression under salt stress confirms its responsiveness to salt stress. Furthermore, *OsMYB2* can enhance salt tolerance by increasing the expression of stress-responsive genes, such as *OsLEA3*, *OsRab16A*, and *OsDREB2A* [17]. R1 identified in castor bean (*Ricinus communis*) has been confirmed as a MYB transcription factor participating in the ABA-mediated salt stress response pathway [18]. Additionally, studies have found that MYB transcription factors can respond to salt stress by regulating the reactive oxygen species (ROS) balance. For example, *AtMYB49* in Arabidopsis thaliana activates the expression of downstream peroxidase genes to enhance plant salt tolerance [19], and overexpression of the *SlMYB102* gene in tomato (*Solanum lycopersicum* L.) helps accumulate a large amount of ROS scavenging enzymes, reducing the rate of ROS generation and improving tomato's tolerance to salt stress [20]. In addition, MYB transcription factors can respond to salt stress by activating osmotic regulation-related genes. The overexpression of the *OsMYB2* gene in rice, as mentioned earlier, leads to metabolic changes in plants, including an increase in the content of soluble sugars and free proline, which are involved in plant osmotic regulation. This indicates that MYB transcription factors play a role in regulating osmolytes to enhance plant osmotic regulation capacity. *GhMYB73* responds to salt stress by activating the expression of downstream osmotic stress-responsive genes such as *AtNHK1*, *AtSOS3*, and *AtP5CS1* [17]. It is evident that MYB transcription factors play a crucial role in a plant's response to salt stress and have significant research value in studying plant stress tolerance mechanisms.

In previous studies, we utilized high-throughput sequencing technology to detect the transcriptome of salt-tolerant eggplant (ML41) seedlings, at 0 and 24 h under high salt stress conditions [21]. Through the analysis of transcriptome sequencing data, we discovered a gene that showed a significant induction of expression in response to salt stress. Sequence

analysis revealed that this gene belongs to the MYB transcription factor family, and we named it *SmMYB39* [22]. However, the specific function of this gene remains unclear. To investigate the role of *SmMYB39* in salt stress response in eggplant, we conducted transcript expression level detection, subcellular localization, transcriptional self-activation verification, and VIGS experiments. The results revealed that SmMYB39 is localized in the cell nucleus and exhibits transcriptional self-activation activity, suggesting a positive regulatory role in the salt stress response of eggplant. In conclusion, our study provides evidence supporting the crucial role of the transcription factor *SmMYB39* in the response to salt stress and highlights its potential to enhance salt tolerance in eggplant.

2. Materials and Methods

2.1. Plant Materials and Treatments

The plant materials used in this experiment were of the salt-tolerant eggplant variety "ML41" and *N. benthamiana*. ML41 seeds were sterilized and treated with gibberellic acid (500 mg/L) for germination. The germinated seeds were sown on moist filter paper in petri dishes and placed in a growth chamber for cultivation. The growth chamber was maintained under a photoperiod of 16 h of light (800 μmol m^{-2} s^{-1}) and 8 h of darkness, with a temperature of 25 °C and relative humidity maintained at 60–70%. After germination, the seedlings were transplanted into seedling trays and then transferred to small pots after the expansion of cotyledons for further growth. The plants were grown at 25 °C and a photoperiod of 16 h (800 μmol m^{-2} s^{-1}) light and 8 h dark until they reached the 4–6 leaf stage for subsequent experiments. *N. benthamiana* seeds were vernalized and directly sown in small pots. After germination, the seedlings were transplanted into seedling pots and placed in a growth chamber under a photoperiod of 16 h light (800 μmol m^{-2} s^{-1}) and 8 h dark. The plants were grown at 25 °C and a photoperiod of 16 h light (800 μmol m^{-2} s^{-1}) and 8 h dark until they reached the 6–8 leaf stage for later experiments. For the salt treatment, the roots of 4–6 leaf-stage eggplant seedlings were immersed in a 150 mM NaCl solution for 12 h, with ddH_2O treatment used as a control. After the treatment, the eggplant roots were washed with ddH_2O, frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis [21].

2.2. Bioinformatic Analysis of SmMYB39

Based on the analysis of transcriptomic data from the ML41 eggplant, we have identified a gene that is significantly upregulated under salt stress. The full-length sequence of this gene was obtained by searching the Eggplant Genome Database "http://eggplant-hq.cn/Eggplant/home/index (accessed on 23 May 2023)", and it was named *SmMYB39* (Smechr0701996). The conserved domains within the amino acid sequence of *SmMYB39* were analyzed using the SMART online tool "http://smart.embl-heidelberg.de/ (accessed on 25 May 2023)". Multiple sequence alignments between *SmMYB39* and homologous genes from other species were performed using the BLAST online tool "https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 25 May 2023)", and an evolutionary tree was constructed using DNAMAN 10 "https://www.lynnon.com/ (accessed on 25 May 2023)". The construction of the phylogenetic tree utilized the maximum likelihood method, with a bootstrap value set at 1000. The cis-acting elements on the promoter region of *SmMYB39* were predicted using the PlantCARE online tool "https://bioinformatics.psb.ugent.be/webtools/plantcare/html/ (accessed on 27 May 2023)".

