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ARTICLE

Effects of Manganese on the Antioxidant System and Related Gene Expression Levels in the "Hong Yang" Kiwifruit Seedlings

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ABSTRACT

To explore how manganese affects the antioxidant system and the expression levels of related genes of "Hong yang" seedlings, the leaves of its tissue cultured seedlings were taken as test materials, and single factor treatment was performed by changing the manganese chloride ($MnCl_2{\cdot}4H_2O$) solution concentration when spraying the leaves. The expression levels of *Mn-SOD*, *POD64* and *POD27* genes in leaves were quantitatively analyzed by real-time quantitative PCR (qRT-PCR) at different determination times. Meanwhile, the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), the activities of antioxidant enzymes, including catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). The results showed that the SOD, CAT, POD, ascorbate peroxidase (APX), and reduced glutathione (GSH) activities in leaves were the highest at 12 h post-treatment with 50 μM $MnCl_2{\cdot}4H_2O$. Furthermore, the contents of MDA and H_2O_2 in leaves also peaked when the concentration of H_2O_2 is 50 μM, which is the minimum value. Additionally at 50 μM Mn^{2+}, the *Mn-SOD* and *POD27* expression was up-regulated as compared to the control, which promoted the expression of their respective enzyme activities. However, *POD64* expression increased with the increasing Mn^{2+} concentration. Therefore, 50 μM is the optimal concentration of Mn when exogenously applied on "Hong yang", which improve the antioxidant enzyme activity and regulate the plant's physiological and biochemical functions.

KEYWORDS

"Hong yang" seedlings; manganese; antioxidant system; related gene expression

1 Introduction

Although manganese (Mn) is one of the essential trace metal elements for plants [1], it is needed in small quantities [2]. Mn deficiency in plants leads to low efficiency of their antioxidant system and chlorophyll degradation [3], with Mn being irreplaceable in plant photosynthetic oxygen release, maintaining normal organellar structure, and activating enzyme activities.

"Hong yang" is a unique red flesh kiwi variety in China, with a large neatly shaped fruit that was tender, delicious, and has a small coefficient of variation [4]. Although its market demand increasing, its production is plagued by some problems, including weak growth, concave fruit top, and easy-to-bruise fruit peel [5]. In order to solve the related problems, people mostly through the increase of different organic fertilizers, while

the increase of fertilizer before and after the kiwifruit leaf nutrition and fruit quality of the corresponding determination and comparative analysis, to screen out the best treatment method to improve the kiwifruit leaf nutrition and fruit quality [6,7].

Another reason is that copper (Cu), zinc (Zn), manganese (Mn), nickel (Ni), and iron (Fe) are essential metal elements, and they can serve as co-factors of enzymes that are involved in important regulatory processes of many organisms [8]. One study [9] found that the Fe-application on soil and foliage during drought stress can increase the plant's enzymatic antioxidant activity. This test shows that antioxidant levels of essential metals can raise enzyme activity in plants. At the same time, related studies on grapes [10] and peaches [11] have found that exogenously applying the trace element Mn on their roots can promote fruit growth, nutrient balance, and improve fruit quality. Therefore, studying the effect of Mn on the physiological and biochemical functions of plants is important. Currently, existing studies on the effects of Mn on the physiological and biochemical functions of plants have mainly focused on manganese tolerant plants, including soybean [12], paper mulberry [13], Masson's pine [14], etc. Furthermore, very few reports have studied the effects of Mn on the antioxidant system of kiwi seedlings. To explore the effects of different Mn concentrations on the antioxidant system of kiwi seedlings, it is important to select the appropriate concentration.

In this study, the "Hong yang" seedlings were taken as the study material. With water taken as the control, Mn concentration gradients were set as different treatments, followed by evaluating the antioxidant system related gene expression in "Hong yang" leaves under different treatments. Furthermore, the determination time was also modulated to verify the sustained effect of the treatment. Thus, the optimal treatment concentration formula and spraying time of antioxidant enzymes in the leaves of "Hong yang" seedlings under the action of exogenous Mn were selected. Therefore, this study can provide a theoretical basis for the rational and effective application of Mn fertilizer, or the use of exogenous substances Mn to improve the activity of antioxidant enzymes in plants, regulate the physiological and biochemical functions of plants, optimize their fruit quality, and enhance their stress resistance.

