Silencing of the SlZF-31 gene decreases the salt stress tolerance and drought tolerance of tomato

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Abstract
The SlZF-31 gene is a member of the tomato C2H2 transcription factor family. Previous studies have shown that SlZF-31 gene expression is upregulated under drought stress and salt stress, but the specific function of this gene in tomato plants in response to these two kinds of stress is still unclear. To further explore the function of the SlZF-31 gene in tomato under drought stress and salt stress, we employed the virus-induced gene silencing method to reduce the expression of the SlZF-31 gene in tomato. The results showed that TRV2-SlZF-31 plants had higher levels of wilt and stem bending than CK and CK-TRV2 plants under drought and salt stress. The ABA content of TRV2-SlZF-31 plants were lower than those of CK and CK-TRV2 plants. The analysis of physiological indexes showed that the SOD and POD activity and the proline content of TRV2-SlZF-31 plants were lower than those of CK and CK-TRV2 plants, while the MDA content of TRV2-SlZF-31 plants was higher than those of CK and CK-TRV2 plants. The accumulation of H2O2 and O2− in TRV2-SlZF-31 plants was greater than those in CK and CK-TRV2 plants. The values of the chlorophyll fluorescence parameters (ΦII and qL) of TRV2-SlZF-31 plants were significantly lower than those of CK and CK-TRV2 plants. These results showed that the silencing of the SlZF-31 gene reduces the drought resistance and salt tolerance of tomato. The finding of this study are expected to provide theoretical support for the study of abiotic stress in tomato.

Keywords SlZF-31 gene · VIGS · Salt stress · Drought stress

Introduction
Tomatoes are one of the important vegetables in the world, and they inevitably encounter adverse environmental factors during their growth and development, including both biological factors and nonbiological factors. Among these factors, drought causes the greatest economic losses in plants among all abiotic stresses (Placide et al. 2014) and can have a significant effect on water metabolism in tomato plants. For example, drought stress can reduce the stomatal opening rate to cause morphological, physiological and biochemical changes in tomato plants, thus hindering the normal growth and development of tomatoes (Torres-Ruiz et al. 2015). Tomatoes are a plant that is sensitive to salt. Salt treatment at an appropriate concentration can modulate the flavor, color and soluble substance content of tomato, which is beneficial for improving the sugar-acid ratio of tomato fruit and, thus, the quality of tomato. However, high salt concentrations often lead to slow growth and decrease the yield and quality of tomato (Xu et al. 2015). Therefore, salt stress disrupts the ion balance and cell structure in tomato, limits the absorption and utilization of water and nutrients in tomato roots, and thereby affects the growth and development of tomato. When plants are subjected to drought and salt stress, the content of abscisic acid (ABA) in plants increases, which activates the ABA hormone signaling pathway and induces the expression of downstream transcription factors (TFs). TFs activate gene expression through cis-acting elements to improve the tolerance of plants to the stress response...
pathway (Wang et al. 2011). There are many TFs related to abiotic stress in the whole tomato genetic system, among which the C2H2 zinc finger protein family is composed of TFs associated with abiotic stress.

The C2H2 zinc finger protein forms a zinc finger structure composed of approximately 25–30 amino acids. The conserved amino acid sequence is C-X2~4-C-X3-P-X5-L-X2-H-X3~5-H (C: cysteine, H: histidine, X: amino acid, P: phenylalanine, L: leucine) (Shimeid 2008). Two cysteines and two histidines combine with zinc ions to form a coordination bond. These amino acid sequences can form a compact finger-like tetrahedral structure in the presence of coordination bonds and remain stable (Pabo et al. 2001). Within the zinc finger structure, there is a highly conserved sequence (QALGGH) located at the junction with DNA, and this conserved amino acid sequence is unique to plant zinc finger proteins, which indicates that C2H2 zinc finger proteins may play a regulatory role in the unique growth and development of plants (Huang et al. 2005).

In 1992, the first EPF1 gene of the C2H2 zinc finger was cloned from the petunia, and in recent years, the study of plant-type C2H2 zinc finger proteins in tobacco, Arabidopsis and tomato has been reported in plants; for example, C2H2-type zinc finger proteins have been found to regulate plant growth and development and to play an important role in abiotic stress (Amarjeet et al. 2013; Englbrecht et al. 2004). The overexpression of the TaZFP1B gene of C2H2 type zinc indicating protein in wheat, can stimulate oxidative reactions and hormone signal transduction pathways in plants and increase the drought tolerance of wheat (Cheuk et al. 2020). We previously performed a genome-wide analysis of the C2H2-ZFP TF family and found that 7 and 5 genes were specifically expressed during drought stress and salt stress, the C2H2-ZFP TF family and found that 7 and 5 genes were specifically expressed during drought stress and salt stress, respectively (Zhao et al. 2020). In recent years, the use of VIGS technology in tomato has also been increasing. In the present study, VIGS technology was used to reduce the expression level of the SlZF-31 gene to analyze and study the function of this gene under abiotic stress (drought stress and salt stress). This study will provide a theoretical basis and is of practical significance for future research on the function of this gene and C2H2 zinc finger proteins in general.