2.3. Subcellular Localization

To validate the subcellular localization of SmMYB39, an *N. benthamiana* transient overexpression system was employed. The subcellular localization method mentioned in previous studies was employed [21]. *SmMYB39* was cloned into the pBinGFP2 plant overexpression vector, resulting in a fusion construct with 35S:*SmMYB39*-GFP. The constructed plasmid was then transformed into the *Agrobacterium tumefaciens* strain *GV3101*. The suspension formulation used for *Agrobacterium* consists of the following components:

10 mM MES, 10 mM $MgCl_2$, 200 mM acetosyringone; pH = 5.4. The experimental materials selected were healthy strains of *N. benthamiana*. The bacterial fluid of *GV3101* cells harboring 35S:H2B-RFP constructs was mixed with cells carrying 35S:*SmMYB39*-GFP or 35S:GFP constructs at a ratio of 1:1. Using a sterile syringe, the leaves of *N. benthamiana* were injected on the abaxial surface with mixed bacterial fluid, respectively, avoiding the leaf veins. This process was repeated three times to ensure replicates. The injected *N. benthamiana* leaves were then cultured in a growth chamber under appropriate light conditions for 48 h. Afterward, the leaves were cut into suitable-sized leaf segments and immersed in a solution of 4',6-diamidino-2-phenylindole (DAPI) for staining. The leaf segments were incubated at 37 °C for 1 h, followed by rinsing with sterile water. Subsequently, the leaf segments were mounted on glass slides and observed using a confocal laser scanning microscope (LSM 880NLO; Leica Microsystems, Wetzlar, Germany) to visualize the fluorescence signals of the expressed gene and determine the subcellular localization of SmMYB39.

2.4. SmMYB39 Transcriptional Activation Activity Validation

Based on our previous research, we employed the yeast GAL4 system to assess the transcriptional activation activity [21]. The open reading frame (ORF) of *SmMYB39* was amplified by PCR and cloned into the pGBKT7 bait vector using molecular cloning techniques. The resulting plasmid, pGBKT7-*SmMYB39*, was transformed into the *Y187* yeast strain along with the pGADT7 empty vector. The transformed yeast cells were then plated on solid yeast peptone dextrose adenine (YPDA) medium lacking tryptophan (Trp) and incubated at 30 °C for approximately 2 days. Subsequently, the pGBKT7-*SmMYB39* plasmid and empty vector were co-transformed into yeast cells and spread on solid YPDA medium supplemented with X-α-Gal, but deficient in both tryptophan and histidine (Trp/His). Positive clones displaying a blue color indicated transcriptional activation activity.

2.5. Functional Analysis of SmMYB39 Based on VIGS Method

The VIGS method was based on previous studies [23,24]. A specific 300 bp DNA fragment derived from the full-length ORF of *SmMYB39* was PCR amplified. The amplified fragment was then cloned into the entry vector pDONR207 using molecular cloning techniques. Subsequently, the cloned fragment was transferred from the entry vector pDONR207 into the destination vector pTRV2 [21]. The pTRV2-*iSmMYB39* construct was transformed into the *Agrobacterium tumefaciens* strain *GV3101*. Positive clones were propagated by inoculating the bacterial suspension in 5 mL liquid LB medium containing kanamycin and rifampicin and incubating at 28 °C with agitation at 220 rpm for 3–5 h. The suspension was then centrifuged at room temperature at 8000 rpm for 10 min, the supernatant was discarded, and the bacterial pellet was resuspended in an appropriate volume of infiltration medium. The concentration of the bacterial suspension was adjusted to OD_{600} = 0.8. The pTRV2-*iSmMYB39*, pTRV2, and pTRV2-*SmPDS* constructs were mixed in a 1:1 ratio with pTRV1, and the mixture was incubated at 28 °C with gentle agitation at 60 rpm for 3 h [23]. For the experiment, young leaves at the pre-expanded stage of ML41 eggplant variety were used. Using a sterile syringe, the bacterial suspension was injected into the abaxial surface of the leaves. The plants were then placed in a growth chamber at 16 °C in the dark for 48 h, followed by transfer to a growth chamber with a temperature of 28 °C and a light cycle of 16 h light (800 μmol m^{-2} s^{-1}) and 8 h darkness. After approximately 15 days, obvious whitening symptoms were observed in the positive control plants injected with pTRV2-*SmPDS*, indicating successful silencing. RNA was extracted from the leaves of pTRV2-*SmMYB39* silenced plants and pTRV2 negative control plants. The efficiency of *SmMYB39* silencing was evaluated using quantitative real-time PCR (qRT-PCR) [21].

2.6. qRT-PCR Assay

The qRT-PCR assay was conducted following previously established protocols to evaluate the expression levels of the selected genes at the transcript level [25]. In order to

ensure the robustness and reliability of the results, four independent biological replicates were employed to determine the transcript expression levels of the target genes.

2.7. Statistical Calculations

The statistical analysis data were processed using Microsoft Office Excel 2019 and IBM SPSS Statistics 26. The differences between samples were tested using one-way analysis of variance (ANOVA) and Tukey's test ($p < 0.01$) [21].