2 Materials and Methods

2.1 Material

The shoots, stems and leaves of 5-year-old "Hong yang" kiwifruit were selected for tissue culture, and the "Hong yang" seedlings obtained by tissue culture were taken as the experimental material. The explants were collected from the kiwifruit production-study-research Base (E:104°57'58.48"E; N:26°24' 55.04"N; 1,100 m above the sea level), in the Shuicheng District, Guizhou Province, China. These were then cultured by the kiwifruit team of Institute of Mountain Resources, Guizhou Academy of Sciences, Guizhou Province, China.

2.2 Design and Treatment

In the experiment, the "Hong yang" seedlings with the same growth potential were selected and moved to the canopy in the field, where the potted matrix had the perlite: vermiculite ratio was 2:1. When the seedlings grew to have six leaves of one heart, relevant treatment was started. During the 21 days of the experiment, $MnCl_2{\cdot}4H_2O$ solution was sprayed on the leaf surface of tissue culture seedlings at 9 AM or 4 PM on three sunny days. Field temperature is 25°C ± 2°C and humidity is 80%. Each spraying interval should be at least three days. Five treatments were set up in the experiment, and the treatment concentration gradient was designed and improved according to a previous experimental design [15]: 5, 10, 50, and 100 μM. The water treatment was taken as the control. The amount of each spray was 10 ml until the leaves were fully wetted by hand-held mist sprayer [16]. Treated leaves were sampled and

measurements were taken at 6, 12, 24, and 48 h post spraying, with the preserved samples being immediately frozen at −80°C with liquid nitrogen until further use.

2.3 Measuring Items and Methods

2.3.1 Determination of Physiological and Biochemical Indexes

The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), along with reduced glutathione (GSH), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents were determined in the leaves to study the effects of Mn^{2+} on the antioxidant system of leaves of "Hong yang" tissue culture seedlings. POD activity was determined by the guaiacol oxidation method [17]. CAT activity was determined using visible light spectrophotometry [18]. The SOD activity was determined by the nitrogen blue tetrazole (NBT) photoreduction method [19]. The APX activity was determined by determining the oxidation rate of ascorbic acid [20]. The GSH content was determined using 2-nitrobenzoic acid (DTNB) [21]. The MDA content was determined by the thiobarbituric acid (TBA) method [22]. The H_2O_2 content was determined by the titanium salt-based spectrophotometry method [23].

2.3.2 Determination of Relative Gene Expression in Leaves of "Hong Yang" Seedlings Treated with $MnCl_2{\cdot}4H_2O$

The relative expression of the genes was determined using real-time quantitative PCR (qRT-PCR). The reverse transcription product cDNA synthesized from the total RNA of the "Hong yang" seedling leaves was used as the template for qRT-PCR. According to the conserved region sequences of *POD27* and *POD64* genes of the *Mn-SOD* and *POD* gene family members in the kiwifruit genome database, combined with the transcriptome sequencing results of "Hong yang" Kiwifruit, gene-specific PCR primers were designed accordingly (see Table 1), which were then synthesized by Shanghai Weiqing Biotechnology Co., Ltd. (China). Using cDNA as a template and polyubiquitin (*UBQ*) as the internal reference gene, qRT-PCR was performed.

Table 1: Primer sequence of real-time PCR

Primer	*Primer ID*	*Primer sequence 5′→3′*	Primer Pos	Annealing temperature (TM)/°C
MnSOD	*MnSOD-2F*	*AATCTTGCTCCAGTTCGTG*	396	62.8
	MnSOD-2R	*CACCTTCAGCATTCATCTTCT*	513	62.9
POD27	*POD27-2F*	*GACTCTTGCAGCTCCTTTGT*	170	65.2
	POD27-2R	*GCATCTTTCTCAGCTTGGTT*	280	63.4
POD64	*POD64-1F*	*ATTCTCTCTCGCTCGCTTT*	63	63.8
	POD64-1R	*ATAACTGACTCTGCCTTGGG*	225	64.1
UBQ	*UBQ-3F*	*TGGAGAGTTCCGATACCATT*	1253	62.9
	UBQ-3R	*TGTTGTAGTCAGCCAAAGTCC*	1384	65.1

2.4 Data Processing

Microsoft Excel 2010 software was used to process the data, while SPSS16.0 software was used for variance analysis and correlation analysis, with the significance level set at 0.05. The Origin 2018 software was used for plotting.

