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Research Article

Reactive Oxygen Species Metabolism and Diacetoxyscirpenol Biosynthesis Modulation in Potato Tuber Inoculated with Ozone-Treated *Fusarium sulphureum*

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Potato dry rot, caused by *Fusarium* species, is a devastating fungal decay that seriously impacts the yield and quality of potato tubers worldwide. *Fusarium sulphureum* is a major causal agent causing potato tuber dry rot that leads to trichothecene accumulation in Gansu Province of China. Ozone (O_3), a strong oxidant, is widely applied to prevent postharvest disease in fruits and vegetables. In this study, *F. sulphureum* was first treated with 2 mg L^{-1} ozone for 0, 30 s, 1 min, and 2 min, then inoculated with the potato tubers. The impact of ozone application on dry rot development and diacetoxyscirpenol (DIA) accumulation and the possible mechanisms involved were analyzed. The results showed that ozone treatment significantly inhibited the development of potato tuber dry rot by activating reactive oxygen species (ROS) metabolism and increased the activities of antioxidant enzymes NADPH oxidase (NOX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) by 24.2%, 13.1%, 45.4%, and 15.8%, respectively, compared with their corresponding control. The activities of key enzymes involved in ascorbate-glutathione cycle (AsA-GSH) of ascorbic peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR) also increased by 26.6%, 41.5%, 56%, and 24.1%, respectively, compared with the control group, and their corresponding gene expressions. In addition, ozone treatment markedly suppressed DIA accumulation in potato tubers by downregulating the expression of genes associated with DIA biosynthesis pathway. These results suggest that ozone treatment inhibited the occurrence of potato dry rot and the accumulation of DIA in potato tubers inoculated with *F. sulphureum* by promoting ROS metabolism and modulating DIA biosynthesis pathway.

1. Introduction

Potato (*Solanum tuberosum*) is listed as one of the world's major food crops, and China ranks number one, accounting for more than a quarter of world total potato production [1]. Despite their high yield, potato tubers often suffer a variety of disease due to pathogenic microorganism infection during postharvest storage. Potato dry rot caused by *Fusarium* species is a devastating fungal decay that impacts the yield and quality of potato tubers worldwide [2, 3]. Among them, *Fusarium sulphureum* is the main pathogen causing potato tuber dry rot in Gansu Province of China [4, 5], which not only results in economic losses but also accumulates trichothecenes that could be detrimental to human health [6]. Dry rot of potato is currently managed mainly through the application of chemical synthesis fungicides such as thiabendazole, carbendazim, and flusilazole [4, 7–9]. However, these drugs have a variety of problems such as chemical residues, environmental pollution, and pathogen resistance. Therefore, effective, green, and eco-friendly strategies are warranted.

Ozone (O_3) has a high oxidizing activity and has been approved by the Food and Drug Administration (FDA) for its excellent antimicrobial properties [10]. It is widely used to control postharvest diseases in fruits and vegetables. Matłok et al. [11] found that O_3 treatment effectively controlled the growth of microorganisms on saskatoon berry surface to reduce disease during storage. What is more, García-Martín et al. [12] reported that O_3 treatment effectively reduced the occurrence of postharvest diseases of citrus varieties caused by *Penicillium digitatum* and *P. italicum*. Ozone management in postharvest disease is attributed to two aspects; on the one hand, ozone can act directly on the fungi by inhibiting their growth and reducing plant decay; on the other hand, ozone application can stimulate reactive oxygen species (ROS) metabolism to generate ROS, which can act as a signal molecule to induce resistance against disease by responding to biotic and abiotic stresses. It is certain that ROS concentration plays a crucial role in postharvest disease. Also, high concentrations of ROS can destroy the cell membrane lipid structure of the host and result in oxidative damage by causing membrane lipid peroxidation, which leads to cell membrane integrity disruption and pathogen invasion [13]. Low concentrations of ROS act as signal molecules to regulate host defense responses [14]. Interestingly, plants have evolved a set of antioxidation system to mitigate excessive ROS accumulation and consequence oxidative damage. The antioxidant system mainly includes antioxidant enzymes and ascorbate glutathione (AsA-GSH) cycle. Previous report revealed that ozone application can control postharvest disease of kiwifruit by increasing the activity of host resistance-related enzymes, such as peroxidase (POD), and enhancing the activities of defense-related enzymes Luo et al. [15]. Nevertheless, how ozone treatment activates ROS metabolism in inoculated potato tubers to control dry rot development is not well understood.

In addition, it was reported that ozone treatment reduced mycotoxin accumulation; for instance, ozone application reduced neosolaniol (NEO) content in *F. sulphureum* infected muskmelon fruit [16]. The possible mechanism involved could be attributed to two aspects; first, ozone, as a strong oxidant, can directly destroy the chemical structure of mycotoxins. Xue et al. [16] found that ozone could react with double bond in the chemical structure of NEO, which led to the reduction of NEO accumulation. Meanwhile, ozone can influence mycotoxin biosynthesis by disturbing the expression of genes involved in mycotoxin biosynthesis pathway. For diacetoxyscirpenol (DIA) (a kind of trichothecenes) biosynthesis, the biosynthesis pathway of trichothecenes has been established, and almost all the *Tri* genes involved have been confirmed in *F. sporotrichioides* and *F. graminearum* [17]. The *Tri* gene located in one genomic locus is referred to as the "*FgTri5* cluster" [18]. In the clustered genes, *Tri5*, *Tri4*, *Tri6*, *Tri10*, and *Tri101* encode key factors in trichothecene synthesis [19, 20]. Nevertheless, information on how ozone influences DIA biosynthesis by regulating gene expression is limited.

In this study, we treated the pathogen of *F. sulphureum* with gaseous ozone ($2\,mg\,L^{-1}$) and then inoculated with potato tuber; we found that potato dry rot caused by ozone-treated *F. sulphureum* was markedly suppressed. Therefore, the aim of this study was to evaluate the effects of ozone treatment on the inhibition of potato dry rot and the accumulation of diacetoxyscirpenol (DIA). The detailed mechanism of action was analyzed.

2. Materials and Methods

2.1. Fungal Pathogen and O_3 Treatment Method. The *Fusarium sulphureum* pathogen was provided by the Institute of Plant Protection, Gansu Academy of Agricultural Sciences, Lanzhou, China. The pathogen was grown on potato dextrose agar (PDA) for 7 days for further use. The spore suspension was prepared and adjusted to a concentration of $1 \times 10^6\,CFU\,mL^{-1}$ according to the method of Li et al. [21].

Ozone treatment was based on the method of Li et al. [21]. Ozone generator (OSAN, Aoshan Huanbao Technology Industry Co. Ltd., Dalian, China) was used to produce gaseous ozone.