3. Results

3.1. Expression Patterns of SmMYB39 in Response to Salt Stress

To investigate the expression patterns of *SmMYB39* in response to salt stress, the gene expression was analyzed using two different approaches: FPKM values obtained from transcriptome data [21] and relative gene expression measured by quantitative real-time polymerase chain reaction (qRT-PCR). Firstly, the FPKM values of *SmMYB39* at 0 h and 24 h of salt stress were examined. The FPKM values showed a significant difference between the two time points, indicating a dynamic regulation of *SmMYB39* expression under salt stress. At 0 h, the expression level was relatively low, while at 24 h, the FPKM value increased to approximately 40-fold higher than the baseline level (Figure 1A). The statistical difference in expression between the two time points was confirmed by statistical analysis. Secondly, qRT-PCR analysis was performed to validate the expression patterns of *SmMYB39* under salt stress at different time intervals (0, 3, 6, 12, 24, and 36 h). The relative gene expression levels were determined and plotted accordingly (Figure 1B). The results demonstrated a distinctive trend with an initial increase followed by a decrease in expression. At 12 h, *SmMYB39* exhibited the highest expression level, reaching a value approximately 50-fold higher than the control. The subsequent time points, 3, 6, and 24 h, displayed expression levels around 8-fold higher. In contrast, the expression levels at 0 h and 36 h were relatively lower. Statistical analysis revealed significant differences among the time points, indicating the time-dependent regulation of *SmMYB39* expression under salt stress.

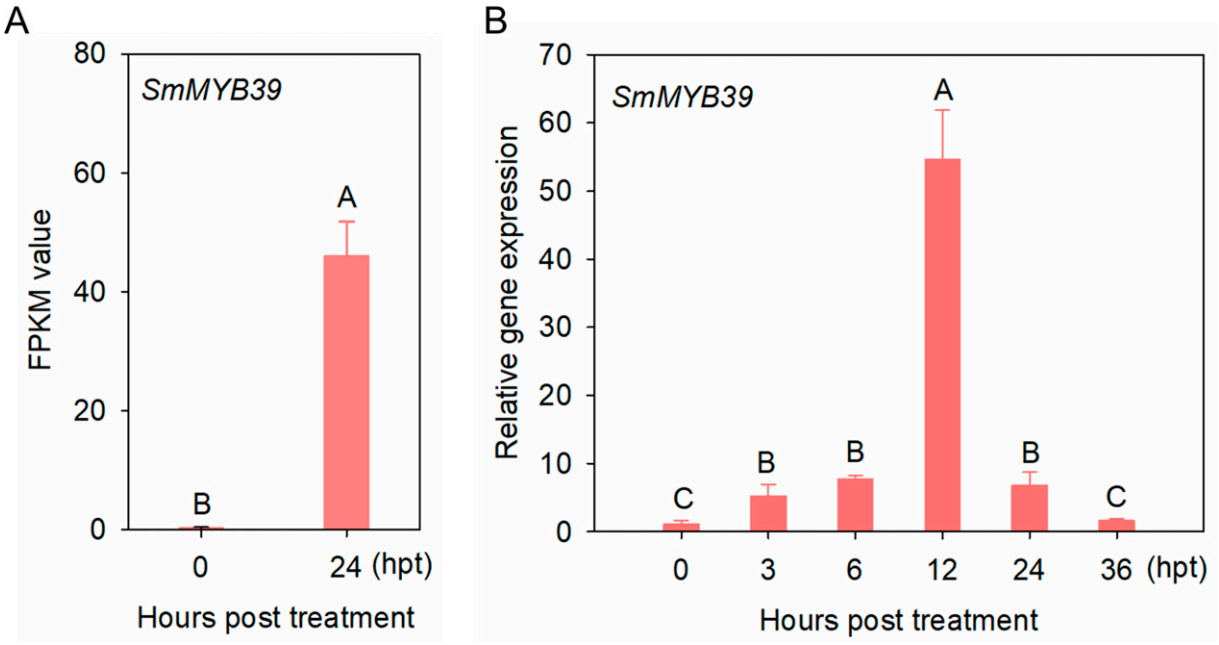

Figure 1. The expression level of *SmMYB39* under salt stress in eggplant was evaluated. Data are means ± standard deviation from four biological replicates. Different capital letters between samples denote significant differences according to one-way ANOVA and Tukey's test ($p < 0.01$). The error bars represent the standard deviation. (**A**) FPKM values of *SmMYB39* at 0 h and 24 h of salt stress. (**B**) Relative gene expression levels of *SmMYB39* at different time intervals under salt stress.

3.2. Sequence, Phylogenetic and Promoter Analysis of SmMYB39

Through searching the Eggplant Database, the gene sequence of *SmMYB39* was obtained, and homologous genes with high similarity were collected. Analyses were performed on the conserved domains of the amino acid sequence of *SmMYB39*, multiple sequence alignment, phylogenetic tree construction, and cis-regulatory elements in the promoter sequence. The results revealed that *SmMYB39* possesses two conserved SANT domains (Figure 2A). Additionally, through multiple sequence alignment and phylogenetic tree construction of the amino acid sequences, it was found that *SmMYB39* was the most closely related to tomato's *SlMYB41* (Figure 2B). Utilizing the PlantCARE online tool to predict cis-regulatory elements in the promoter sequence of *SmMYB39*, multiple stress-responsive elements were discovered, including two ABA-responsive elements (ABRE), one jasmonic acid (JA)-responsive element (CGTCA-motif), two ethylene (ET)-responsive elements (ERE), two MYB transcription factor binding elements (MYB), two drought-induced MYB transcription factor binding elements (MBS), two salicylic acid (SA)-responsive elements (TCA-element), and one WRKY transcription factor binding element (Figure 2C). These findings indicated that *SmMYB39* was likely to be involved in the response of eggplant to salt stress by responding to hormone signals.

