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Iron homeostasis disruption suppresses viral infection via ferroptosis-like cell death and RNA interference in plants

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Abstract. Ferroptosis-like cell death in plants is a recently described mechanism triggered by iron accumulation and lipid peroxidation; however, its role in viral infection remains unclear. In this study, we investigated the effect of iron (Fe-EDTA) treatment on *Nicotiana benthamiana* plants infected with wild-type tomato bushy stunt virus (wtTBSV). Morphological and biochemical analyses under combined stress revealed pronounced symptoms, including growth inhibition, necrosis, leaf yellowing, chlorosis, and leaf deformation, while the expression of the viral suppressor protein P19 was reduced. Biochemical assays showed that low Fe-EDTA concentrations maintained the photosynthetic apparatus and increased chlorophyll content, whereas high concentrations induced lipid peroxidation and ferroptosis-like cell death. Our results suggest that excess iron activates RNA interference and ferroptosis-like death, thereby suppressing viral infection, while low iron concentrations preserve photosynthetic activity and alleviate symptoms. In addition, combined stress led to reduced levels of HSP70 and HSP90, indicating that iron homeostasis may interfere with cellular chaperones essential for viral replication. These findings highlight the key role of ferroptosis-like cell death in plant-virus interactions.

Keywords: ferroptosis, plant virus, combined stress, ROS, TBSV

Introduction

Abiotic stress refers to a set of unfavorable non-living factors whose impact disrupts the physiological processes of plants, alters their metabolism, and suppresses growth and development. The major abiotic factors include extreme temperatures, water deficit or excess, soil salinization, as well as disturbances in ion balance caused by mineral deficiency or overaccumulation. Metals act as significant abiotic stressors: although plants require them in small amounts for normal growth, excessive accumulation can poison cells [1]. Metals of this type include iron (Fe). Within plant cells, it can exist in two oxidation states, Fe^{2+} and Fe^{3+}, and serves as a cofactor in respiration, DNA synthesis, photosynthesis, and chlorophyll formation

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[2]. However, under neutral and especially alkaline soil conditions, most iron exists in the form of poorly soluble compounds, which limits its availability to the root system. To overcome this problem, chelated forms of iron are widely used in crop production. In particular, the Fe-EDTA complex stabilizes iron ions in a soluble form, prevents their precipitation, and facilitates their transport to plant cells. Chelates provide a convenient and effective tool for fertigation, alleviating iron deficiency [3]. Despite its clear advantages, an excessive influx of iron into the cell is associated with certain risks. One of the most serious consequences is ferroptosis, a form of cell death induced by iron overload [4].

Ferroptosis is an iron-dependent form of regulated necrotic cell death, triggered by excessive lipid peroxidation and leading to the destruction of cellular membranes [5]. Ferroptosis is characterized by a decrease in the level of reduced glutathione (GSH) and inactivation of glutathione peroxidase 4 (GPX4). Under normal conditions, GPX4 reduces lipid hydroperoxides to non-toxic alcohols using GSH, thereby preventing the accumulation of lipid peroxidation products. When ferroptosis begins, cells suppress GPX4 activity or degrade the enzyme, which rapidly accumulates lipid peroxides and triggers cell death. Stress induces other antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which temporarily limit ROS accumulation; in plants, in particular, ethylene enhances the activity of these enzymes, while transcription factors of the ERF family increase the expression of antioxidant defense genes. Nevertheless, it is the inactivation of GPX4 that defines the specificity of ferroptosis, since the activation of other antioxidants reflects only a general stress response and does not prevent cell death [6,7]. At the same time, the molecular mechanisms underlying changes in the expression and activity of antioxidant enzymes in plants under ferroptosis conditions remain largely unexplored, highlighting the relevance of further research.

Heat shock proteins (HSPs) represent an evolutionarily conserved family of molecular chaperones that play a key role in maintaining protein homeostasis. Initially described as proteins induced by elevated temperatures, they are now known to be upregulated under a wide range of abiotic and biotic stresses [8,9]. During ferroptosis in animal cells, the HSP70 family exhibits a protective role: HSP70 has been shown to enhance GPX4 expression and antioxidant activity, thereby increasing resistance to ferroptosis. In contrast, HSP90 may promote ferroptosis by binding to timosaponin AIII, thereby inducing ubiquitination and degradation of GPX4, which enhances lipid peroxidation [7,10]. In plants, the direct roles of HSP70 and HSP90 in ferroptosis remain poorly understood and require further investigation.

Viral infections trigger ROS generation and cell death by employing virus-encoded suppressor proteins that inhibit RNA interference, antioxidant systems, and hormone-mediated defense pathways in plants. One such suppressor protein is p19, encoded by the tomato bushy stunt virus (TBSV). This protein binds double-stranded siRNAs, thereby suppressing post-transcriptional gene silencing and facilitating the spread of the viral genome within the plant. Previous studies have shown that p19 forms dimers and interacts with the host RNA-binding protein Hin19. The resulting complex plays a key role in the effective suppression of RNA interference and promotes viral progression in plant cells [11]. As viruses spread through plant cells, they cause significant changes in the activity of antioxidant enzymes. For example, cucumber mosaic virus (CMV) disrupts the plant's antioxidant defense by binding its 2b protein to catalase CAT3, initiating its degradation via the ubiquitin–proteasome pathway. This process leads to the accumulation of H_2O_2 and the development of necrosis [12].