### Materials and methods

#### Plant materials

Seeds of the tomato variety “Moneymaker” were provided by the Tomato Research Institute of Northeast Agricultural University (Harbin, China) and were seeded in a multihole tray and incubated in the dark at 25 °C with 75% humidity for 5 d until seedling emergence. The seedlings were cultured in incubators at 30 °C with 75% humidity under a 16 h light, 8 h dark cycle until the first real leaf emerged, and the tomato seedlings were used for the VIGS experiment.

#### VIGS vector construction for the SlZF-31 gene and preparation of the Agrobacterium suspension

Total RNA was extracted from tomato leaves using the Trizol method described previously (Zhao et al. 2018a, b). The specific steps were modified according to the instructions provided for the reagent by Invitrogen. cDNA extraction was performed in accordance with the cDNA Synthesis Kit of Vazyme Biotech Co., Ltd. The primers for the gene SlZF-31 were designed according to the mRNA sequence of the SlZF-31 gene (Solyc08g063040.2.1) in the SGN tomato database. All primers employed in this study were designed using primer 5.0 software (Table 1). The target fragment of the SlZF-31 gene was subjected to homologous recombination with the tobacco rattle virus (pTRV2) vector, in which the specific steps were performed according to the CloneExpressII One Step Cloning Kit of Vazyme Biotech Co., Ltd.

| Primer name                      | (Restriction site sequence) Primer sequence (5′–3′) |
|----------------------------------|-----------------------------------------------------|
| **SLZF-31** primer-F             | (CGGAATTCG)AGGTCTACCCCTGGGGTGTCAT                  |
| **SLZF-31** primer-R             | (CGGATCCG)AGGCGAGCGTGTGTTGTAG                      |
| **SLZF-31** primer-F(qRT-PCR)    | GATGAAAGATTGCTGCGG                                |
| **SLZF-31** primer-R(qRT-PCR)    | AAGACGCAGCGTGTGTTAG                               |
| Actin primer-F                   | GTCCTCTTCCAGCCATCCAT                              |
| Actin primer-R                   | ACCACTGAGCAATGTATCCG                              |

Table 1 Primmers used for target fragment amplification and qRT-PCR analysis
L. The ligated vector was transferred to an Escherichia coli DH5a culture in accordance with the instructions of the Escherichia coli transformation kit, and single colonies were selected for growth in liquid LB medium (50 µg/mL kanamycin) and then sequenced. The sequenced vector pTRV2-SIZF-31 was transferred to Agrobacterium GV3101 for the preparation of the immersion solution according to the method of Huang et al. (Huang et al. 2014). Single colonies were transferred to liquid LB medium (50 µg/mL kanamycin, 50 µg/mL rifampicin). Then, the bacteria were transferred to IM medium (50 µg/mL kanamycin, 50 µg/mL rifampicin, 200 µM acetylenegonone) for 24 h, after which they were collected and transferred to IM medium (200 µM acetylenegonone) containing MgCl₂ to obtain an infection solution with an OD₆₅₀ of 0.2–0.3.

Infection of tomato plants to reduce the SIZF-31 gene expression level

Agrobacterium infection was carried out on tomato seedlings via vacuum infiltration with a syringe; 30 seedlings were infected at a time, among which 10 were used as the control plant (CK), 10 were used at the empty vector control plants (CK-TRV2), 10 were used as the SIZF-31 plants (SIZF-31-TRV₂). The experiment was repeated 3 times. The experimental steps were performed in accordance with the method described by Velasquez et al. (Velasquez et al. 2009).

Drought and salt stress treatment and sample collection

SIZF-31-silenced tomato plants at 25 d after infection were subjected to drought stress and salt stress (Zhang et al. 2014). After silenced SLZF-31 gene, tomato plant samples (CK, CK-TRV2 and SIZF-31-TRV2) were taken for 0 h, then the roots of these tomato plants were washed clean and the roots were inserted into 15% PEG 6000 solution for drought stress, and tomato leaves were collected at 1.5 h, 3 h, 6 h and 12 h after treatment. Meanwhile, tomato plants were inserted into 100 mM NaCl solution in the same way as above for salt stress, and tomato leaves were collected at the same 4 time points after treatment. The collected tomato leaves were used to detect various indicators in the VIGS experiment.