3.3. Nuclear Localization of SmMYB39

To investigate the subcellular localization of SmMYB39 and its functional role in plant cells, the fusion of *SmMYB39* with the pBinGFP2 plant expression vector was performed. This vector contained the 35S promoter and the GFP reporter gene (Figure 3A). The recombinant constructs, 35S:*SmMYB39*-GFP and 35S:GFP (control), were co-infiltrated into *N. benthamiana* epidermal cells along with a nuclear localization marker (35S:H2B-RFP), using *Agrobacterium*-mediated transformation. Confocal laser scanning microscopy was employed for visualization. The results revealed that the fluorescence signals of *SmMYB39*-GFP and H2B-RFP co-localized specifically within the nuclei of the *N. benthamiana* epidermal cells. In contrast, the fluorescence signal of the control group (GFP) was distributed throughout the entire cell, indicating that SmMYB39 localizes to the nucleus (Figure 3B).

3.4. SmMYB39 Exhibits Transcriptional Activation Activity

To investigate the transcriptional activation activity of *SmMYB39*, the yeast *GAL4* system was employed for transcriptional activation assays. The yeast cells were transformed with pGBKT7-*SmMYB39* and the empty pGBKT7 vector, leading to the formation of colonies on YPDA solid medium lacking Trp. Additionally, the yeast cells were inoculated on YPDA solid medium containing X-α-Gal but lacking Trp and His. The yeast cells carrying pGBKT7-*SmMYB39* exhibited robust growth on the aforementioned medium and formed blue colonies, whereas the yeast cells carrying pGBKT7 could not survive. These findings indicated the transcriptional activation activity of *SmMYB39* (Figure 4).