2.2. Potato Tuber and Treatment. Potato tuber (*Solanum tuberosum*) of the original seed "Longshu No. 3" was harvested commercially from Dingxi, Gansu Province, China, in October 2020. The tubers were pretreated as described by Xing-dong and Hua-li [22], then kept at -80°C for further experiments.

2.3. Determination of Lesion Area and Disease Incidence. The sterilized potato tubers were inoculated with ozone-treated *F. sulphureum* spore suspension ($20\,\mu L$, 1×10^6 spores mL^{-1}), then incubated in darkness (24°C, 90% RH). Afterwards, the lesion diameter was measured by the crossover method after 0, 1, 2, 3, 5, and 7 days calculated based on πr^2. At the same time, the disease incidence rate was statistically recorded and calculated according to the following formula (1). Each treatment in the experiment was performed in three replicates of 30 potato tubers.

$$\text{Disease incidence}(\%) = \frac{\text{number of diseased tubers}}{\text{number of total tubers}} \times 100\%. \quad (1)$$

2.4. Analysis of DIA Accumulation in Inoculated Potato Tuber. The determination of DIA content was based on the method of Xue et al. [23] with slight modifications. Firstly, *F. sulphureum* was treated with ozone ($2\,mg\,L^{-1}$) for 0, 1 min, and 2 min, respectively, according to the method of Section 2.1, and the ozone-treated pathogens were centrally inoculated into potato tubers. After 0, 2, 3, 5, and 7 days of incubation, the lesion part of the inoculated potato tubers was cut with a sterile knife. Subsequently, DIA was extracted with acetonitrile/water (84 : 16, *v*/*v*) from the lesion part, then purified with a PriboFast MFC270 column, and finally analyzed by using UPLC-MS/MS (Waters Acquity Ultra Performance LC System, Waters, Milford, MA) based on our previous publication [23]. Each treatment in the experiment was performed in three replicates of 30 potato tubers.

2.5. Malondialdehyde (MDA) Content and Cell Membrane Integrity Assay. Malondialdehyde (MDA) content and cell membrane permeability are important indexes that reflect cell membrane integrity. The determination was based on the

method of Yang et al. [24], and the MDA content in the fresh potato tuber was expressed as μmol g^{-1} FW (fresh weight).

2.6. The Generation Rate of $O_2\cdot^-$ and H_2O_2 Content Assays. The production rate of $O_2\cdot^-$ and H_2O_2 contents was determined by referring to the method of Bao et al. [25]. The $O_2\cdot^-$ production rate was expressed as μmol min^{-1} g^{-1} FW, and H_2O_2 content was expressed as mmol g^{-1} FW.

2.7. Enzymatic Activity Assay

2.7.1. Determination of the Activities of Enzymes Involved in ROS Generation. The activities of NADPH oxidase (NOX) and superoxide dismutase (SOD) were assayed based on the kits (Nanjing Jiancheng Bioengineering Institute, A116-1-1 and A001-4-1). The NOX and SOD activities were indicated as U mg^{-1} protein.

2.7.2. Assay of the Activities of Enzymes Involved in ROS Scavenging. The activities of POD and catalase (CAT) were assayed based on the kits (Nanjing Jiancheng Bioengineering Institute, A084-3-1 and A007-1-1). The POD and CAT activities were indicated as U mg^{-1} protein.

2.7.3. Assay of the Activities of the Key Enzymes Involved in AsA-GSH Cycle. The activities of ascorbic peroxidase (APX) and glutathione reductase (GR) were determined using the kits (Nanjing Jiancheng Bioengineering Institute, A123-1-1 and A062-1-1). The APX and GR activities were indicated as U mg^{-1} protein.

The activity of dehydroascorbate reductase (DHAR) was determined using the kit (Suzhou Keming Co., Ltd., Suzhou, China); DHAR activity was indicated as U mg^{-1} protein. Based on the reaction of catalytic reduction of NADH to ASA and NAD^+, the activity of monodehydroascorbate reductase (MDHAR) was determined according to the kit (Suzhou Keming Co., Ltd., Suzhou, China). The MDHAR activity was indicated as U mg^{-1} protein.

2.8. Gene Expression Analysis. Real-time quantitative PCR (RT-qPCR) was employed to evaluate the expression level of the related genes involved in ROS metabolism and DIA biosynthesis pathway. Total RNA was extracted by RNAiso Plus kit (B511321, Sangon Biotech) according to the manufacturer's instructions. Then, the first strand of complementary DNA was synthesized by gDNA Eraser Reverse Transcription Kit (TaKaRa, RR047A). Real-time PCR uses SYBR Premix Ex Taq™ II kit (TaKaRa Biotechnology). RT-qPCR primers were designed with Primer Premier 5.0 (Tables 1 and 2). The relative expression abundance of gene was calculated by the $2^{-\Delta\Delta Ct}$ method [26]. The experiments were repeated three times.

2.9. Statistical Analysis. Each treatment in the experiment was repeated three times. Excel 2019 was adopted to calculate the average value and standard deviation of all the data, and SPSS 21.0 was used to analyze the difference significance ($p < 0.05$). The figures were made with Origin 8.5 (Northampton, MA, USA).

Table 1: Primers for ROS metabolic pathway genes for RT-qPCR analysis.

Gene	Sequence forwards (5′-3′)
GADPH	F: AACGGCAACACCAACTCCTC
	R: TAAAACACACGACCGACTGGAA
SOD	F: CCAGTCAACCCTCACAACAA
	R: TGACAGAGCCCTTAGCATTTC
NOX	F: ATTGTTACGACGAAGGATCAGG
	R: GGCAGCCTTTCCGTTAAGATA
CAT	F: CCATCACCGATAAGGACTTTGA
	R: CGCTCAGCGTTGGAAGAATA
POD	F: CTCAAGACAGAACAGCAGGAA
	R: CAATCGTAGCGAGACTCTTATGG
APX	F: CAGTTCCCGCTGGCTTAATA
	R: CTGGTGAGGACTGTTGTGTT
GR	F: CAGAAGGGACCAACAAACTACA
	R: GCATCTCACCACTACCCAATC

Table 2: Primers for DIA synthesis pathway gene RT-qPCR analysis.