SIZF-31 gene expression pattern analysis

The silencing efficiency of the SIZF-31 gene was detected in tomato leaves collected at 25 d after infection. In this study, we analyzed SIZF-31 gene expression patterns in tomato leaves at different time points (0 h, 1.5 h, 3 h, 6 h and 12 h) after the silencing efficiency and expression levels of the SIZF-31 gene in TRV2-SIZF-31 tomato leaves were also analyzed by qRT-PCR at different time points (0 h, 1.5 h, 3 h, 6 h and 12 h) under the initiation of drought stress and salt stress. The same methods described above were employed for RNA extraction and cDNA synthesis from tomato leaves. The qRT-PCR method specifically followed that described by Zhao et al. (Zhao et al. 2015; Zhao et al. 2016). The primers used in the qRT-PCR analysis were designed by using the primer design software Primer 5.0 (Table 1). According to the CT values obtained with the qTOWER3G system (Analytik Jena, Germany), the relative expression of genes was calculated according to the $2^{-\Delta\Delta CT}$ method with Actin as the internal reference gene (Livak 2001; Rotenberg et al. 2006). Bar charts were generated with Office 2016 software.

Determination of ABA content

The content of ABA hormone in CK, CK-TRV2 and TRV2-SIZF-31 tomato plants under drought and salt stress (0 h, 3 h and 12 h) was determined by high performance liquid chromatography (HPLC). For specific ABA hormone detection methods, refer to the 2018 article by Li et al. (2018). Weighed 0.1 g tomato samples, ground them and added 70–80% methanol solution (pH 3.5); Centrifuge overnight after extraction; The supernatant was vaporized at 40 °C under reduced pressure, and petroleum ether was added for static stratification; After extraction and decolorization for 2–5 times, triethylamine, PVPP and other solutions were added for shock incubation, and the supernatant was extracted after centrifugation; Adjust pH to 3.0 and extract with ethyl acetate; The product was evaporated at 40 °C under reduced pressure and dissolved by adding mobile phase solution in shock. After filtration, the product was placed under ultraviolet wave of 254 nm for detection.

Determination of physiological indexes (SOD, POD, proline and MDA)

The physiological indexes of superoxide dismutase (SOD) and peroxidase (POD) activity and proline and malondialdehyde (MDA) content were determined in tomato leaves (CK, CK-TRV2, TRV2-SIZF-31) after drought stress and salt stress. The SOD activity in tomato leaves was determined according to Giannopolitis and Ries (1977). The POD activity in tomato leaves was determined according to Chance and Maehly (1955). The proline content in tomato leaves was determined according to Cakmak and Horst (1991). The MDA content was determined with the thiobarbital acid method (Ali et al. 2017). These kits were purchased from Suzhou Keming Biotechnology Co., Ltd. A. 0.2 g of tomato samples were weighed for the determination of SOD, POD, proline and MDA indexes, add 1 ml of extraction solution to tomato samples and grind the samples to homogenate; The supernatant was extracted after centrifugation at 8000 × g at 4 °C for 10 min; Add
different reagents to the liquid and place at room temperature for rest, water bath, centrifugation; The colorimetric results were quantified at OD560 nm for SOD, 470 nm for POD, 520 nm for proline and 532 nm and 600 nm for MDA.

**NBT and DAB staining**

Tomato leaves (CK, CK-TRV2, TRV2-*SlZF-31*) under drought stress and salt stress were assessed by NBT and DAB staining (Kumar et al. 2014). NBT (0.1 g) was dissolved in 50 ml of phosphate buffer, and 50 mg DAB was dissolved in 50 ml of distilled water, after which the pH was brought to 7.5. The leaves were placed in the NBT and DAB solutions overnight, and an appropriate amount of ethanol was added, followed by boiling in a water bath at 100 °C for 15 min. Finally, the results were observed on glass slides. A blue precipitate was observed in the tomato leaves after NBT staining, where a greater intensity of the blue precipitate indicated a higher O$_2^-$ content. A brown precipitate was observed in the tomato leaves after DAB staining, where a greater intensity of the brown precipitate indicated a higher H$_2$O$_2$ content.

**Chlorophyll fluorescence detection**

This evaluation was conducted under drought stress and salt stress at different time points (0 h, 1.5 h, 3 h, 6 h and 12 h) in CK, CK-TRV2 and TRV2-*SlZF-31* plants with a PhotosynQ MultispeQ multifunction plant measurement instrument made in the United States to measure the chlorophyll fluorescence parameters (PSII quantum yield (ΦII), non-photochemical quenching (Φ(NPQ)), photochemical quenching (qL) and electron transfer efficiency (ETR)).

**Results**

**Results of *SlZF-31* gene silencing efficiency analysis**

The expression level of the *SlZF-31* gene in TRV2-*SlZF-31* plants was detected by qRT-PCR. Figure 1 shows the gene expression levels of partially silenced plants, and the results showed that the expression level of the *SlZF-31* gene in 10 TRV2-*SlZF-31* plants was lower than that in CK and CK-TRV2 plants. *SlZF-31* gene expression in TRV2-*SlZF-31* plants was decreased by 42.6–85%, where the greatest decrease was 85%, and the average decrease was 79.4%. Tomato plants in which gene expression was decreased by more than 50% were selected for subsequent drought stress and salt stress treatment.