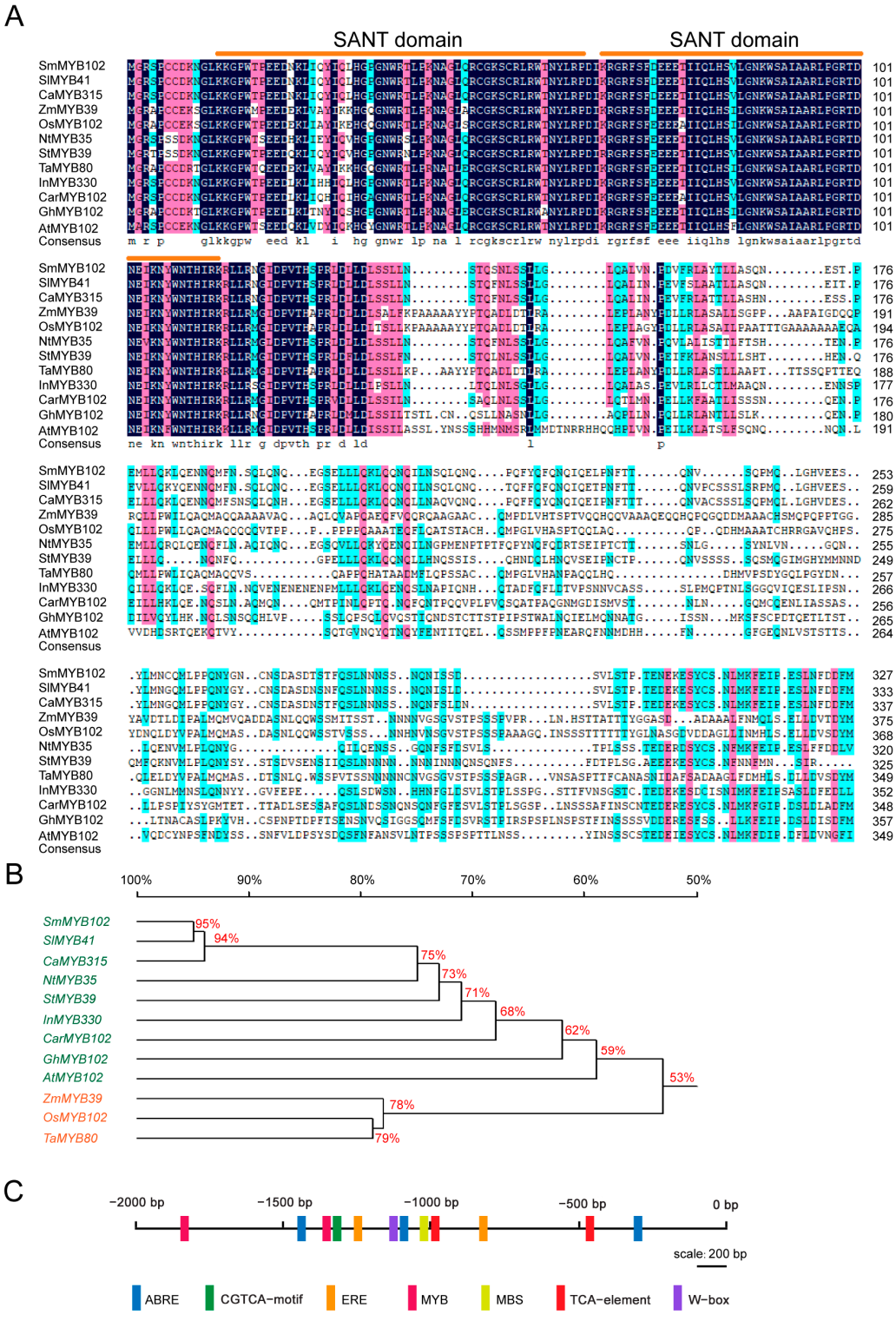

Figure 2. Sequence and phylogenetic analysis of *SmMYB39*. (**A**) Comparison results of *SmMYB39* and its highly homologous MYB protein amino acid sequences. Proteins from *Solanum lycopersicum* L. (SlMYB41); *Capsicum annuum* L. (CaMYB315); *Zea mays* L. (ZmMYB39); *Oryza sativa* L. (OsMYB102); *Nicotiana tabacum* (NtMYB35); *Solanum tuberosum* (StMYB39); *Triticum aestivum* (TaMYB80); *Ipomoea nil* (InMYB330); *Daucus carrot* (CarMYB102); *Gossypium hirsutum* (GhMYB102); *Arabidopsis thaliana* (AtMYB102). The red line denotes the SANT domain. (**B**) The phylogenetic tree was showed 11 other MYB proteins that showed high homology with SmMYB39. (**C**) Analysis of cis-regulatory elements in the promoter sequence of *SmMYB39*.

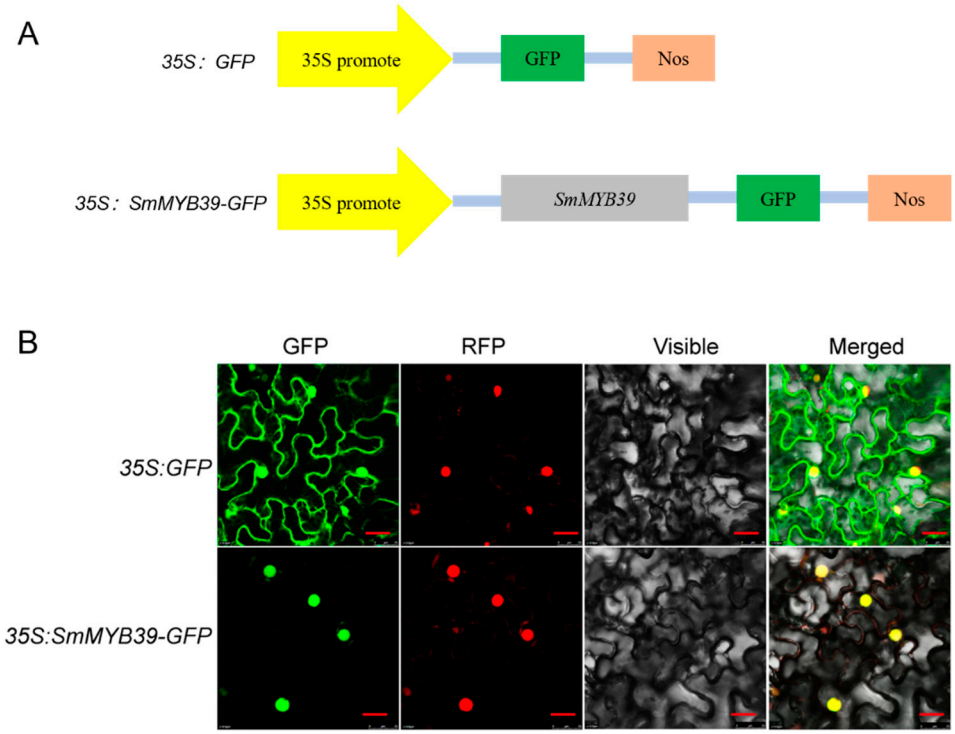

Figure 3. Subcellular localization of SmMYB39. (**A**) Construction of *SmMYB39* vector. (**B**) 35S:*SmMYB39*-GFP and 35S:GFP were co-infiltrated into *N. benthamiana* epidermal cells along with a nuclear localization marker (35S:H2B-RFP) using *Agrobacterium*-mediated transformation. Results were visualized using confocal microscopy 48 h after transformation. Bars = 50 μm.

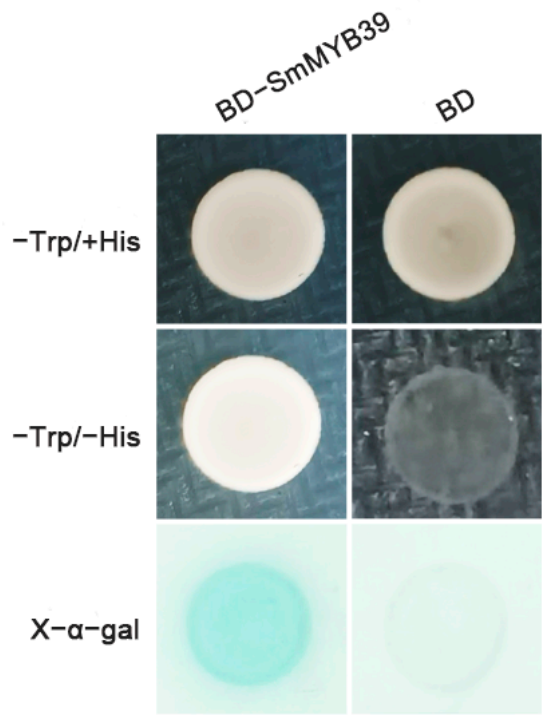

Figure 4. Verification of *SmMYB39* transcriptional activation activity. Growth of yeast cells transformed with pGBKT7-***SmMYB39*** and the empty pGBKT7 vector in medium lacking Trp and His, supplemented with X-α-Gal. Colonies of pGBKT7-***SmMYB39*** exhibited a blue color, while the empty vector control did not show any blue color, indicating the transcriptional activation activity of ***SmMYB39***. BD: binding domain.