Simultaneous exposure to multiple stressors usually worsens plant damage as signaling pathways interact in complex ways. The relationship between viral infections and ferroptosis has been studied in detail in animals: viruses can both activate and suppress iron-dependent cell

death by altering Fe metabolism, enhancing lipid peroxidation, and influencing ROS-dependent mechanisms of cell death, thereby promoting either viral replication or evasion of the immune response [13].

In plants, these processes have been studied to a limited extent. It is known that they involve mechanisms similar to those in animals but also exhibit specific features, such as the presence of multiple GPX isoforms and the involvement of chloroplasts and peroxisomes. Suppression of GPX4 in *Nicotiana benthamiana* has been shown to enhance ferroptosis-like cell death during infection with a mutant *tobacco mosaic virus;* however, systematic data remain scarce [6]. Studying such interactions is essential for understanding multistress responses, plant immunity, and adaptation, as well as for breeding resistant cultivars. This is particularly important in the context of combined viral infections and toxic metals, which are critical factors for predicting crop yield and ensuring food security. Identifying common signaling nodes of multistress responses may provide a basis for discovering genetic markers of resistance [14].

Materials and research methods

Plant preparation

In this study, *Nicotiana benthamiana* plants were used. Soil and pots were sterilized by autoclaving, after which the soil was mixed with vermiculite at a ratio of 3:1 and watered every other day for 30 days. We grew plants under controlled conditions with a 16-hour light/8-hour dark photoperiod at 25°C during the day and 23°C at night.

Plant treatment

On day 30, plants were mechanically inoculated with tomato bushy stunt virus (TBSV) in 10 mM PBS buffer (1:3). One day post-inoculation, plants were irrigated with iron chelate (Fe-EDTA) solutions at various concentrations: 0.05 mM, 0.1 mM, 0.5 mM, 0.8 mM, and 1 mM. Morphometric measurements were performed on day 7 post-inoculation (dpi). Plants were harvested, their root systems rinsed with water, and then fixed for measurement of shoot and root length.

Chlorophyll content determination

Chlorophyll content in plant leaves was determined according to Arnon's method [15]. We measured chlorophyll using a Multiskan SkyHigh spectrophotometer (Thermo Fisher Scientific) with SkanIt Software at wavelengths of 664 nm and 647 nm. The contents of chlorophyll a, chlorophyll b, and total chlorophyll were calculated using the equations proposed by Lichtenthaler and Wellburn (1985) [16].

SDS-PAGE

For protein analysis, 0.1 g of plant material was collected and homogenized in 200 µL of extraction buffer (1×TE: 10 mM Tris, pH 7.4–7.6; 1 mM EDTA, pH 8.0). SDS-PAGE was performed according to the protocol developed by Laemmli (1970) using a polyacrylamide gradient gel with a concentration range of 5–20% [17]. The gel was prepared from a separating gel (5 mL 30% acrylamide/bisacrylamide solution [29:1], 2.5 mL 1.5 M Tris, pH 8.8, 2.3 mL dH_2O, 100 µL 10% SDS, 100 µL 10% APS, 5 µL TEMED) and a stacking gel (1.35 mL 30% acrylamide/bisacrylamide solution [29:1], 1 mL 1 M Tris, pH 6.8, 7.2 mL dH_2O, 100 µL 10% SDS, 100 µL 10% APS, 5 µL TEMED). Samples were mixed with β-mercaptoethanol (1:3). Electrophoresis was carried out at 120 V, 110 mA, and 50 W using 1×TGS buffer (25 mM Tris-HCl, 192 mM glycine, 0.1% SDS) for 2–3 hours.

Protein transfer and immunodetection

Proteins were transferred onto nitrocellulose membranes using a Mini Trans-Blot® Cell (Bio-Rad). Transfer efficiency was verified by staining the membranes with Ponceau S. Non-specific binding sites were blocked with 7.5% non-fat dry milk in TBS-Tween-20 (50 mM Tris, 200 mM NaCl, 0.25% Tween-20). Membranes were then washed three times for 7 min each in 1× TBS-Tween-20.

Immunodetection was performed using monoclonal primary antibodies against HSP70 (Agrisera, #AS08371) and HSP90 (Agrisera, #AS08346), as well as polyclonal primary antibodies against P19. Secondary anti-rabbit antibodies (Rockland Immunochemicals, 611-1502) were applied. Protein bands were visualized by incubating the membranes with NBT/BCIP substrate in the dark for 5 min.