**Expression pattern analysis of the *SlZF-31* gene after drought stress and salt stress**

The expression pattern of the *SlZF-31* gene at different time points (0 h, 1.5 h, 3 h, 6 h, 12 h and 24 h) after drought stress and salt stress was detected by qRT-PCR. The results showed that the expression of the *SlZF-31* gene in CK, CK-TRV2 and TRV2-*SlZF-31* plants first increased and then decreased under drought stress and salt stress treatment, and that its expression was always lower in TRV2-*SlZF-31* plants than in CK and CK-TRV2 plants. These results indicated that VIGS exerted a significant silencing effect on tomato *SlZF-31* (Fig. 2).

**Phenotypic observations of tomato plants under drought stress and salt stress**

The phenotypes of CK, CK-TRV2 and TRV2-*SlZF-31* plants were observed after PEG 6000 drought stress treatment, as shown in Fig. 3. CK and CK-TRV2 plants showed leaf wilting after 6 h of drought stress, which was aggravated after 12 h and was more serious after 24 h. The leaves of TRV2-*SlZF-31* plants were wilted after 1.5 h of drought treatment, and the leaves were wilted and dried from 3 to 12 h. After 24 h of drought stress, stem bending degree increased gradually, and leaf wilting degree reached the highest level. Some of the leaves have dried up and some of the stalks have wilted and fallen.

CK, CK-TRV2 and TRV2-*SlZF-31* plants were observed after NaCl stress treatment. The CK plants showed leaf
**Fig. 2** Expression levels of *SlZF-31* under drought stress and salt stress. **a** Relative expression of the *SlZF-31* gene under drought stress; **b** relative expression of the *SlZF-31* gene under salt stress.

**Fig. 3** Phenotypic observation of tomato plants under drought stress and salt stress. **a** Phenotypic observation of tomato plants (CK, CK-TRV2 and TRV2-*SlZF-31*) under drought stress; **b** Phenotypic observation of tomato plants (CK, CK-TRV2 and TRV2-*SlZF-31*) under salt stress.
wilting after 12 h of treatment, and leaf wilting worsened after 24 h of treatment. CK-TRV2 plants showed leaf wilting after 3 h of treatment, and leaf wilting increased from 6 to 24 h, with no obvious change in the stem. The leaves of TRV-SIZF-31 plants began to wilt, and the stems were slightly bent after 3 h of salt stress. The degree of leaf wilting gradually increased from 6 to 12 h, and after 24 h of treatment, the leaves had dried out, and the stems were severely bent. Thus, the SIZF-31 gene plays a role in both drought and salt tolerance in tomato, and reducing the expression of the SIZF-31 gene decreases the drought and salt tolerance of tomato.

Determination of ABA content in tomatoes

As shown in Fig. 4, the ABA content of TRV2-SLZF-31 for 0 h under drought and salt stress was slightly lower than that of CK and CK-TRV2, which indicates that TRV2-SLZF-31 gene may be involved in the synthesis and accumulation of plant ABA hormone. After drought stress and salt stress, the ABA content in tomato plants (TRV2-SLZF-31, CK and CK-TRV2) showed a trend of first increasing and then decreasing, and the ABA content of TRV2-SLZF-31 tomato plants was compared with CK and CK-TRV2 plants are significantly reduced.

![Fig. 4 Determination of ABA content under drought stress and salt stress. a ABA content under drought stress; b ABA content under salt stress](image)

![Fig. 5 Quantification of SOD, POD, proline and MDA under drought stress and salt stress. a SOD activity under drought stress; b POD activity under drought stress; c proline content under drought stress; d MDA content under drought stress; e SOD activity under salt stress; f POD activity under salt stress; g: proline content under salt stress; h MDA content under salt stress](image)
Results of SOD, POD, proline and MDA quantification

As shown in Fig. 5, the SOD and POD activities and the contents of proline and MDA in CK, CK-TRV2 and TRV2-SIZF-31 plants increased after drought and salt treatment. At all time points (0 h, 1.5 h, 3 h, 6 h and 12 h) during drought treatment, the SOD and POD activities and the content of proline in TRV2-SIZF-31 plants were lower than those in CK and CK-TRV2 plants, while the MDA content in TRV2-SIZF-31 plants was higher than those in CK and CK-TRV2 plants. The results for the SOD activity, POD activity, proline content and MDA content in plants (CK, CK-TRV2 and TRV2-SIZF-31) subjected to salt stress were similar to those obtained under drought stress. Therefore, the degree of cell damage was more severe in TRV2-SIZF-31 plants than in CK and CK-TRV2 plants.