3.5. Silencing of SmMYB39 Reduced Eggplant Tolerance to Salt Stress

Gene silencing refers to the process by which the expression of specific genes is suppressed or inhibited, leading to a decrease or absence of their corresponding protein or RNA production [26]. To further investigate the function of *SmMYB39* in salt stress response in eggplant, VIGS was employed to silence *SmMYB39*.The *Agrobacterium* infiltration solution mentioned in Section 2.5 was injected into the leaves of ML41 seedlings at the 2–4 leaf stage. When eggplant leaves infected with TRV:*SmPDS Agrobacterium* exhibited whitening, the silencing efficiency of *SmMYB39* under salt stress was detected using qRT-PCR for TRV:*SmMYB39* and TRV:*00* plants (with four biological replicates for each treatment). Compared to the control plants (TRV:*00*), the expression level of *SmMYB39* in the root system of eggplants silenced for *SmMYB39* (TRV:*SmMYB39*) was significantly reduced by approximately 97%. The salt stress treatment method mentioned in Section 2.1 was used to treat the eggplant seedlings in each group. The results showed that after 12 h of salt stress treatment, the silenced plants exhibited more severe wilting symptoms and a lower survival rate compared to the control plants, indicating that the silencing of *SmMYB39* reduced the salt tolerance of ML41 (Figure 5B,C).

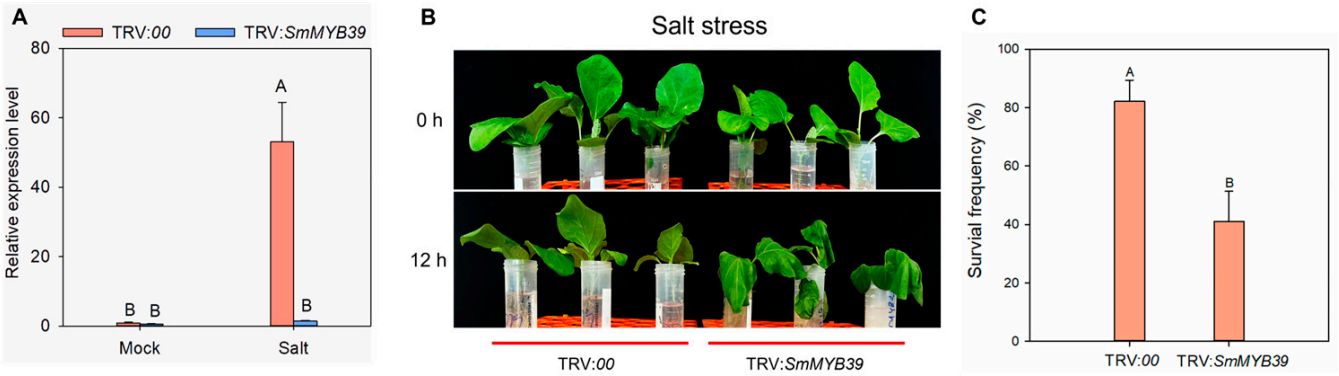

Figure 5. Effects of *SmMYB39* silencing on eggplant responses to salt stress **(TRV:*SmMY39*, *SmMYB39*-silenced; TRV:*00*, control).** (**A**) Silencing efficiency of *SmMYB39* in plants under salt stress based on a qPCR assay. (**B**) Phenotype of *SmMYB39*-silenced and control plants challenged with salt stress at 12 h post-treatment. There were no significant differences in the phenotype of plants between TRV:***SmMY39*** and TRV:***00*** when no salt stress treatment was applied. After 12 h of salt stress, the TRV:***SmMY39*** plants exhibited more severe wilting compared to TRV:***00***. (**C**) Survival frequencies of *SmMYB39*-silenced and control plants subjected to salt stress at 12 h post-treatment.

3.6. Analysis Interaction Network of SmMYB39 in Eggplant

To further investigate the function of SmMYB39, the interaction network in eggplant was predicted and analyzed using STEING v11.5 "https://version-11-5.string-db.org/" (accessed on 12 June 2023). The analysis revealed that SmMYB39 interacts with other proteins in eggplant (Figure 6). SmMYB39 showed interactions with serine/threonine-protein kinases (Smechr0600214.1, Smechr0603008.1, Smechr0800498.1, Smechr1000803.1), proteins associated with thiosulfate sulfurtransferase activity and ubiquitinyl hydrolase activity (Smechr0100346.1, Smechr0202180.1), and proteins related to the SWI/SNF complex and anaerobic respiration (Smechr0101466.1, Smechr0402591.1). Additionally, the proteins Smechr0800498.1, Smechr1000803.1, and Smechr0603008.1 that interact with SmMYB39 show co-occurence across genomes. According to the predicted interacting proteins' KEGG pathways, their potential involvement was indicated in the citrate cycle (TCA cycle), nicotinate and nicotinamide metabolism, 2-oxocarboxylic acid metabolism, alanine, aspartate and glutamate metabolism, and MAPK signaling pathway. The results of the STEING local network cluster analysis suggested that these interacting proteins might have a role in the citrate cycle, pyruvate metabolism, and the metabolic process of uridine triphosphate (UTP).