Native-PAGE

A 7% polyacrylamide gel was prepared under native conditions to determine enzyme activity. The separating gel contained 3.75 mL N3 buffer (pH 8.5; 1 M Tris base, 2 M Tris-HCl), 2.82 mL N5 buffer (40% acrylamide/bisacrylamide solution, 19:1), 8.44 mL dH_2O, 100 µL APS, and 5 µL TEMED. The stacking gel contained 5 mL N6 buffer (6.24% acrylamide/bisacrylamide solution, 4:1), 2.5 mL N4 buffer (pH 6.9; 18 mM Tris base, 2 mM Tris-HCl), 2.5 mL dH_2O, 100 µL APS, and 5 µL TEMED.

Electrophoresis was carried out at 150 V, 120 mA, and 50 W in the UPPER buffer (pH 8.88; 50 mM Tris, 7 mM Tris-HCl, 60 mM glycine) for 2–3 h. To determine AO activity, gels were incubated in a solution containing 4 mM Tris-HCl, 3 mM indole-3-carboxyaldehyde, 0.6 mM MTT, and 0.7 mM PMS at 37 °C for 40 min [18].

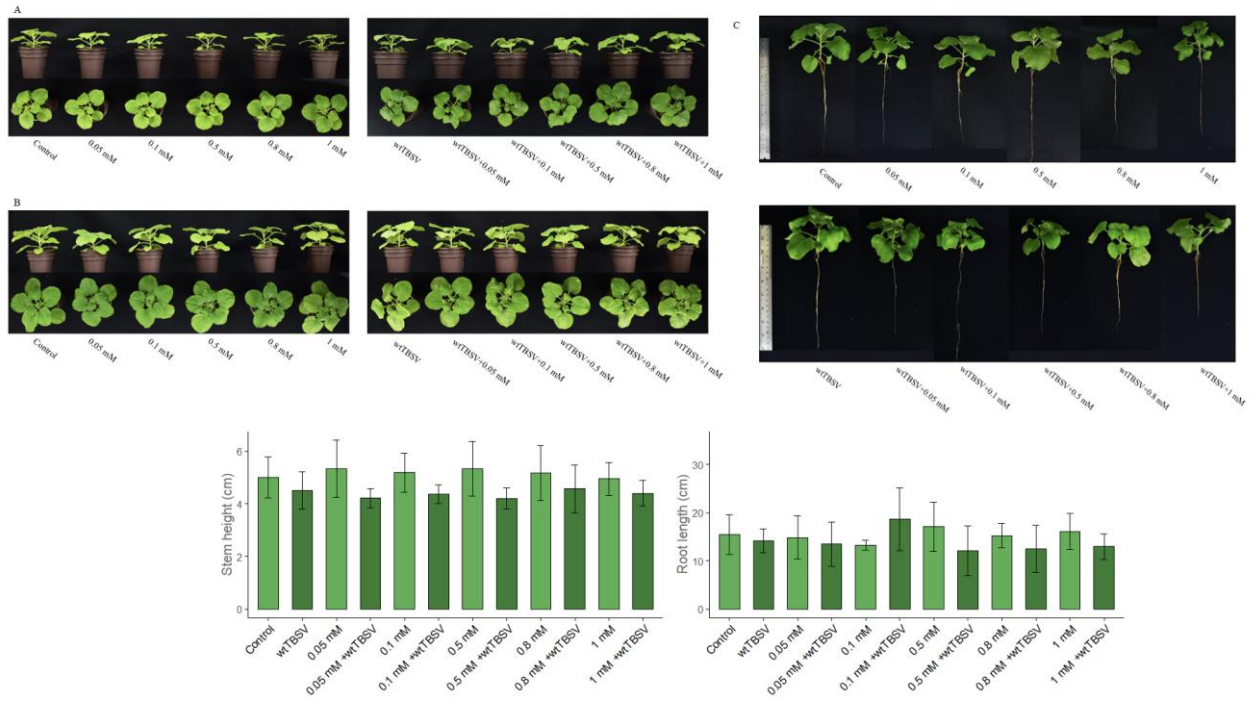
Catalase activity was determined by incubating samples with 0.03% hydrogen peroxide solution (500 µL H_2O_2 in 49.5 mL distilled water) for 10 min. Subsequently, 0.6 g $K_3[Fe(CN)_6]$ dissolved in 26.4 mL distilled water and 0.6 g $FeCl_3$ dissolved in 26.4 mL distilled water were added, and the reaction was incubated in the dark for 3 min [19].

Results

Nicotiana benthamiana plants were inoculated with wtTBSV, followed by irrigation with Fe-EDTA solution starting at 2 days post-inoculation (dpi) (Figure 1A). At 7 dpi, plants exhibited symptoms such as chlorosis and leaf wilting. Under combined stress, however, symptoms of viral infection were alleviated compared with control plants inoculated only with wtTBSV. The most pronounced improvement was observed at 0.8 mM and 1 mM Fe-EDTA.

Plants inoculated with wtTBSV displayed typical symptoms of TBSV infection, including leaf curling, chlorosis, growth suppression, necrotic spots, and wilting (Figure 1B). Morphometric analysis showed that the average shoot height of control plants was 5.0 ± 0.7 cm. Irrigation with Fe-EDTA at concentrations ranging from 0.05 to 1 mM did not significantly affect shoot growth ($p > 0.05$) (Figure 1C). In wtTBSV-infected plants, shoot height decreased to 4.5 ± 0.6 cm, and under combined stress, the reduction was more pronounced.