Results of NBT and DAB staining

As shown in Fig. 6, DAB and NBT staining was performed on CK, CK-TRV2 and TRV2-SIZF-31 at different time points (0 h, 1.5 h, 3 h, 6 h, 12 h and 24 h) during drought stress and salt stress, respectively. The results showed that leaf color gradually deepened with the extension of the treatment time. The brown precipitate and the blue precipitate appeared in the leaves of CK, CK-TRV2 and TRV2-SIZF-31 plants stained with DAB and NBT, respectively, after drought stress. The area and color of the precipitates in the leaves of TRV2-SIZF-31 plants were larger and darker than those in CK and CK-TRV2 plants after salt treatment.

Fig. 6 Images of the NBT and DAB staining of CK, CK-TRV2 and TRV2-SIZF-31 plants after drought stress and salt stress. a DAB staining images of CK, CK-TRV2 and TRV2-SIZF-31 plants after drought treatment; b NBT staining images of CK, CK-TRV2 and TRV2-SIZF-31 plants after drought treatment; c DAB staining images of CK, CK-TRV2 and TRV2-SIZF-31 plants after salt treatment; d NBT staining images of CK, CK-TRV2 and TRV2-SIZF-31 plants after salt treatment
CK and CK-TRV2 plants, indicating that the accumulation of H$_2$O$_2$ and O$_2^-$ under drought stress was greater in TRV2-SIZF-31 plants than in CK and CK-TRV2 plants. Under salt stress, the CK, CK-TRV2 and TRV2-SIZF-31 plants stained with DAB and NBT showed similar characteristics, which proved that the accumulation of H$_2$O$_2$ and O$_2^-$ in SIZF-31 plants was higher than that in CK and CK-TRV2 plants after salt stress. Therefore, reducing the expression level of the SIZF-31 gene causes more damage to tomato under drought stress and salt stress.

**Chlorophyll fluorescence parameter detection under drought stress and salt stress**

As shown in Fig. 7, under drought stress, the chlorophyll fluorescence parameters (ΦII, qL and ETR) of CK, CK-TRV2 and TRV2-SIZF-31 plants were reduced, among which the ΦII and qL values of TRV2-SIZF-31 plants were significantly lower than those of CK and CK-TRV2 plants, and the ETR of TRV2-SIZF-31 plants decreased the most. The Φ (NPQ) values of CK, CK-TRV2 and TRV2-SIZF-31 plants were increased. The changes in the chlorophyll fluorescence parameters of CK, CK-TRV2 and TRV2-SIZF-31 plants under salt stress were similar to those under drought stress.

**Discussion**

In the abiotic stress response, the regulatory network generated by the signal transduction pathways of different hormones is complex and diverse (Verma et al. 2016). ABA is one of the key hormones regulating plant growth, development and physiological activities. It not only regulates stomatal opening and controls organ shedding but also plays an important role in the response to adverse conditions and various types of stress (Yamaguchi-Shinozaki and Shinozaki 2006). When plants encounter drought, salt damage and other stresses, ABA levels increase, activating the ABA signal transduction pathway and inducing the expression of many genes related to stress resistance to reduce the damage to plants caused by stress and protect their normal growth (Wang et al. 2011). In this study, the ABA content of TRV2-SIZF-31 plants was lower than that of CK and CK-TRV2 plants under drought and salt stress. Therefore, we speculate that the SIZF-31 gene may be involved in the ABA hormone synthesis or metabolism pathway, so that this gene affects the drought and salt tolerance of tomato plants by affecting the signal transduction pathway of ABA hormone. Tian also found that STZFP1, a C2H2 type zinc finger protein gene in potato, may respond to plant salt and drought stress through ABA signaling pathway (Tian et al. 2010).