Gene	Sequence forwards (5′-3′)
B-Tubulin	F: GGTAACCAAATCGGTGCTGCTTTC
	R: GATTGACCGAAAACGAAGTTG
Tri 4	F: TAAACGCCCGCGAAGTTCACA
	R: TGGTGATGGTTCGCTTCGAG
Tri 5	F: TGCAAGTTCTTTGAGCAGGC
	R: CTCCACTAGCTCAATTGAACTTAG
Tri 6	F: FAGCGCCTTGCCCCTCTTTG
	R: RAGCCTTTGGTGCCGACTTCTTG
Tri 10	F: FTCTGAACAGGCGATGGTATGGA
	R: RCTGCGGCGAGTGAGTTTGACA
Tri 101	F: TTGGGTTTGGACTGGGTAAG
	R: TTGCGTACTTTGTCCACTCCT

3. Results

3.1. Ozone Treatment Inhibited Potato Tuber Dry Rot Development and DIA Accumulation. Ozone treatment significantly reduced the incidence of dry rot in potato tubers inoculated with *F. sulphureum* in a time-dependent manner. The inhibition effect became more obvious with the extension of treatment time, and 2 min treatment showed the best inhibition effect. For example, when compared with the control, the lesion area of tubers treated with ozone for 30 s, 1 min, and 2 min decreased by 33.5%, 58.9%, and 89.3%, respectively, on the 7 d after inoculation (Figure 1(a)). The incidence of dry rot potato decreased by 26.9%, 62.5%, and 87.8% after 30 s, 1 min, and 2 min of ozone treatment, respectively, on the 7 d after inoculation (Figure 1(b)). Similarly, ozone treatment reduced DIA accumulation in potato tuber inoculated with *F. sulphureum*, and the longer the treatment time, the more obvious the inhibitory effect. For example, on the 7th day after inoculation, compared with the control, the

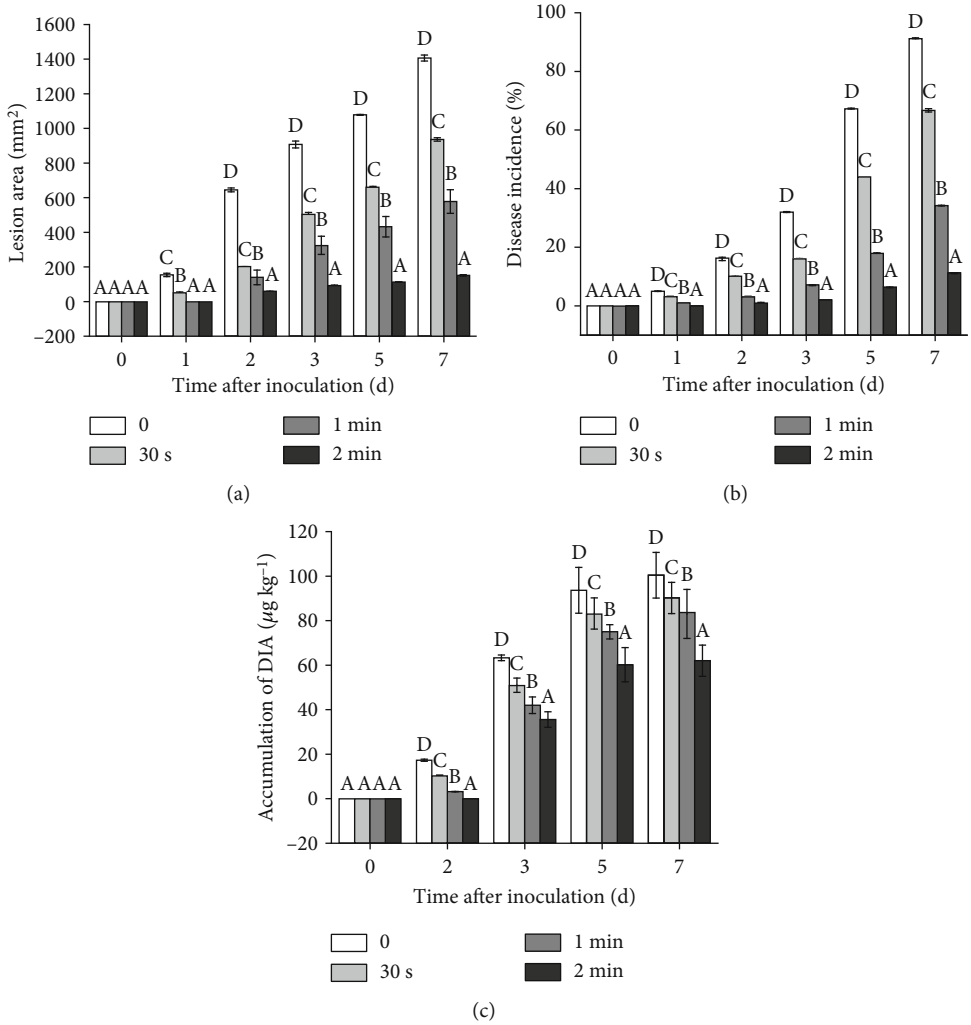

Figure 1: The effect of ozone treatment on the potato lesion area (a), disease incidence (b), and accumulation of DIA (c). Bars indicate standard errors (±SE). Different letters represent as significant difference of same day ($p < 0.05$).

content of DIA decreased by 17.4% and 38.2% after ozone treatment for 1 and 2 min, respectively (Figure 1(c)).

3.2. Ozone Treatment Decreased Cell Membrane Permeability and MDA Content in Inoculated Potato Tubers. Cell membrane permeability and MDA content are two important indicators to evaluate cell membrane integrity. It can be seen from Figure 2 that the cell membrane permeability and MDA content of potato tubers inoculated with ozone-treated *F. sulphureum* were significantly lower than those of the control group. For example, after 7 d of inoculation, compared with the control, the cell membrane permeability of tubers treated with ozone for 30 s, 1 min, and 2 min decreased by 4.5%, 8.4%, and 11.5%, respectively (Figure 2(a)). Similarly, the content of MDA in potato tubers inoculated with ozone-treated *F. sulphureum* decreased by 7.3%, 14.7%, and 10.7% after ozone treatment for 30 s, 1 min, and 2 min, respectively (Figure 2(b)).

3.3. Ozone Treatment Increased $O_2^{\cdot -}$ Production Rate and H_2O_2 Content in Inoculated Potato Tubers. The $O_2^{\cdot -}$ production rate and H_2O_2 content of potato tubers inoculated with ozone-treated *F. sulphureum* were significantly higher ($p < 0.05$) than those in the control group. For instance, after 7 days of inoculation, when compared with control, $O_2^{\cdot -}$ production rate in potato tubers inoculated with ozone-treated *F. sulphureum* increased by 22.4%, 53.0%, and 56.3% after treatment for 30 s, 1 min, and 2 min, respectively (Figure 3(a)). The content of H_2O_2 in inoculated potato tubers increased significantly ($p < 0.05$) after ozone treatment. After ozone treatment for 30 s, 1 min, and 2 min, the content of H_2O_2 in potato tubers increased by 7.7%, 45.2%, and 45.4%, respectively, when compared with the control (Figure 3(b)).