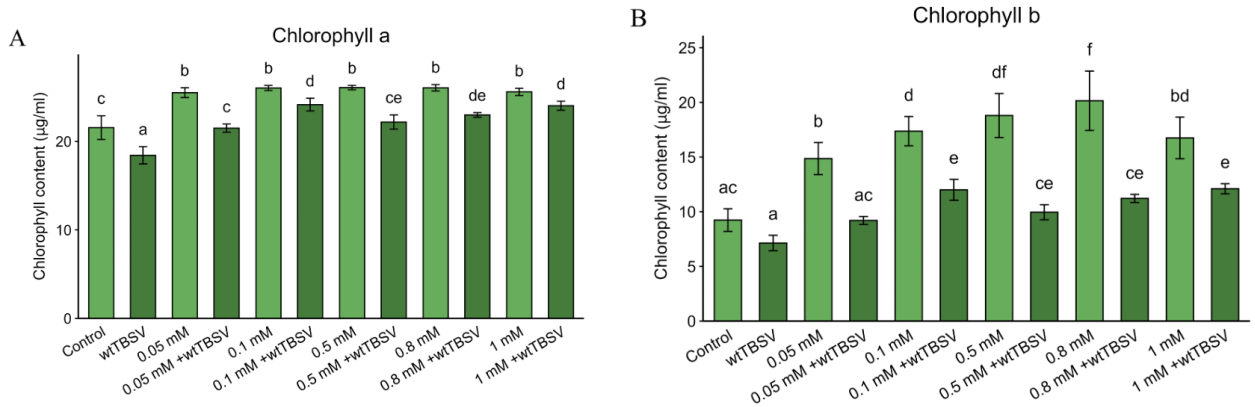
Analysis of root length revealed an average of 15.8 ± 4.2 cm. Fe-EDTA irrigation at concentrations from 0.05 to 1 mM had no significant effect compared with the control ($p > 0.05$), although an increase was observed at 0.5 mM (17.1 ± 4.8 cm) and a decrease at 0.1 mM (13.2 ± 0.9 cm). In wtTBSV-infected plants, root length moderately decreased to 14.2 ± 2.3 cm. Under combined stress, root length increased to 18.9 ± 6.8 cm at wtTBSV+0.1 mM, whereas at wtTBSV+0.8 mM it decreased to 10.0 ± 5.2 cm. However, these changes did not reach statistical significance ($p > 0.05$).

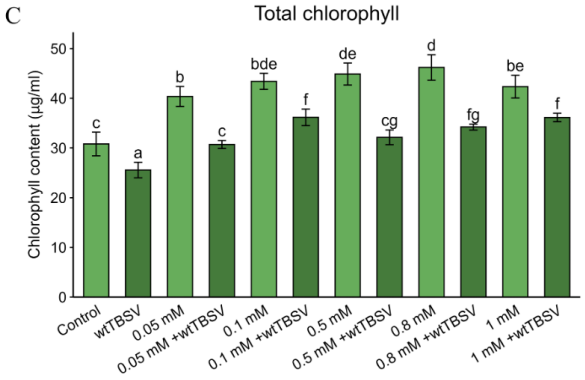


Note: Differences between groups were considered statistically significant at $p < 0.001$. Data were analyzed using one-way ANOVA followed by Tukey's HSD test. All experiments included at least three biological replicates.

Figure 1. Morphometric parameters of *Nicotiana benthamiana*. **(A)** Day 1 after inoculation with wtTBSV and Fe-EDTA irrigation; **(B)** Day 7 after inoculation with wtTBSV and Fe-EDTA irrigation; **(C)** Morphometric measurements of plant shoots and roots

To assess the activity of the photosynthetic apparatus, the contents of chlorophyll a, chlorophyll b, and total chlorophyll were analyzed in *N. benthamiana*. It was found that infection with wtTBSV significantly reduced chlorophyll levels compared to control plants. Treatment with Fe-EDTA markedly increased chlorophyll levels, with the highest values observed at 0.1 mM and 0.5 mM Fe-EDTA. Under combined stress, chlorophyll content was restored to control levels or even exceeded them (Figure 2).





Note: Differences between groups were considered statistically significant at $p < 0.001$. We performed the analysis using one-way ANOVA followed by Tukey's HSD test. All experiments included at least three biological replicates.

Figure 2. Analysis of chlorophyll content in the leaves of *N. benthamiana* plants. **(A)** Chlorophyll a content; **(B)** Chlorophyll b content; **(C)** Total chlorophyll content

Differences between groups were considered statistically significant at $p < 0.001$. We performed the analysis using one-way ANOVA followed by Tukey's HSD test. All experiments included at least three biological replicates.