TRV2-SIZF-31 plants showed no significant change in phenotype before stress treatment, indicating that the SIZF-31 gene had no significant effect on the plant phenotype after silencing. We observed the phenotypes of CK, CK-TRV2 and TRV2-SIZF-31 plants under drought and salt stress. It was found that the degrees of leaf wilting, drying and stem bending in TRV2-SIZF-31 plants were more serious than those in CK and CK-TRV2 plants under drought stress and salt stress. Therefore, we speculate that the SIZF-31 gene may play a role in both drought and salt tolerance in tomato, where reducing the expression of the gene SIZF-31 will decrease the drought and salt tolerance of tomato.
ROS production is one of the earliest responses of plants to drought stress and salt stress. ROS damage the cell membrane, thus affecting cell metabolism. To remove ROS from plant cells and protect macromolecules in plant cells, a series of antioxidant systems involving reactive oxygen scavenging enzymes, such as SOD and POD, are activated in plants (Apel and Hirt 2004). SOD can convert superoxide in plants into H₂O₂ and O²⁻ to resist oxidative damage to plant cells (Ajithkumar and Panneerselvam 2014). POD can protect the cell structure and reduce damage to the membrane system and plays a highly significant role in the maintenance of normal physiological activities under adverse conditions (Apel and Hirt 2004). Proline is an osmotic regulator that is accumulated by plants in the process of drought resistance and salt resistance and reflects the resistance abilities of plants to some extent. MDA is the final product of membrane peroxidation in plants, and the accumulation of MDA damages the structure and function of the cell membrane. The MDA content represents the degree of membrane lipid peroxidation and the degree of damage to the membrane system and is therefore an important indicator of stress resistance in the plant (Kishor and Sreenivasulu 2014). The results of this experiment showed that under drought stress and salt stress, the SOD and POD activities and the proline content of TRV2-SIZF-31 plants were higher than those of CK and CK-TRV2 plants. The MDA content of TRV2-SIZF-31 plants was also higher than that of CK and CK-TRV2 plants, indicating that tomato plant cells suffered more damage after the expression of the SIZF-31 gene was reduced. Similar results were found in a study by Zhao et al., who reported that the SOD and POD activity and the proline content were lower in TRV2-SL-ZH13 plants than in CK plants after SL-ZH13 gene expression was reduced, while the content of MDA was higher than that of CK plants (2018a, b; 2019).

The accumulation of superoxide radicals (O²⁻) in tomato leaves was examined by NBT staining. The higher the O²⁻ accumulation is, the darker the blue precipitate will be in leaves stained with NBT (Ivan et al. 2009). DAB staining was used to detect the accumulation of H₂O₂. The higher the accumulation of H₂O₂ is, the more intense the brown precipitate will be in leaves stained with DAB (Fryer et al. 2002). The experimental results showed that after drought stress and salt stress, the blue precipitate in the leaves of TRV2-SIZF-31 plants stained with NBT was darker than that in the leaves of CK and CK-TRV2 plants, and the brown precipitate in the leaves of TRV2-SIZF-31 plants stained with DAB was darker than that in the leaves of CK and CK-TRV2 plants. Therefore, the accumulation of O²⁻ and H₂O₂ in TRV2-SIZF-31 plants was higher than those in CK and CK-TRV2 plants. This phenomenon indicated that SIZF-31 gene silencing did not directly affect the accumulation of reactive oxygen species in leaves, but after drought and salt stress, SIZF-31 gene silencing led to more intense accumulation of reactive oxygen species in leaves.

Drought, salt and other abiotic stresses can degrade chlorophyll in plants, thus reducing the absorption and transmission of light energy by chloroplasts (Kuang et al. 1980). Chlorophyll fluorescence parameters can reflect the PSII plant photosynthetic system of light absorption and utilization (Demmig-Adams and Adams 1996). ФII represents the actual photosynthetic efficiency of PSII (the PSII quantum efficiency of energy conversion). QL reflects the proportion of open PSII reaction centers (i.e., the degree of openness of the PSII reaction center). Ф (NPQ) represents the PSII regulatory energy dissipation quantum yield. ETR is the relative linear electron flow rate through the PSII optical system. In this study, the values of the chlorophyll fluorescence parameters (ФII and QL) of TRV2-SIZF-31 plants were found to be significantly lower than those of CK and CK-TRV2 plants under drought stress and salt stress, and the ETR of TRV2-SIZF-31 plants showed the greatest decrease. We speculated that this might have occurred because of the decreased drought resistance and salt resistance of TRV2-SIZF-31 plants after the SIZF-31 gene was downregulated. Therefore, the chloroplast photosynthetic mechanism of TRV2-SIZF-31 plants under abiotic stress was more severely damaged than those of CK and CK-TRV2 plants, and the photosynthetic capacity of TRV2-SIZF-31 plants was significantly decreased. Lu and others show that the plant chloroplast photosynthetic mechanism is damaged under abiotic stress, the PSII original light energy conversion efficiency (ФII) is reduced, the photosynthetic ETR is reduced, the QL light energy available for photosynthesis is reduced, and the excess excitation energy (Ф (NPQ)) is increased, leading to a decrease in PSII function and photosynthetic capacity (Lu and Zhang 1999).

Prior to the abiotic stress treatment (drought stress and salt stress), there were no significant differences in the phenotype, physiological indexes (SOD, POD, proline and MDA), NBT staining or DAB staining between control plants and TRV2-SIZF-31 plants. After drought stress and salt stress, the phenotypes and related indexes of the control plants and TRV2-SIZF-31-silenced plants were significantly different, indicating that SIZF-31 gene silencing had no significant influence on the growth and development of tomato plants, while this gene did play a role in plant resilience to stress.