3 Results and Analysis

3.1 Effects of Foliar Spraying $MnCl_2{\cdot}4H_2O$ on the Antioxidant System of "Hong Yang" Seedlings

3.1.1 Effect of Foliar Spraying $MnCl_2{\cdot}4H_2O$ on the SOD Activity of "Hong Yang" Seedlings

SOD is a widely existing metalloenzyme in organisms. It can catalyze the dismutation of superoxide anions and is vital in the biological antioxidant system. As shown in Table 2, as compared with the control treatment (water spraying), spraying $MnCl_2{\cdot}4H_2O$ solution on the leaf can improve the SOD activity of "Hong yang" seedlings. Under the same measurement time, with the increase in the $MnCl_2{\cdot}4H_2O$ concentration, the SOD activity of leaves of "Hong yang" seedlings first increased and then decreased. Under 50 μM $MnCl_2{\cdot}4H_2O$, the SOD activity was significantly higher than that of other concentrations, especially, 12 h post treatment, the SOD activity was the highest, with the difference being the most significant. Furthermore, at the same treatment concentration and different determination times, the SOD activity also showed a trend of first increasing and then decreasing, with the SOD activity at 12 h being the highest. In conclusion, the SOD activity of "Hong yang" leaves can be improved by using 50 μM $MnCl_2{\cdot}4H_2O$, with it being the most significant at 12 h post treatment.

Table 2: SOD activities ($U{\cdot}g^{-1}$) of "Hong yang" seedlings under different treatment concentrations and determination times

Treatment concentration (μM)	Determination time (h)			
	6	12	24	48
0	0.722 ± 0.548 cγ	8.399 ± 4.36 eα	5.430 ± 0.79 cαβ	1.014 ± 0.72 eαβ
5	4.010 ± 1.793 cβ	34.543 ± 2.19 cα	6.554 ± 0.81 cβ	5.986 ± 0.40 dβ
10	13.769 ± 2.22 bβ	40.182 ± 0.56 bα	12.919 ± 0.91 bβ	10.187 ± 0.25 bγ
50	35.757 ± 1.96 aβ	59.535 ± 1.81 aα	35.177 ± 1.30 aβ	23.080 ± 0.31 aγ
100	16.041 ± 0.96 bα	28.121 ± 1.17 dα	11.301 ± 0.88 bβ	8.731 ± 0.24 cγ

Note: In the same row of data, different letters (a, b, c, d, e) indicated that there were significant differences among different treatment concentrations (0, 5, 10, 50, 100 μM) in the same determination time ($p < 0.05$); In the same column of data, different Greek letters (α, β, γ, θ) indicated that under the same treatment concentration, different determination times (6, 12, 24, 48 h) had significant differences ($p < 0.05$).

3.1.2 Effect of Foliar Spraying $MnCl_2{\cdot}4H_2O$ on POD Activity of "Hong Yang" Seedlings

POD catalyzes diverse oxidative reactions involving H_2O_2, and is closely related to various physiological and biochemical processes, including respiration, photosynthesis, and auxin oxidation. It is vital in the cellular redox metabolism. As shown in Table 3, as compared with the control treatment, foliar spraying with $MnCl_2{\cdot}4H_2O$ improved the POD activity of "Hong yang" seedlings. POD activity at 50 μM $MnCl_2{\cdot}4H_2O$ was significantly higher than those of the control group and 5 μM treatment groups, with the most significant difference observed at 12 h. However, when $MnCl_2{\cdot}4H_2O$ was (≤50 μM), the POD activity in the leaves first increased, and then decreased with the increasing determination time, but it only decreased at 100 μM. With the increasing concentration, the POD activity of the leaves of "Hong yang" showed a trend of first increasing and then decreasing during the same determination time, with its peak being observed at 50 μM at 12 h post treatment. Therefore, $MnCl_2{\cdot}4H_2O$ at 50 μM was the most beneficial for improving the POD activity of the "Hong yang" leaves.