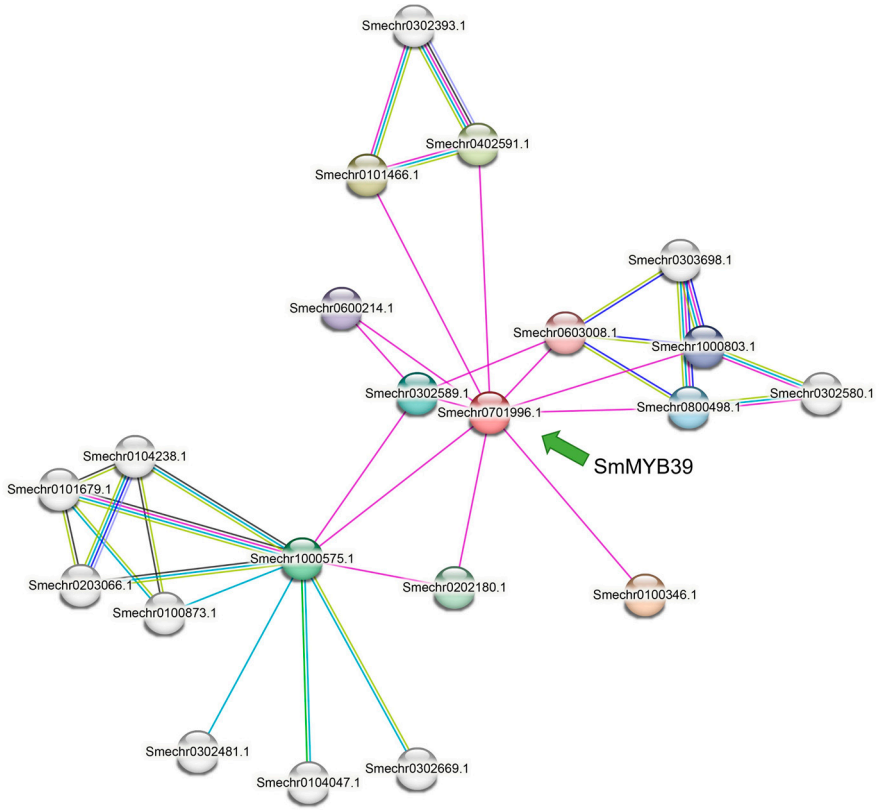

Figure 6. Interaction network of SmMYB39 protein in response to salt stress in eggplant. Nodes represent different proteins in eggplant. Blue lines indicating interactions predicted from homologous genes in other species, red lines indicating interactions that have been experimentally validated, green lines indicating interactions predicted using text mining methods, and purple lines indicating the presence of homology between the connected proteins.

4. Discussion

MYB transcription factors play important roles in the response of Solanaceae plants to biotic and abiotic stresses. Research findings have indicated that the fruit-specific expression of *SlMYB1* improved the tomato fruit quality and resistance to B. cinerea [27]. The impact of ectopic expression of *MdoMYB121* under salt stress on tomato seedlings was investigated by researchers. The response of tomato seedlings to high salt concentrations was determined based on seed germination and seedling growth. The results showed that overexpression of *MdoMYB121* increased the salt tolerance of seed germination and early seedling development. Subsequently, the effects of ectopic expression of *MdoMYB121* on adult tomatoes under salt, drought, and cold conditions were tested. The results showed that transgenic *MdoMYB121* tomato plants exhibited lower electrolyte leakage and malondialdehyde (MDA) levels, and higher accumulation of the osmoprotectant proline under salt, drought, and low temperature stress. It is hypothesized that *MdoMYB121* can reduce membrane damage and promote the accumulation of protective compounds against environmental stress [28]. *ARS1* encodes an R1-MYB-type transcription factor whose expression is influenced by leaf salt content. Identification of silenced and overexpressing *ARS1* tomato transgenic lines confirmed that this gene can regulate water loss through transpiration under salt stress [29]. Tomato plants overexpressing *MYB49* exhibited reduced accumulation of reactive oxygen species (ROS), malondialdehyde (MDA) content, and relative electrolyte leakage under salt and drought stress. The activities of peroxidase, superoxide dismutase, chlorophyll content, and photosynthetic rate increased. As a positive regulator, *SlMYB49* can enhance tomato's ability to scavenge ROS, inhibit cell membrane damage and cell death, and protect chloroplasts, thereby improving salt stress resistance [30]. After the transfer of the *TaPIMP1* gene into tobacco, the transgenic tobacco plants overexpressing