**Conclusion**

In conclusion, the degrees of withering and stem bending in TRV2-SIZF-31 plants were greater than those in CK and CK-TRV2 plants under drought stress and salt stress. The ABA content of TRV2-SIZF-31 plants were lower than those
of CK and CK-TRV2 plants. The analysis of physiological indexes showed that the SOD and POD activity and the proline content of TRV2-SIZF-31 plants were lower than those of CK and CK-TRV2 plants, while the MDA content of TRV2-SIZF-31 plants was higher than those in CK and CK-TRV2 plants. The accumulation of H₂O₂ and O₂⁻ in TRV2-SIZF-31 plants was higher than those in CK and CK-TRV2 plants. The values of the chlorophyll fluorescence parameters (ΦII and qL) of TRV2-SIZF-31 plants were significantly lower than those of CK and CK-TRV2 plants, and the ETR of TRV2-SIZF-31 plants showed the greatest decrease. These results indicated that the downregulation of the SIZF-31 gene affected the response of tomato under drought and salt stress and reduced the drought and salt resistance of tomato plants.

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References

Ajitkumar IP, Panneerselvam R (2014) ROS scavenging system, osmotic maintenance, pigment and growth status of Panicum sumatrense Roth. under drought stress. Cell BiochemBiophys 68:587–595
Ali Q, Javed MT, Noman A, Haider MZ, Waseem M, Iqbal N, Waseem M, Shah MS, Shahzad F, Perveen R (2017) Assessment of drought tolerance in mung bean cultivars/lines as depicted by the activities of germination enzymes, seedling’s antioxidative potential and nutrient acquisition. Arch Agron Soil Sci 64:84–102
Amarjeet S, Poonam K, Amita P, Tyagi AK, Sopory SK, Sanjay K (2013) Comprehensive genomic analysis and expression profiling of phospholipase c gene family during abiotic stresses and development in rice. PLoS ONE 8:62494
Anand A, Vagbhshippawala ZR, Ryo CM, Kang L, Wang K, Del-Pozo O MGB, Myrose KS (2007) Identification and characterization of plant genes involved in Agrobacterium-mediated plant transformation by virus-induced gene silencing. MPMI 187:41–54
Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399
Becker A, Lange M (2009) VIGS genomics goes functional. Trends Plant Sci 15:1–4
Benedito VA, Visser PB, Angenent GC, Krens FA (2004) The potential of virusinduced gene silencing for speeding up functional characterization of plant genes. Genet Mol Res 3:323–413
Cakmak I, Horst WJ (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiol Plant 83:463–468
Chance B, Maehly A (1955) Assay of catalases and peroxidases. Methods Enzymol 2:764–775
Cheuk A, Ouellet F, Houde M (2020) The barley stripe mosaic virus expression system reveals the wheat c2h2 zinc finger protein tazfp1b as a key regulator of drought tolerance. BMC Plant Biol 20(1):1–34
Demmin-Adams B, Adams WW (1996) Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. Planta 198:460–470
Englebrecht CC, Schoof H, Bohm S (2004) Conservation, diversification and expansion of C2H2 zinc finger proteins in the Arabidopsis thaliana genome. BMC Genom 5:39
Fryer MJ, Oxborough K, Mullineaux PM, Baker NR (2002) Imaging of photo-oxidative stress responses in leaves. J Exp Bot 53:1249–1254
Giannopolitis CN, Ries SK (1977) Superoxide dismutase occurrence in higher plants. Plant Physiol 59:309–314
Huang J, Wang J, Zhang H (2005) Rice ZEP15 gene encoding for a novel C2H2-type Zinc Finger protein lacking DLN box, is regulated by spike development but not by abiotic stresses. MolBiol Rep 32:177–183
Huyn H, Mei M, Mao Z, Lv S, Zhou J, Chen S (2014) Molecular cloning and virus induced gene silencing of MiASB, in the southern root-knot nematode, Meloidogyne incognita. Eur J Plant Pathol 138:181–193
Ivan C, Matthieu B, Cécile SC, Fanny R, Gwenola G (2009) Differential patterns of reactive oxygen species and antioxidative mechanisms during atrazine injury and sucrose-induced tolerance in Arabidopsis thaliana plantlets. BMC Plant Biol 9:28
Kishor PBK, Sreenuvasulu N (2014) Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue. Plant Cell Environ 37:300–311
Kuang TY, Zuo PY, Chang CT, Lon SJ, Li TS, Hao HP, Lin SC (1980) Structure and function of chloroplast membranes. Effects of potassium and magnesium ions on the structure and the absorption spectrum of two kinds of chloroplast membranes. Photos biochem-istryPhoto phys 1:73–82
Kumar D, Yusuf MA, Singh P, Sardar M, Sarin NB (2014) Histochemical detection of superoxide and H₂O₂ accumulation in brassica juncea seedlings. Bio Protocol 4:1–4
Li XY, Zhao QQ, Liu Y, Liu ZL, Yang JM, Zhang GJ (2018) Determination of abscisic acid in begonia fruit by HPLC. North Hortic 4:138–144
Livak KJ (2001) Schmittgen Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:402–408
Lu CM, Zhang JH (1999) Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. J Exp Bot 50:1199–1206
Pablo CO, Peisach E, Grant RA (2001) Design and selection of novel Cys2HIs2 zinc finger proteins. Annu Rev Biochem 70:313–340
Placide R, Hirit GB, Stephan N, Fekadu B (2014) Assessment of drought stress tolerance inrout and tuber crops. Afr J Plant Sci 8:214–224
Rotenberg D, Thompson TS, German TL, Willis DK (2006) Methods for effective real-time RT-PCR analysis of virus-induced gene silencing. J Virol Methods 138:49–59
Senthil-Kumar M, Hema R, Anand A, Kang L, Udayakumar M, Mysore KS (2007) A systematic study to determine the extent of gene silencing in Nicotianabenthamiana and other Solanaceae species when heterologous gene sequences are used for virus-induced gene silencing. New Phytol 176:782–791
Shimeid SM (2008) C2H2 zinc finger genes of the Gli, Zic, KLF, SP, Wilms’ tumour, Hucklebein, Snail, Ovo, Spalt, Odd, Blimp-1, Fez and related gene families from Branchiostoma floridae. Dev Genes Evol 218:639–649