Table 3: POD activities ($U \cdot g^{-1}$) of "Hong yang" seedlings under different treatment concentrations and determination times

Treatment concentration (μM)	Determination time (h)			
	6	12	24	48
0	0 ± 14 cα	5 ± 8 cα	4 ± 8 aα	1 ± 8 aα
5	5 ± 2 cα	14 ± 8 bcα	10 ± 8 aα	5 ± 8 aα
10	13 ± 8 bcα	22 ± 8 bα	13 ± 8 aα	5 ± 8 aα
50	33 ± 8 aα	40 ± 8 aα	16 ± 8 aα	7 ± 8 aα
100	26 ± 8 abα	16 ± 8 bcα	10 ± 8 aα	2 ± 8 aα

Note: In the same row of data, different letters (a, b, c, d, e) indicated that there were significant differences among different treatment concentrations (0, 5, 10, 50, 100 μM) in the same determination time ($p < 0.05$); In the same column of data, different Greek letters (α, β, γ, θ) indicated that under the same treatment concentration, different determination times (6, 12, 24, 48 h) had significant differences ($p < 0.05$).

3.1.3 Effects of Foliar Spraying $MnCl_2 \cdot 4H_2O$ on the CAT Activity of "Hong Yang" Seedlings

CAT assists SOD in removing superoxide free radicals. In plants, the vital coordination between SOD, CAT, and POD maintains the cellular redox homeostasis. Table 4 shows that the CAT activity in the "Hong yang" leaves first increased and then decreased with the increasing $MnCl_2 \cdot 4H_2O$ treatment concentration at the same determination time. At 50 μM, the CAT activity of leaves was the highest among all concentrations ($p < 0.05$). Under different determination times, the CAT activity showed an "N" type trend under control treatment, with the maximum being 4.391 $U \cdot g^{-1}$ at 24 h post treatment. At 5 μM $MnCl_2 \cdot 4H_2O$, the CAT activity first decreased and then increased, with the minimum value of 3.121 $U \cdot g^{-1}$ being reached at 12 h post treatment. Although the opposite trend was seen at 10 or 50 μM, the maximum CAT activities were 7.158 and 11.213 $U \cdot g^{-1}$, respectively, at 12 h post spraying. However, for 100 μM, the CAT activity decreased with the increasing time. Thus, the CAT activity of leaves was significantly affected by the $MnCl_2 \cdot 4H_2O$ treatment. Therefore, spraying $MnCl_2 \cdot 4H_2O$ at 50 μM can promote and improve the CAT activity, with the effect being best at 12 h after spraying.

Table 4: CAT activities ($U \cdot g^{-1}$) "Hong yang" seedlings under different treatment concentrations and determination times

Treatment concentration (μM)	Determination time (h)			
	6	12	24	48
0	1.015 ± 0.038 eγ	1.802 ± 0.029 cβ	4.391 ± 0.044 dα	1.066 ± 0.055 dγ
5	4.150 ± 0.051 dβ	3.121 ± 0.032 cθ	5.416 ± 0.046 cα	3.821 ± 0.059 bγ
10	6.587 ± 0.047 bβ	7.158 ± 0.048 b_2α	6.054 ± 0.049 bγ	3.897 ± 0.054 b
50	8.895 ± 0.053 aγ	11.213 ± 0.060 aα	10.731 ± 0.056 aβ	6.742 ± 0.053 aθ
100	6.258 ± 0.033 cα	7.656 ± 1.551 bα	2.657 ± 0.051 eβ	2.536 ± 0.053 aβγ

Note: In the same row of data, different letters (a, b, c, d, e) indicated that there were significant differences among different treatment concentrations (0, 5, 10, 50, 100 μM) in the same determination time ($p < 0.05$); In the same column of data, different Greek letters (α, β, γ, θ) indicated that under the same treatment concentration, different determination times (6, 12, 24, 48 h) had significant differences ($p < 0.05$).

3.1.4 Effects of Foliar Spraying of $MnCl_2{\cdot}4H_2O$ on the APX Activity of "Hong Yang" Seedlings

APX is a specific peroxidase with ascorbic acid as the electron donor, which directly affects the ascorbic acid content in plants. As one of the most important antioxidant enzymes involved in the metabolism of reactive oxygen species (ROS) in plants, it can remove the damaging H_2O_2 that accumulated and protect chloroplasts and other cellular components. As can be seen from Table 5, at the same measurement time, with the increasing $MnCl_2{\cdot}4H_2O$ concentration, the APX activity in the "Hong yang" leaves first increased and then decreased. The same trend was also seen for the same concentration and different determination times. At 50 μM, the APX activity showed the maximum value, and was significantly higher than the control group ($p < 0.05$). The APX activity in the leaves also increased first and then decreased when the same concentration of $MnCl_2{\cdot}4H_2O$ was sprayed under different. When the treatment concentration was ≤10 μM, the APX activity was the maximum at 24 h post treatment, which when compared with other treatment times, the increase was not significant ($p < 0.05$). Although when the treatment concentration was (>10 $\mu mol{\cdot}L^{-1}$), the APX activity peaked at 12 h post treatment, which was not significant as compared with other treatment times. Therefore, the APX activity in leaves was significantly affected by $MnCl_2{\cdot}4H_2O$ treatment, which significantly differed from the control group. Therefore, spraying 50 μM $MnCl_2{\cdot}4H_2O$ can improve the APX activity, with the effect being the best at 12 h post spraying.