TaPIMP1 showed improved salt tolerance compared to the non-transformed tobacco host plant [31]. A MYB gene, *SpMYB*, was isolated from tomato. Transgenic tobacco plants overexpressing *SpMYB* exhibited improved salt and drought tolerance compared to the wild type [32]. However, to our knowledge, there is limited research on the involvement of MYB transcription factors in the response to salt stress in eggplant. In this study, a significant upregulation of the transcription factor *SmMYB39* was observed after 12 h of salt stress. This rapid upregulation response may represent a mechanism employed by plants to adapt to salt stress by enhancing the expression of specific genes in order to cope with environmental pressures. To gain a deeper understanding of the functionality of *SmMYB39*, sequence and phylogenetic analyses were conducted, revealing the genetic evolutionary relationships and conserved domains of *SmMYB39*. We found that *SmMYB39* shares a conserved SANT domain with its closely related MYB transcription factors. The SANT (SWI3, ADA2, N-CoR, and TFIIIB) domain is a highly conserved DNA-binding domain commonly found in various transcription factors across different organisms [33]. These findings provide important insights into the function and regulatory mechanisms of *SmMYB39* in eggplant. In addition, the nuclear localization of SmMYB39 has been confirmed, and it exhibits transcriptional activation activity, which is crucial for the functional role of transcription factors. This indicates its ability to bind downstream gene expression or interact with other transcription factors. VIGS experiments have demonstrated that suppressing the expression of *SmMYB39* reduces eggplant's tolerance to salt stress, validating the critical role of *SmMYB39* in eggplant's response to salt stress.

Various studies have shown that plant hormones play a crucial role in regulating plants' adaptive growth under stress conditions. Transcriptomic and metabolomic analyses under salt stress in cotton have revealed that ABA is a key salt-responsive biomarker for salt tolerance [34]. Jasmonic acid (JA) also influences plant responses to non-biological stresses such as salt stress. In the study of alfalfa, JA was found to regulate the expression of genes related to energy supply and antioxidant capacity at the transcriptional and post-transcriptional levels following salt stress [35]. Additionally, studies have discovered that salicylic acid (SA), polyamines (PA), and ETI-mediated ethylene synthesis and signaling can regulate salt tolerance in tomatoes. This pathway controls AOX activity and initiates the tomato antioxidant defense system through hormone regulation, thereby enabling plants to cope with the adverse effects of salt stress [36]. In this study, we analyzed the promoter region of *SmMYB39* and identified numerous response elements for ABA, JA, ET, and SA, indicating the potential involvement of *SmMYB39* in hormone signaling in eggplant's salt stress defense response. Furthermore, we found complex connections between SmMYB39 and proteins such as CBL proteins, L-aspartate oxidase, BRM (ATP-dependent helicase), and MAPK (mitogen-activated protein kinase) family proteins in eggplant. CBL proteins are closely related to plant salt tolerance. They are a class of calcium sensors widely present in plants, participating in calcium signaling transduction and ion channel regulation by forming complexes with CIPKs (CBL-interacting protein kinases) [37]. Under salt stress conditions, the activity of CBL protein-CIPK complexes is activated to regulate plant ion balance and salt tolerance response [38]. L-aspartate oxidase is an oxidoreductase that converts L-aspartate to α-ketoglutarate, generating hydrogen peroxide [39]. Hydrogen peroxide is considered an important signaling molecule in plants, activating and regulating a series of stress-responsive genes and signaling pathways. BRM protein, an ATPase, has been found to play a role in ABA-mediated drought stress response in Arabidopsis [40]. Moreover, the Arabidopsis mutant BRM-3 has been found to produce more transcripts of *PP2C* genes under salt stress, which are responsive to salt stress in Arabidopsis [41]. The MAPK family is a highly conserved group of serine/threonine protein kinases widely present in eukaryotes and involved in various cellular signaling pathways. Transcriptomic data in ginger (*Zingiber officinale Roscoe*) under salt stress revealed that differentially expressed genes activate MAPK signaling pathways in response to salt stress [42]. We speculate that *SmMYB39* may interact with the aforementioned proteins, activating salt stress defense-related genes and promoting the transmission of stress-related

hormone pathways, thereby enhancing eggplant's resistance to salt stress. However, this speculation requires further experimental verification. It is important to note that although our study establishes the positive regulatory role of *SmMYB39* in eggplant salt tolerance, there are still many unresolved questions that require further investigation. For instance, a deeper understanding of the regulatory relationships between *SmMYB39* and other genes, as well as the detailed mechanisms of its regulatory pathways, is needed. In conclusion, our study identifies the positive regulatory role of *SmMYB39* in eggplant's response to salt stress, providing a reference for further research on the mechanisms of salt stress resistance in eggplants.

5. Conclusions

This study identified a salt stress-responsive gene, *SmMYB39*, in eggplant and discovered its positive regulatory role in the root response to salt stress. In general, this study provides the first identification and validation of the important role of the eggplant MYB transcription factor in response to salt stress, which will contribute to further elucidating the regulatory mechanism of *SmMYB39* in eggplant's stress response. Our research findings have provided valuable information for the study of salt tolerance in eggplant breeding within the field of vegetable molecular breeding. Further investigations can build upon these findings to explore in depth the functionality and regulatory mechanisms of *SmMYB39*, thereby offering a theoretical basis for the development of salt-tolerant vegetable varieties and breeding strategies aimed at enhancing crop salt tolerance.

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