3.4. Effects of Ozone Treatment on ROS Production-Related Enzyme Activities and Gene Expressions. ROS is a stress response of host plants under unfavorable environmental stress. The excessive accumulation of ROS will lead to oxidative stress in organisms, resulting in oxidative damage and inactivation or even death of biological cells. NOX and SOD are two important enzymes that produce $O_2^{\cdot -}$ and H_2O_2, and NOX is the main producer of $O_2^{\cdot -}$, which acts as an electron donor to convert extramembrane O_2 to

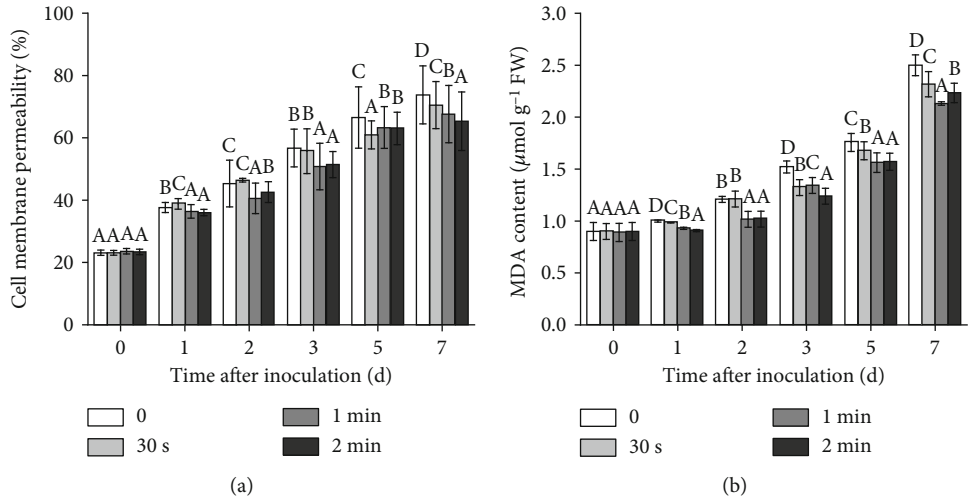

Figure 2: Effects of ozone treatment on cell membrane permeability (a) and MDA content (b) of potato tubers. Bars indicate standard errors (±SE). Different letters represent as significant difference of same day ($p < 0.05$).

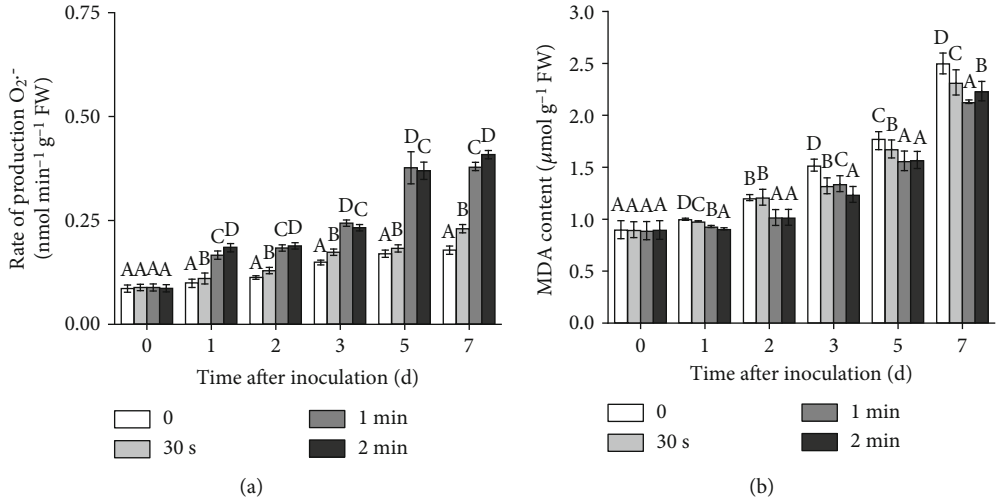

Figure 3: Effects of ozone treatment on $O_2^{\cdot -}$ production rate (a) and H_2O_2 content (b) in potato tubers. Bars indicate standard errors (±SE). Different letters represent as significant difference of same day ($p < 0.05$).

$O_2^{\cdot -}$. It can be seen from Figure 4 that the NOX activity and gene expression increased first and then decreased with the extension of incubation time. After ozone treatment for 2 min, NOX activity and gene expression peaked at 3 d, increasing by 24.2% and 12.5%, respectively, compared with the control (Figures 4(a) and 4(b)). To avoid oxidative stress caused by excessive ROS and keep redox homeostasis, plants have evolved an adaptive mechanism to regulate the metabolic balance of ROS in organisms. SOD catalyzes $O_2^{\cdot -}$ disproportionation to produce H_2O_2 and O_2, which is the first line of defense for oxidative stress. After 5 d of inoculation, the SOD activity and its gene expression were increased by 13.1% and 11.5%, respectively, after 2 min of ozone treatment (Figures 4(c) and 4(d)). These results suggested that ozone treatment increased ROS production-related enzyme activities and gene expressions.

3.5. Effects of Ozone Treatment on ROS Scavenging-Related Enzyme Activities and Gene Expressions. POD and CAT are two key enzymes in scavenging H_2O_2; POD and CAT can convert H_2O_2 into H_2O and O_2. The results indicate that the activities of POD, CAT, and their corresponding gene expressions in tubers inoculated with ozone-treated *F. sulphureum* significantly increased compared with their corresponding control. POD enzyme activity and its gene expression increased by 15.8% and 14.1% on the 7th day, respectively, compared with the control group (Figures 5(a) and 5(b)). The CAT enzyme activity and gene expression were enhanced by 45.4% and 12.3% on the 7th day after ozone treatment for 2 min, compared with the control group (Figures 5(c) and 5(d)). These results suggest that ozone treatment increased ROS scavenging-related enzyme activities and gene expressions, thereby activating the ROS metabolism and avoiding the oxidative damage to cell membrane due to excessive ROS accumulation in tubers.