Table 5: APX activities ($U{\cdot}g^{-1}$) of "Hong yang" seedlings under different treatment concentrations and determination times

Treatment concentration (μM)	Determination time (h)			
	6	12	24	48
0	0.321 ± 0.055 cα	0.405 ± 0.058 bα	1.158 ± 1.048 aα	0.492 ± 0.104 dα
5	0.131 ± 0.068 dβ	1.027 ± 0.814 a_2bα	0.694 ± 0.054 aαβ	0.583 ± 0.062 bdα
10	0.940 ± 0.059 bα	0.873 ± 0.294 bα	0.837 ± 0.038 aα	1.035 ± 0.060 cα
50	1.999 ± 0.056 aαβ	4.006 ± 2.491 aα	0.956 ± 0.072 aα	2.356 ± 0.068 aαβ
100	1.892 ± 0.062 aα	3.364 ± 1.879 abα	0.706 ± 0.328 aα	1.833 ± 0.059 bα

Note: In the same row of data, different letters (a, b, c, d, e) indicated that there were significant differences among different treatment concentrations (0, 5, 10, 50, 100 μM) in the same determination time ($p < 0.05$); In the same column of data, different Greek letters (α, β, γ, θ) indicated that under the same treatment concentration, different determination times (6, 12, 24, 48 h) had significant differences ($p < 0.05$).

3.1.5 Effect of Foliar Spraying of $MnCl_2{\cdot}4H_2O$ on the GSH Content of "Hong Yang" Seedlings

GSH is an important antioxidant participating in the ASA-GSH cycle that is vital to abiotic stress tolerance. It helps maintain the plant immune system, with its antioxidative and integrated detoxification effects. Table 6 shows that, at the same determination time, with the increasing $MnCl_2{\cdot}4H_2O$ concentration, the GSH content in "Hong yang" first increased and then decreased. At 50 μM, the GSH content was the highest, which significantly differed from the control group. However, under the same concentration at different determination times, although it showed a trend of first increasing and then decreasing, it tended to stabilize and was not significantly different. Therefore, the GSH content of leaves was significantly affected by the $MnCl_2{\cdot}4H_2O$, which was improved by spraying at a concentration of 50 μM, and reached the highest (62.22 $\mu g{\cdot}g^{-1}$) at 12 h after spraying.

Table 6: GSH contents ($\mu g \cdot g^{-1}$) of "Hong yang" seedlings under different treatment concentrations and determination times

Treatment concentration (μM)	Determination time (h)			
	6	12	24	48
0	55.976 ± 0.425 dβ	56.397 ± 0.515 eβ	54.231 ± 0.128 eγ	57.480 ± 0.129 eα
5	56.987 ± 0.444 cβ	57.508 ± 0.485 dβ	57.009 ± 0.117 dβ	58.535 ± 0.065 dα
10	58.384 ± 0.436 bγ	58.687 ± 0.386 cγ	59.635 ± 0.189 cβ	59.343 ± 0.088 cβ
50	61.145 ± 0.428 aβ	62.110 ± 0.159 aα	61.925 ± 0.212 aα	61.751 ± 0.076 aα
100	52.929 ± 0.395 eα	61.150 ± 0.229 bα	60.746 ± 0.153 bβ	60.320 ± 0.084 bγ

Note: In the same row of data, different letters (a, b, c, d, e) indicated that there were significant differences among different treatment concentrations (0, 5, 10, 50, 100 μM) in the same determination time ($p < 0.05$); In the same column of data, different Greek letters (α, β, γ, θ) indicated that under the same treatment concentration, different determination times (6, 12, 24, 48 h) had significant differences ($p < 0.05$).