To determine the levels of host proteins HSP70, HSP90, and the viral RNA silencing suppressor P19, western blot analysis was performed. The detection of the viral protein P19 was carried out using polyclonal antibodies against P19. Under combined stress conditions (Fe-EDTA + wtTBSV), the level of the viral protein P19 was significantly reduced compared to the wtTBSV control (Figure 3A). In addition, the levels of host proviral heat shock proteins (HSPs) were examined. The level of HSP70 increased at Fe-EDTA concentrations of 0.05 mM, 0.1 mM, and 1 mM, but decreased at 0.5 mM and 0.8 mM. Under combined stress, the lowest levels were observed at 0.05 mM, 0.5 mM, and 0.8 mM Fe-EDTA, whereas a significant increase was recorded at 1 mM (Figure 3B). Analysis of HSP90 revealed maximum accumulation in control plants and in response to 0.05 mM Fe-EDTA, while the lowest accumulation was observed at 1 mM Fe-EDTA. Under combined stress conditions, HSP90 levels remained moderate compared to wtTBSV but also decreased at 1 mM Fe-EDTA, similar to the results observed with Fe-EDTA treatment alone (Figure 3C).

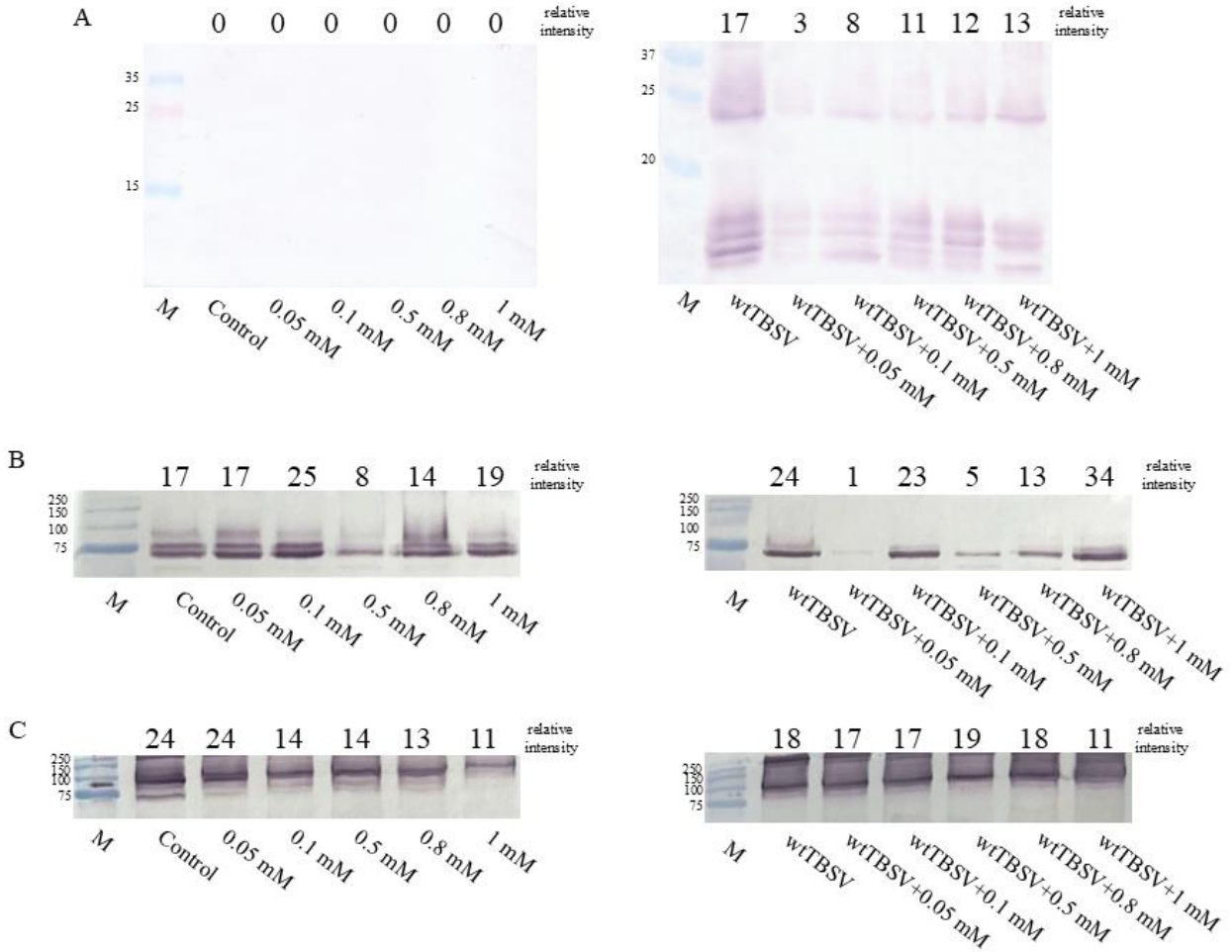


Figure 3. Western blot analysis of P19, HSP70, and HSP90 in *N. benthamiana* leaves. **(A)** Immunoblot detection of the viral protein P19. **(B)** Immunoblot detection of HSP70. **(C)** Immunoblot detection of HSP90. Data were analyzed using Image J. All experiments included at least three biological replicates

Discussion

The term ferroptosis was first described in animals as a novel form of cell death triggered by erastin in tumor cells [20]. Later, ferroptosis was also identified in plants [21], where it is activated in response to various abiotic and biotic stresses [22], including Fe^{2+} overload, accumulation of reactive oxygen species (ROS), and deficiencies in antioxidant defenses, particularly glutathione-dependent antioxidants such as GPX4 [23,24]. Lipid peroxidation in the membranes of mitochondria, chloroplasts, and peroxisomes, where ROS and iron-dependent radicals accumulate, has been shown to act as the key trigger of ferroptosis initiation [25,26]. Damage to membrane lipids disrupts membrane integrity and suppresses respiration and photosynthesis, thereby initiating a cascade of ferroptosis-like cell death.