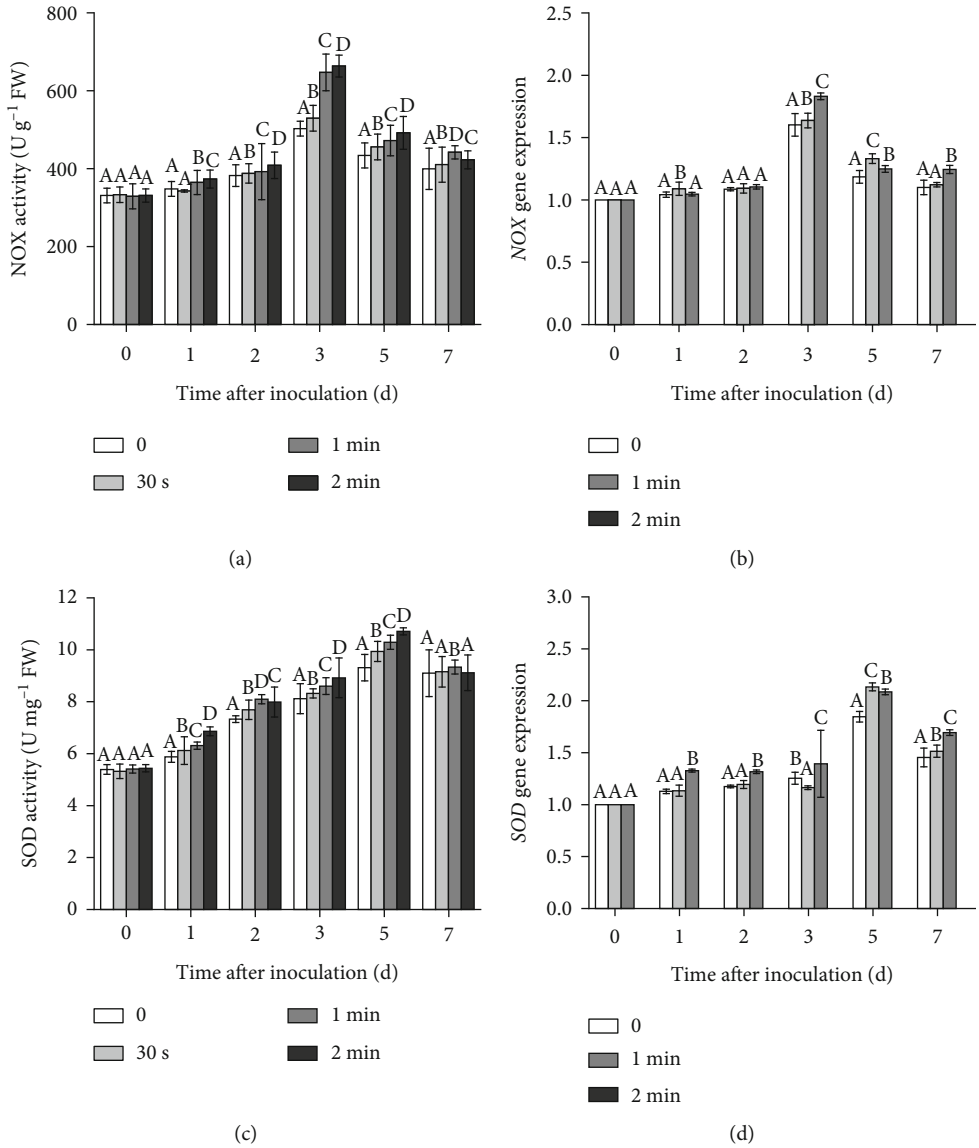

Figure 4: The effect of ozone treatment on the activities of NOX (a) and SOD (c) and gene expressions of *NOX* (b) and *SOD* (d) in potato tubers. Bars indicate standard errors (±SE). Different letters represent as significant difference of same day ($p < 0.05$).

3.6. Ozone Treatment Improved Enzyme Activities and Gene Expressions Involved in AsA-GSH Cycle. APX and GR are key enzymes involved in AsA-GSH cycle and play crucial roles in maintaining ascorbic acid (AsA) and glutathione (GSH) levels; APX is the first enzyme that converts H_2O_2 to H_2O catalyzed AsA. DHAR catalyzed dehydroascorbic acid (DHA) to AsA under the electron-donating condition of GSH and oxidized GSH to oxidized glutathione (GSSG). Under the action of GR, GSSG is then reduced to GSH. APX, MDHAR, DHAR, and GR are involved in AsA-GSH cycle. As a regulator of redox balance in plants, they play crucial roles in maintaining AsA and GSH levels. In this study, the APX activity gradually increased with prolonged ozone treatment time, and the activities of MDHAR, DHAR, and GR increased first and then decreased with the extension of ozone treatment time. After ozone treatment for 2 min, APX activity increased by 26.6% on 7 d, compared with the control group (Figure 6(a)). Gene expression of *APX* increased by 28.0% on the 5th day, compared with the control (Figure 6(e)). After ozone treatment for 2 min, MDHAR activity increased by 41.5% on the 5 d, and the activity of DHAR increased by 56% on 3 d (Figures 6(b) and 6(c)). GR activity peaked on 5 d after 2 min of ozone treatment; the enzyme activity and gene expression of GR increased by 24.1% and 43.5% compared with the control groups (Figures 6(d) and 6(f)).

3.7. Effect of Ozone Treatment on Gene Expressions Involved in DIA Biosynthesis Pathway in Inoculated Potato Tuber. The biosynthesis of DIA begins with farnesyl pyrophosphate (FPP) which is regulated by key genes *Tri4* and *Tri5* and major regulatory genes *Tri6*, *Tri10*, and *Tri101*. Under a