3.1.6 Effects of Foliar Spraying of $MnCl_2 \cdot 4H_2O$ on the MDA and H_2O_2 Contents of "Hong Yang" Seedlings

MDA is the end product of membrane lipid peroxidation, which inhibits the cell protective enzymes. Since the MDA content can reflect the stress-induced cellular stability, increased plant MDA content indicates increased plant cell membrane damage. As can be seen from Table 7, within the same determination time, the MDA content in "Hong yang" showed a trend of first decreasing and then increasing with the increasing $MnCl_2 \cdot 4H_2O$ treatment concentration, with the minimum observed at 50 μM. Meanwhile, as compared with the control group, the MDA content in the "Hong yang" leaves had significantly decreased by 16.4%, 13.3%, 19.2%, and 24.89%. Under the same treatment concentration of $MnCl_2 \cdot 4H_2O$ and different determination times, the MDA content in leaves also showed a trend of first decreasing and then increasing. It can be seen that the MDA content in the leaves is affected by the concentration of $MnCl_2 \cdot 4H_2O$. Foliar spraying of 50 μM $MnCl_2 \cdot 4H_2O$ can significantly reduce the MDA content, with the best effect (5.312 $nmol \cdot g^{-1}$) seen at 12 h post treatment.

Table 7: MDA contents ($nmol \cdot g^{-1}$) of "Hong yang" seedlings under different treatment concentrations and determination times

Treatment concentration (μM)	Determination time (h)			
	6	12	24	48
0	60.438 ± 0.149 aγ	39.893 ± 0.121 aθ	68.070 ± 0.127 aβ	74.158 ± 0.115 aα
5	25.568 ± 0.127 cβ	21.027 ± 0.114 cγ	25.503 ± 0.132 bβ	26.875 ± 0.143 cα
10	21.788 ± 0.116 dβ	16.963 ± 0.139 dγ	23.157 ± 0.118 cα	23.186 ± 0.117 dα
50	9.944 ± 0.135 eγ	5.312 ± 0.133 eθ	13.095 ± 0.129 eβ	18.455 ± 0.141 eα
100	36.119 ± 0.141 bβ	22.126 ± 0.128 bβ	20.782 ± 0.123 dγ	49.715 ± 0.128 bα

Note: In the same row of data, different letters (a, b, c, d, e) indicated that there were significant differences among different treatment concentrations (0, 5, 10, 50, 100 μM) in the same determination time ($p < 0.05$); In the same column of data, different Greek letters (α, β, γ, θ) indicated that under the same treatment concentration, different determination times (6, 12, 24, 48 h) had significant differences ($p < 0.05$).

H_2O_2 is the most common reactive oxygen molecule in living organisms and is the hub of mutual transformation of reactive oxygen species. It is mainly generated as a byproduct by SOD and xanthine oxidase (XOD), which is then degraded by CAT and POD. It can not only directly or indirectly oxidize nucleic acid, protein, and other biological macromolecules, but also damage the cell membrane, thereby

accelerating cellular aging and disintegration. As can be seen from Table 8, H_2O_2 content in the leaves of the "Hong yang" seedlings first decreased and then increased with increasing treatment concentration during the same determination time. At 50 μM $MnCl_2{\cdot}4H_2O$, the H_2O_2 content in leaves was 98.02%, 96.63%, 97.47% and 97.54% of that in control group, and significantly decreased ($p < 0.05$). Under the same treatment concentration and different determination times, the H_2O_2 content of leaves also showed a trend of first decreasing and then increasing. When the treatment concentration was ≤10 μM, the minimum H_2O_2 content was at 24 h post treatment, with the difference not being significant as compared to other determination times. However, when the concentration was >10 μM, the H_2O_2 content was the minimum at 12 h post treatment, which did not significantly differ from other determination times. Therefore, at the same treatment concentration and the increasing determination times, the H_2O_2 content was unaffected, as its enzymatic decomposition remained similar irrespective of the increasing determination time post $MnCl_2{\cdot}4H_2O$ treatment. Therefore, when the spraying time of $MnCl_2{\cdot}4H_2O$ is 12 h, the minimum H_2O_2 content was 72.167 $\mu mol{\cdot}g^{-1}$ when the spraying concentration was 50 μM.