Ferroptosis is considered a relatively recently described type of programmed cell death, and therefore, information about its regulation in plants under abiotic and biotic stresses remains fragmented. Consequently, the influence of viruses on ferroptosis is still unclear.

Our study demonstrated that Fe-EDTA treatment significantly influenced plant development and the symptoms of wtTBSV infection in *N. benthamiana*, as well as the levels of host heat shock proteins (HSPs) and the viral suppressor P19.

To analyze ferroptosis-like cell death, *N. benthamiana* plants were treated with Fe-EDTA solutions at varying concentrations to determine the optimal range. Fe-EDTA was selected as the iron source because its chelated form ensures high bioavailability and promotes the accumulation of Fe^{2+} ions in plant cells. The buildup of free iron ions triggers the Fenton reaction, which generates hydroxyl radicals (·OH):

$$Fe^{2+}+H_2O_2 \longrightarrow Fe^{3+}+OH^-+\cdot OH$$

Subsequently, the hydroxyl radical (·OH) reacts with polyunsaturated fatty acids (PUFAs), generating lipid radicals. This initiates a chain reaction in which the lipid radicals propagate by reacting with other lipids, leading to the accumulation of reactive oxygen species (ROS) [23]. Ultimately, this process results in damage to organelle membranes, proteins, and DNA [24].

In the assessment of physiological and morphometric parameters, we found that the effects of Fe-EDTA treatment and viral infection were accompanied by symptoms such as growth inhibition, leaf mottling, curling, necrosis, and chlorosis (Figure 1A, B). Iron (Fe) is essential for many cellular functions in plants, including chlorophyll biosynthesis, photosynthesis, and respiration [27]. It is well established that viral infection reduces photosynthesis and causes severe alterations in the ultrastructure of chloroplasts [28]. Previous studies have shown that treatment with iron oxide nanoparticles (Fe_3O_4) at high concentrations under high light intensity (300 μM $m^{-2}\cdot s^{-1}$) significantly increased plant biomass, as well as the contents of chlorophyll a, chlorophyll b, and carotenoids in leaves. At the same time, the nanoparticles did not have a noticeable effect on primary photochemical processes or stomatal conductance. Treatment with Fe_3O_4 nanoparticles led to a reduction in malondialdehyde (MDA) levels in roots and leaves, indicating the absence of oxidative stress. This was supported by increased activity of antioxidant enzymes, such as ascorbate peroxidase (APX) and superoxide dismutase (SOD). The authors suggest that elevated iron content in leaves promotes higher chlorophyll levels and enhances the activity of enzymes such as RuBisCO, ultimately increasing the rate of CO_2 assimilation [29]. An increase in chlorophyll content was also observed following foliar application of $Fe_2(SO_4)_3$ and EDTA-Fe·Na fertilizers to potato (Solanum tuberosum L.) tubers [30]. Iron deficiency in pea (Pisum sativum L.) plants led to a reduction in chlorophyll a and b, accompanied by an increase in dry biomass per unit of fresh shoot weight. This resulted in a significant decrease in photosynthetic rate per leaf area, as well as increased stomatal resistance and reduced transpiration rate. However, under partial iron deficiency, the photosynthetic rate per leaf area was not reduced, even though chlorophyll content decreased [31]. This may be because the reduction in chlorophyll likely did not result from the destruction of all components of the photosynthetic apparatus, but rather from an adaptive response to stress conditions. In our study, we also observed an increase in chlorophyll content following both Fe-EDTA treatment and combined stress from wtTBSV infection and Fe-EDTA, compared to control plants (Figure 2). This effect is likely due to the crucial role of iron as a component of various photosynthetic electron carriers, such as cytochrome (Cyt) b_6f and Cyt c_6, and as an integral part of both photosystem I (PSI) and photosystem II (PSII) [32–34]. Iron deficiency induces significant changes in the structure of the thylakoid membrane and in the core processes involved in photochemical energy conversion. The first transcriptomic studies conducted on

P. tricornutum cells under iron-deficient conditions identified novel regulators of the iron deficiency response, such as iron starvation-induced proteins (ISIPs) [35].