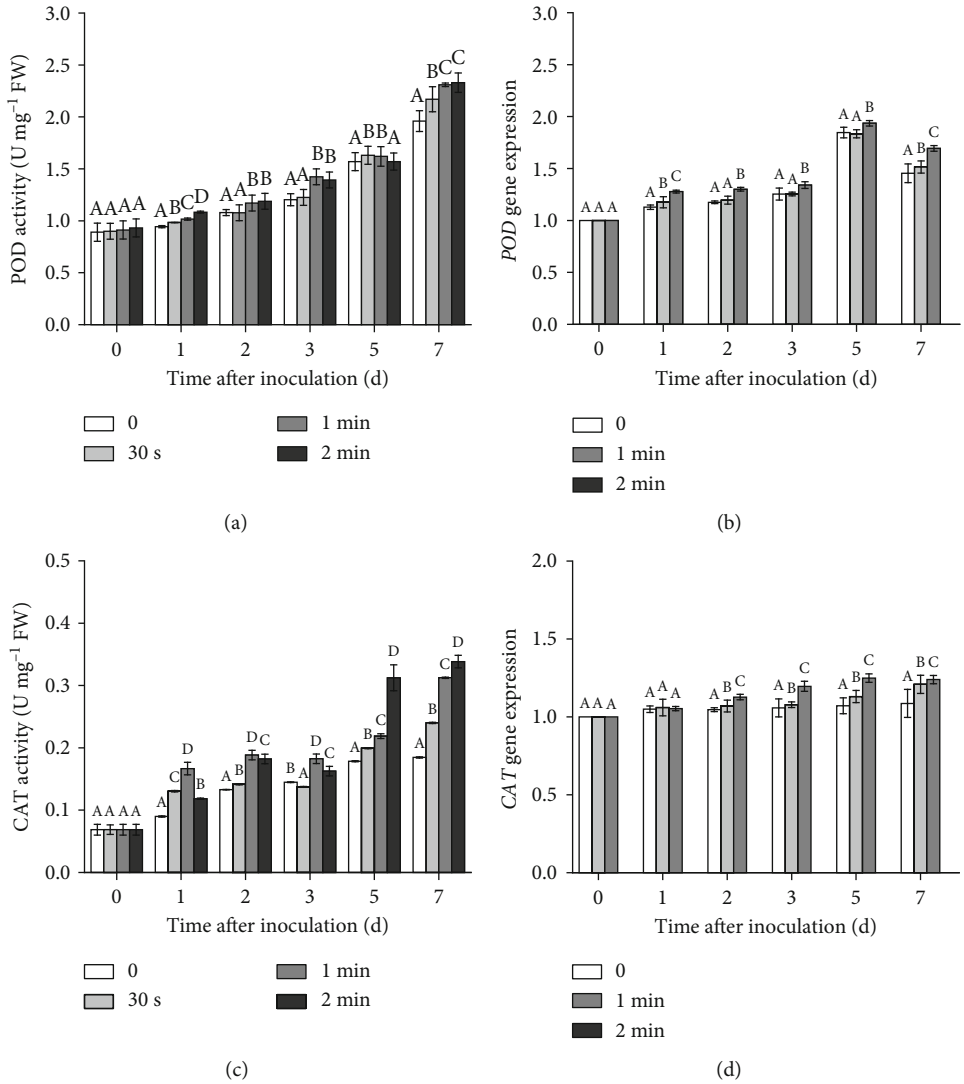

Figure 5: The effect of ozone treatment on the activities of POD (a) and CAT (c) and gene expressions of *POD* (b) and *CAT* (d) in potato tubers. Bars indicate standard errors (±SE). Different letters represent as significant difference of same day ($p < 0.05$).

series of enzymatic reactions, FPP is synthesized through a series of chemical reactions such as oxidation and esterification. Ozone treatment downregulated the expression of genes related to DIA biosynthesis pathway. On 7 d after inoculation, *Tri4*, *Tri5*, *Tri6*, and *Tri10* genes were downregulated by 70.7%, 74.18%, 7.6%, and 51.75%, respectively (Figures 7(a)–7(d)). *Tri101* gene downregulated 50.0% on 3 d after inoculation (Figure 7(e)).

4. Discussion

Ozone, a strong oxidant, can effectively manage the postharvest decay of fruits and vegetables [27]. In this study, we found that ozone treatment inhibited the development of dry rot and accumulation of DIA in potato tubers inoculated with *F. sulphureum* (Figure 1). This result is consistent with Ong's report [28], who suggested that ozone fumigation significantly inhibited anthracnose disease incidence of papaya caused by *Colletotrichum gloeosporioides*. Xue et al. [16] also proved that ozone treatment not only controlled *Fusarium* rot of muskmelon but also reduced NEO accumulation. The possible action mechanism of ozone application can be attributed to the activation of ROS metabolism and the modulation of DIA biosynthesis pathway.

4.1. Ozone Treatment Maintained Cell Membrane Integrity by Activating ROS Metabolism. Cell membrane is a biomembrane with phospholipid bilayer structure, which is the protective barrier of organism cells and plays a significant role in maintaining the balance of intracellular and extracellular environment, protecting the function of cell membrane such as energy supply, material exchange, and information transmission, to orderly coordinate cell life activities [29]. In this study, cell membrane permeability of potato tubers inoculated with ozone-treated *F. sulphureum* was lower than that of untreated group (Figure 2(a)). MDA content is a momentous indicator to evaluate the degree of membrane lipid peroxidation. MDA content was significantly lower than that of the control group after ozone treatment (Figure 2(b)). The above results pointed out that ozone treatment significantly

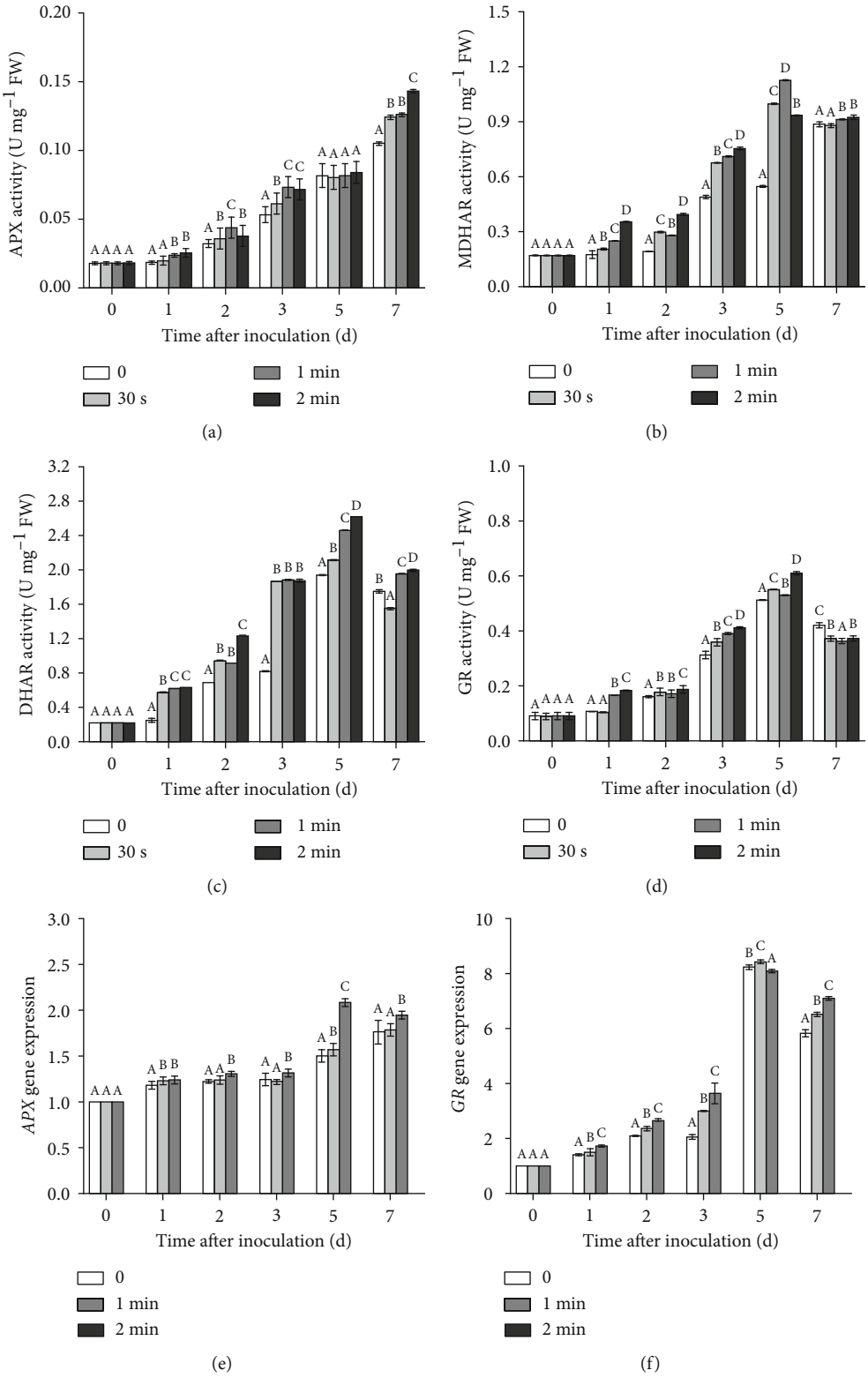

Figure 6: The effect of ozone treatment on the activities of APX (a), MDHAR (b), DHAR (c), and GR (d) and gene expressions of *APX* (e) and *GR* (f) in potato tubers. Bars indicate standard errors (±SE). Different letters represent as significant difference of same day ($p < 0.05$).