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Tian ZD, Zhang Y, Liu J, Xie CH (2010) Novel potato C2H2-type zinc finger protein gene, StZFP1, which responds to biotic and abiotic stress, plays a role in salt tolerance. Plant Biol 12:689–697
Torres-Ruiz JM, Antonio DE, Alfonso PM, Virginia HS (2015) Role of hydraulic and chemical signals in leaves, stems and roots in the stomatal behaviour of olive trees under water stress and recovery conditions. Tree Physiol 4:415–424
Velásquez AC, Chakravarthy S, Martin GB (2009) Virus-induced gene silencing (VIGS) in Nicotianabenthamiana and tomato. J Vis Exp 28:e1292
Verma V, Ravingran P, Kumar PP (2016) Plant hormone-mediated regulation of stress responses. BMC Plant Biol 16:86
Wang ZY, Xiong LM, Li WB, Zhu JK, Zhu JH (2011) The plant cuticle is required for osmotic stress regulation of abscisic acid biosynthesis and osmotic stress tolerance in Arabidopsis. Plant Cell 23:1971–1984
Xu JN, Xu YQ, Yan Z, Zhang LY, Gao JW (2015) Research progress on response mechanism of tomato to abiotic stress. Shandong AgricSci 47:120–124
Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57:781–803
Zhang ZZ, Chen XL, Guan X, Liu Y, Chen HY, Wang TT, Mouekkoub LD, Li JF, Wang AX (2014) A genome-wide survey of homeodomain-leucine zipper genes and analysis of coldresponsive HD-Zipl members’ expression in tomato. BiosciBiotechnolBiochem 78:1337–1349
Zhao TT, Liu G, Li S, Li JF, Jang JB, Zhang H, Chen X, Xu XY (2015) Differentially expressed gene transcripts related to the Cf-19-mediated resistance response to Cladosporium fulvum, infection in tomato. PhysiolMol Plant Pathol 89:8–15
Zhao TT, Jiang JB, Liu G, He S, Zhang H, Chen X, Li JF, Xu XY (2016) Mapping and candidate gene screening of tomato Cladosporium fulvum-resistant gene Cf-19, based on high-throughput sequencing technology. BMC Plant Biol 16:51
Zhao TT, Hu JK, Gao YM, Wang ZY, Xu XY (2018a) Silencing of the SL-ZH13 transcription factor gene decreases the salt stress tolerance of tomato. J Am SocHorticSci 143:1–6
Zhao TT, Yang HH, Jiang JB, Liu G, Zhang H, Xiao D, Chen XL, Li JF, Xu XY (2018b) Silencing of the SAMDC gene decreases resistance of tomato to Cladosporium fulvum. PhysiolMol Plant Pathol 102:1–7
Zhao TT, Wang ZY, Bao YF, Zhang XC, Yang HH, Zhang DY, Jiang JB, Zhang H, Li JF, Chen QS, Xu XY (2019) Downregulation of SL-ZH13 transcription factor gene expression decreases drought tolerance of tomato. J IntegrAgric 18:1579–1586
Zhao TT, Wu TR, Wang ZY, Pei T, Yang HH, Li JF, Xu XY (2020) Genome-wide analyses of the genetic screening of C2H2-Type Zinc finger transcription factors and abiotic and biotic stress responses in tomato (Solanum lycopersicum) based on RNA-Seq data. Front Genet 11:540

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