Tomato bushy stunt virus (TBSV) is a typical member of the genus Tombusvirus within the family Tombusviridae. The P19 protein encoded by TBSV acts as a potent suppressor of RNA interference (RNAi) and blocks a key post-transcriptional defense mechanism in plants. RNAi is a conserved eukaryotic mechanism that degrades RNA with high sequence specificity [36–38]. A DICER-containing complex recognizes and cleaves double-stranded RNA (dsRNA) or single-stranded RNA (ssRNA) with hairpin structures, producing short interfering RNAs (siRNAs) of approximately 20–25 nucleotides. The RNA-induced silencing complex (RISC) incorporates these siRNAs and directs the endonucleolytic cleavage of complementary viral RNAs, thereby limiting viral replication. P19, however, binds viral siRNAs (vsiRNAs) with high affinity, preventing their incorporation into RISC. By doing so, P19 inhibits the RNAi pathway, allowing the virus to evade degradation [39–41].

In our study, combined stress from wtTBSV infection and Fe-EDTA treatment led to a reduction in the level of the viral suppressor P19 compared to virus-infected control plants. The decrease in P19 protein levels indicates an effective RNAi response, resulting in a reduced wtTBSV viral titer in N. benthamiana (Figure 3A). Interestingly, at higher Fe-EDTA concentrations, P19 protein levels increased compared to 0.1 mM Fe-EDTA, highlighting the dose-dependent role of iron in regulating cellular metabolism. Similar studies in Cucumis sativus L. under combined stress with Glomus mosseae showed significant improvements in key physiological parameters, including chlorophyll content, photosynthetic rate, stomatal conductance, and accumulation of phenolic compounds. These results also demonstrated that combined stress increased the activity of antioxidant enzymes such as SOD, POD, CAT, and APX, mitigating oxidative stress and promoting plant health [42]. Previous studies have shown that foliar application of Fe-EDTA at a concentration of 3.36 mg·L^{-1} in N. benthamiana plants infected with potato virus Y (PVY) also reduced symptom severity and suppressed the accumulation of viral RNA and proteins, particularly during the early stages of infection [43]. These findings support our results showing a reduction in viral titer in plants undergoing ferroptosis-like cell death. As noted earlier, viral infection suppresses photosynthesis. Iron treatment may assist the plant by regulating genes associated with cytochromes, chlorophyll, and photosystems (PSI and PSII). Similar observations were reported by Bwalya, Alazem, and Kim (2021), where photosynthesis-related genes (PSaC and ATPsyn-α) conferred resistance to soybean mosaic virus (SMV) in N. benthamiana through RNAi [44]. Additionally, Xu et al. (2023) employed virus-induced gene silencing (VIGS) and found that suppressing the expression of the NbHSP90 gene in N. benthamiana significantly increased PVY accumulation [43].

Heat shock proteins (HSPs), particularly HSP70, play a key role in the formation of the viral replication complex. HSP70 interacts with the viral protein p33 and activates RNA-dependent RNA polymerase (RdRp), ensuring efficient viral genome replication. HSP90 also contributes by stabilizing and activating the viral RdRp. Together with the cofactor CDC37, HSP90 forms a complex with the viral RdRp p92, which is essential for initiating replication [45–48]. The role of HSP proteins in host cells is critical, as they participate in protein folding, prevent the aggregation of denatured proteins, and maintain cellular homeostasis under stress conditions [9]. Analysis of HSP70 protein expression showed a significant decrease under combined stress compared to control plants, except for wtTBSV + 1 mM Fe-EDTA (Figure 3B). These results suggest possible degradation of host proteins, in which HSPs normally play a protective role, as also indicated by HSP70 expression under single stress with 1 mM Fe-EDTA. Interestingly, treatment with 0.5 mM Fe-EDTA caused a marked reduction in HSP70 expression compared to

control plants. Analysis of HSP90 expression revealed that under combined stress (wtTBSV + 1 mM Fe-EDTA), its level significantly decreased (Figure 3C).

Based on all our experiments, we propose that the combined stress of wtTBSV infection and 1 mM Fe-EDTA causes more severe damage to plant cells. We suggest that high iron concentrations induce an enhanced form of programmed cell death, ferroptosis. Accumulation of Fe^{2+} and the activation of lipid peroxidation may trigger localized necrosis by disrupting cellular membranes, which in turn reduces viral titer in the plants. In contrast, lower Fe concentrations help maintain the photosynthetic apparatus in N. benthamiana.

Conclusion

Our study provided new insights into the role of ferroptosis-like cell death in plant-virus interactions with TBSV. We showed that Fe-EDTA treatment alters the accumulation of wtTBSV in *N. benthamiana*. High concentrations of Fe-EDTA induced oxidative stress and ferroptosis-like cell death, accompanied by membrane damage, necrosis, and growth inhibition, while simultaneously leading to a decrease in the level of the suppressor protein P19. At the same time, low concentrations of Fe-EDTA maintained photosynthetic activity and alleviated symptoms. In addition, a decrease in HSP70 and HSP90 was observed under combined stress, suggesting a possible interaction of iron with host chaperones required for viral replication.

Thus, viral infection accumulation is regulated by iron availability in the cell: limited iron supply supports photosynthesis and RNA interference activation, whereas its excess may trigger ferroptosis-like cell death pathways. These findings highlight the importance of iron homeostasis in studying plant defense mechanisms and open perspectives for the practical application of iron-containing treatments to control viral diseases. However, further studies are needed to clarify the precise mechanisms of interaction between viral infection and ferroptosis-like cell death in plants.