inhibited the oxidative damage of cell membrane, reduced membrane lipid peroxidation, and maintained the cell membrane integrity of the inoculated potato tubers. This result was in accordance with the report by Liu et al. [30].

ROS has a variety of forms and mainly includes the single-electron state of $O_2^{\cdot -}$ and the double electron reduction state of H_2O_2. High concentrations of ROS can attack the host plasma membrane and react with the unsaturated

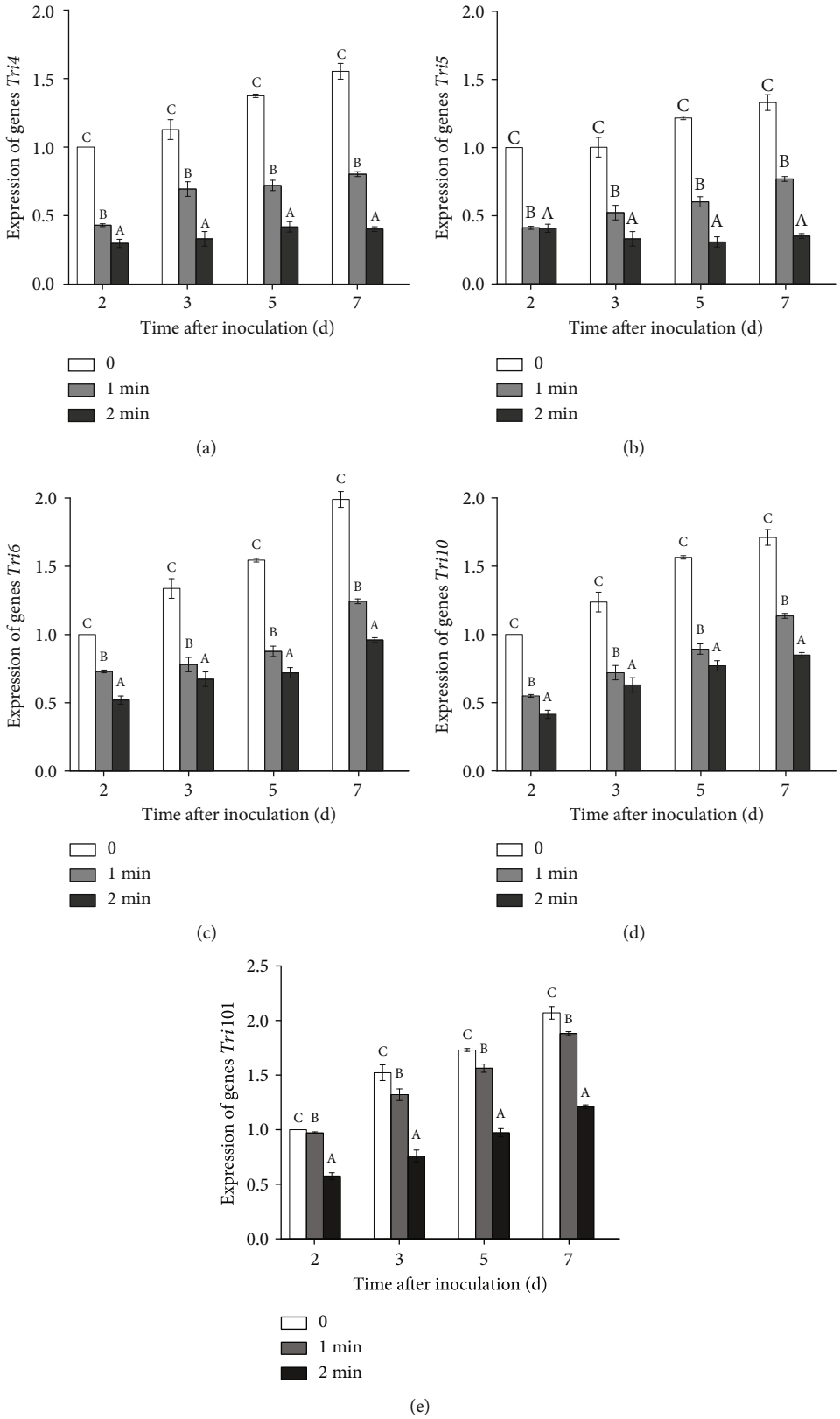

Figure 7: Effects of ozone treatment on expression of genes *Tri4* (a), *Tri5* (b), *Tri6* (c), *Tri10* (d), and *Tri101* (e) related to DIA synthesis pathway in potato tubers. Bars indicate standard errors (±SE). Different letters represent as significant difference of same day ($p < 0.05$).

fatty acids of phospholipid bilayer structure to cause oxidative damage through lipid peroxidation, resulting in cell membrane damage and pathogen invasion [13]. Low concentrations of ROS act as signaling molecules regulating the host defense responses. ROS concentration is a crucial factor to maintain physiological activities in organisms and plays a significant role in cell signal transduction and homeostasis [14]. In this study, we found that $O_2\cdot^-$ production rate in potato tubers inoculated with ozone-treated *F. sulphureum* was higher than that of the control group, and a similar trend was found in H_2O_2 content (Figure 3), indicating that ozone treatment activated ROS metabolism through increasing $O_2\cdot^-$ production rate and H_2O_2 accumulation. These findings are consistent with the report of Zhao et al. [31], who suggested that ozone-treated soybean roots led to higher $O_2\cdot^-$ production rate and H_2O_2 content than the control group, which was ascribed to ozone-induced oxidative stress. $O_2\cdot^-$ mainly comes from NOX that can reduce oxygen molecules into superoxide anion through NADPH-dependent single electron reduction, thereby regulating the production of superoxide anion, which is the main source of ROS in the cell and the only enzyme that directly produces ROS in the cell [18]. This study showed that the activity and gene expression level of NOX in tubers inoculated with ozone-treated *F. sulphureum* significantly increased compared with the control group (Figures 4(a) and 4(b)), which was in accordance with Kangasjarvi et al. [32], who suggested that the ozone treatment activated NOX activity. SOD is a metal-containing enzyme that catalyzes the disproportionation reaction of $O_2\cdot^-$ to produce H_2O_2 and O_2, which is the first line of defense for organisms to respond to oxidative stress. In this experiment, we observed that the activity and gene expression level of SOD in tuber inoculated with ozone-treated *F. sulphureum* significantly enhanced compared with the control group (Figures 4(c) and 4(d)). A similar study by Piechowiak and Balawejder [33] pointed out that ozone treatment increases SOD activity in fruit.