Author Contributions

N.I. and **Z.M. –** conceptualization; **N.I.** and **D.A. –** methodology; **N.I., D.A., T.E., S.B., Z.B.,** and **Z.T. –** validation; **N.I.** and **Z.M. –** investigation; **N.I., D.A.,** and **Z.B. –** data curation; **N.I., D.A., T.E., S.B.,** and **Z.B. –** writing-original draft preparation; **N.I.** and **D.A. –** writing-review and editing; **D.A., T.E., S.B.,** and **Z.T. –** visualization; **N.I. –** supervision; funding acquisition **– N.I.** All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain any description of studies performed by the authors involving human subjects or using animals as objects.

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Темір гомеостазының бұзылуы, ферроптоз тәрізді жасушалық өлімі және өсімдіктердегі РНҚ интерференциясы арқылы вирустық инфекцияны басуы

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Аңдатпа. Ферроптоз-тәрізді жасушалық өлім - бұл өсімдік жасушаларында темірдің жиналуы мен липидтердің асқын тотығуынан қоздырылатын жаңадан сипатталған механизм. Алайда оның вирустық инфекциядағы рөлі әлі толық анықталмаған. Осы зерттеуде *Nicotiana benthamiana* өсімдіктеріне темірмен (Fe-EDTA) өңдеудің, wild type tomato bushy stunt virus (wtTBSV) инфекциясы жағдайындағы ықпалы талданды. Кешенді стресс жағдайында жүргізілген морфологиялық және биохимиялық талдау өсімдіктер өсуінің тежелуі, некроз, жапырақтардың сарғаюы, хлороз және жапырақтардың жиырылуы сияқты айқын симптомдардың байқалғанын көрсетті, ал супрессорлық P19 ақуызының экспрессиясы төмендеді. Биохимиялық талдау жағынан Fe-EDTA төмен концентрациялары өсімдіктердің фотосинтетикалық аппараттың тұрақтылығын қамтамасыз етіп, хлорофилл құрамын арттыратыны анықтады, ал жоғары концентрациялар липидтердің асқын тотығуын және ферроптоз тәрізді жасуша өлімін тудырды. Алынған нәтижелер темірдің артық мөлшерінде РНҚ-интерференциясының белсенуі мен ферроптоз тәрізді жасушалық өлімнің іске қосылатынын, сондай-ақ осы жағдайда вирустық инфекцияның тежелетінін дәлелдейді. Ал темірдің төмен концентрацияларында фотосинтетикалық

белсенділік сақталып, симптомдардың айқындылығы төмендеді. Сонымен қатар, кешенді стресс жағдайында HSP70 және HSP90 жылулық шок ақуыздарының деңгейі төмендегені анықталды, бұл микроэлементтік гомеостаздың вирус репликациясына қажетті жасушалық шаперондардың жұмысына араласуын көрсетуі мүмкін. Бұл зерттеу ферроптозға ұқсас жасушалық өлімнің вирус пен өсімдік арасындағы өзара әрекеттесудегі негізгі рөлін көрсетеді.
Түйін сөздер: ферроптоз, өсімдік вирустары, құрама стресс, белсенді оттек түрлері (БОТ), TBSV

Нарушение гомеостаза железа подавляет вирусную инфекцию посредством клеточной смерти, подобной ферроптозу, и РНК-интерференции в растениях

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Аннотация. Ферроптоз-подобная клеточная гибель у растений [1] это недавно описанный механизм, запускаемый накоплением железа и перекисным окислением липидов, однако его роль в вирусной инфекции остаётся неясной. В данном исследовании было проанализировано влияние обработки железом (Fe-EDTA) на растения *Nicotiana benthamian*а, инфицированные wild type tomato bushy stunt virus (wtTBSV). Морфологические и биохимические анализы растений под воздействием комбинированного стресса показали более выраженные симптомы, такие, как угнетение роста растений, некроз, пожелтение листьев, хлороз и сморщивание листьев, тогда как экспрессия супрессорного белка P19 снижалась. Биохимический анализ выявил, что низкие концентрации Fe-EDTA поддерживали фотосинтетический аппарат растений и повышали содержание хлорофиллов, тогда как высокие концентрации индуцировали перекисное окисление липидов и ферроптоз-подобную гибель клеток. Результаты указывают на активацию РНК-интерференции и ферроптоз-подобной клеточной гибели в условиях избытка железа, при котором вирусная инфекция подавлялась. В то время как при низких концентрациях железа сохранялась фотосинтетическая активность и снижалась выраженность симптомов. Кроме того, было выявлено снижение уровня белков теплового шока HSP70 и HSP90 при комбинированном стрессе, что может отражать вмешательство микроэлементного гомеостаза в работу клеточных шаперонов, необходимых для репликации вируса. Это указывает на ключевую роль ферроптоз-подобной клеточной гибели во взаимодействии вирус-растение.
Ключевые слова: ферроптоз, вирус растений, комбинированный стресс, активные формы кислорода (АФК), TBSV

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