To avoid the excessive accumulation of ROS and the aggravation of membrane lipid peroxidation, the plant stimulates its own antioxidant enzyme system to remove ROS and maintain the homeostasis of oxidation. CAT and POD can further decompose H_2O_2 into H_2O and O_2, so that cells are protected from ROS damage. CAT exists in the peroxisome of cells and is a marker enzyme of peroxisome and can catalyze the decomposition of hydrogen peroxide into H_2O and O_2 [34]. POD can catalyze many reactions, with the elimination of hydrogen peroxide and phenols, amines, and aldehydes toxicity of the dual roles. In this experiment, we observed that the activities and gene expressions of POD and CAT were markedly higher than those in the control groups (Figures 5(a)–5(d)). The result was in accordance with the research report by Zhang et al. [34], who proved that low concentrations of ozone treatment stimulated the activities of CAT and POD, maintained the cell membrane integrity, and reduced disease in strawberries during storage. Moreover, Ong and Ali [28] also indicated that ozone treatment enhanced the activity of ROS metabolic enzymes in fruit and improved the stress response of the host, thereby inhibiting the decay of fruits. Therefore, ozone treatment promoted the accumulation of ROS, activated ROS metabolism, promoted enzyme activity and gene expression, and inhibited oxidative stress caused by the accumulation of ROS.

4.2. Ozone Treatment Activated AsA-GSH Cycle in Inoculated Potato Tubers. APX, MDHAR, DHAR, and GR are important enzymes in AsA-GSH cycle, which can be used as regulators of redox balance in plants and play important roles in maintaining AsA and GSH levels. APX, MDHAR, DHAR, and GR are also ROS scavenging enzymes that play an indispensable role in protecting cells from H_2O_2 damage. APX and GR are key enzymes. APX exists in chloroplast matrix and is the main enzyme of ROS scavenging enzymes and reduces H_2O_2 to H_2O through ASA-GSH cycle reaction under the action of ascorbic acid salt, thereby alleviating the toxicity of ROS to plants [35]. At the same time, APX is the first enzyme in AsA-GSH cycle that specifically catalyzes the reaction of AsA with H_2O_2 to produce MDHA. DHAR uses the electrons produced by GSH to reduce dehydroascorbic acid (DHA) and supply it to AsA. GSH can be oxidized by DHAR to GSSG. Under the action of GR, GSSG was subsequently reduced to GSH [35]. The results of this study showed that the activities of APX, MDHAR, DHAR, and GR in potato tuber inoculated ozone-treated *F. sulphureum* were significantly higher than those in the control group (Figure 6), indicating that APX and GR were involved in H_2O_2 removal in early tuber treatment.

4.3. Ozone Treatment Modulation DIA Biosynthesis in Inoculated Potato Tubers. The occurrence of potato dry rot is accompanied by DIA accumulation. DIA is a kind of trichothecene, and the mevalonate pathway supplies a substrate for FPP during the DIA synthesis pathway. For the synthetic pathway, *Tri5* gene encodes trichothecene synthase which catalyzes the cyclization of FPP to form trichothecene, which is the first precursor for the synthesis of trichothecene compounds [36]. After that, under the catalysis of multifunctional P450 monooxygenase encoded by *Tri4* gene, isotriol is synthesized by four-step oxygenation reaction. The *Tri101* gene encodes isotriol to form isotrichodermin (ITD) [37, 38]. The results of this study indicate that ozone treatment downregulated the expressions of *Tri4*, *Tri5*, *Tri10*, *Tri6*, and *Tri101* genes, which led to lower DIA biosynthesis. In addition, some studies showed that H_2O_2 can oxidize the HMGR protein located in membrane. The HMGR protein is involved in the mevalonate pathway, which led to downregulating the mevalonate pathway, thus reducing FPP accumulation [39–41]. FPP is the first substrate for DIA biosynthesis pathway, and lower FPP accumulation will lead to a reduction in the supply of DIA synthesis substrate, thus reducing DIA biosynthesis [42, 43].

5. Conclusion

The current results demonstrated that $2\,mg\,L^{-1}$ ozone treatment significantly increased the activities of NOX, SOD,

POD, and CAT, activated AsA-GSH cycle, and upregulated the gene relative expression abundance of *NOX*, *SOD*, *POD*, *CAT*, *APX*, and *GR* in potato tubers inoculated with *F. sulphureum*, which maintained the cell membrane integrity of the inoculated tuber, thus inhibiting the occurrence of potato dry rot. In addition, ozone treatment significantly inhibited the DIA accumulation by downregulating the relative expressions of *Tri4*, *Tri5*, *Tri10*, *Tri6*, and *Tri101* genes involved in DIA biosynthesis pathway. Further studies are needed to reveal the correlation mechanism between ozone treatment and DIA accumulation in *F. sulphureum*.

Abbreviations

DIA: Diacetoxyscirpenol
ROS: Reactive oxygen species
POD: Peroxidase
DHAR: Dehydroascorbate reductase
APX: Ascorbic peroxidase
CAT: Catalase
SOD: Superoxide dismutase
MDHAR: Monodehydroascorbate
GR: Glutathione reductase.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Additional Points

Novelty Impact Statement. (i) Ozone treatment promoted the productions of $O_2 \cdot^-$ and H_2O_2 by increasing the activities and gene expressions of *NOX* and *SOD* in inoculated potato tubers. (ii) Ozone treatment maintained the cell membrane integrity of the inoculated tubers by promoting ROS metabolism and AsA-GSH cycle, thus inhibiting the occurrence of dry rot. (iii) Ozone treatment inhibited the DIA accumulation by downregulating the expressions of genes involved in DIA biosynthesis pathway.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Zhiguang Liu was responsible for writing the original draft and wrote, reviewed, and edited the manuscript. Huali Xue was responsible for the data curation and funding acquisition. Yang Bi was responsible for the project administration and supervision. Xi Yang was responsible for the methodology. Qianqian Zhang was responsible for the conceptualization. Qili Liu was responsible for the software. Jiangyang Chen was responsible for the validation. Mina Nan was responsible for the formal analysis. Dov Prusky was responsible for the visualization.

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