Table 8: H_2O_2 contents ($\mu mol{\cdot}g^{-1}$) of "Hong yang" seedlings under different treatment concentrations and determination times

Treatment concentration (μM)	Determination time (h)			
	6	12	24	48
0	75.531 ± 0.018 aα	74.681 ± 0.028 aγ	74.775 ± 0.032 bβ	75.550 ± 0.036 bα
5	75.380 ± 0.038 bα	74.170 ± 0.016 bβ	73.169 ± 0.030 cγ	75.323 ± 0.014 cα
10	74.870 ± 0.034 cα	73.944 ± 0.045 cβ	73.131 ± 0.026 cθ	73.849 ± 0.042 dγ
50	74.038 ± 0.026 eα	72.167 ± 0.038 eθ	72.885 ± 0.017 dγ	73.698 ± 0.022 cβ
100	74.718 ± 0.020 dγ	72.791 ± 0.021 dγ	76.003 ± 0.024 aβ	76.381 ± 0.038 aα

Note: In the same row of data, different letters (a, b, c, d, e) indicated that there were significant differences among different treatment concentrations (0, 5, 10, 50, 100 μM) in the same determination time ($p < 0.05$); In the same column of data, different Greek letters (α, β, γ, θ) indicated that under the same treatment concentration, different determination times (6, 12, 24, 48 h) had significant differences ($p < 0.05$).

3.2 Effects of Foliar Spraying of $MnCl_2{\cdot}4H_2O$ on the Expression of Antioxidant System-Related Genes in "Hong Yang" Seedlings

3.2.1 3.2.1 Effects of Foliar Spraying of $MnCl_2{\cdot}4H_2O$ on Relative Expression Level of Mn-SOD in "Hong Yang" Seedlings

Mn-SOD mainly exists in prokaryotic cells and a few plant cells, where most are located in their mitochondria. Besides chloroplasts, plant mitochondria are the second site producing ROS, with *Mn-SOD* located in them being vital in clearing ROS. As can be seen from Fig. 1, with the increase in the Mn^{2+} treatment concentration, the *Mn-SOD* expression level first increased and then decreased under different determination times. However, when the Mn^{2+} was >50 μM), the *Mn-SOD* expression showed a decreasing trend under different determination times. Therefore, with the increasing Mn^{2+} concentration, the *Mn-SOD* expression was significantly up-regulated under the same determination time, as compared to the control group. However, when the Mn^{2+} concentration was too high, it had greater toxicity to the leaves of "Hong yang" seedlings.

At the same concentration of Mn^{2+}, the *Mn-SOD* expression in the leaves also showed a trend of first increasing and then decreasing with the increasing determination time, while peaking at 12 h post treatment, which was higher than other treatments. Therefore, at 50 μM $MnCl_2{\cdot}4H_2O$ and 12 h post

treatment, the *Mn-SOD* expression was the highest. But when the determination time was >12 h, the promoting effect of Mn^{2+} on the antioxidant system of "Hong yang" seedling leaves would decline, and the effect would be gradually weakened with the extension of time.

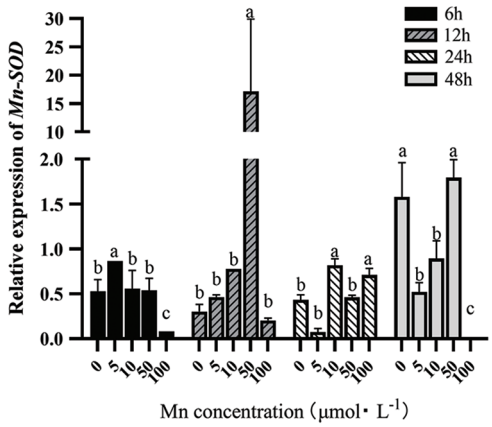

Figure 1: Effects of different treatment concentrations and determination time on the relative expression of *Mn-SOD* in "Hong yang" leaves. Data were expressed as the mean ± SD of three replicates. Values designated over the bars in different letter are significant differences at $p < 0.05$

3.2.2 Effects of Foliar Spraying with $MnCl_2 \cdot 4H_2O$ on Relative Expression Levels of POD27 and POD64 in "Hong Yang" Seedlings

POD27 and *POD64* are mainly involved in hydrogen peroxide decomposition, auxin metabolism, lignin synthesis, and stress response in intercellular spaces and vacuoles. Fig. 2 shows that with the increasing Mn^{2+} concentration, the *POD27* expression in leaves first increased and then decreased when the determination time was ≤24 h. However, when Mn^{2+} concentration ≥50 μM, the expression level of the *POD27* expression in leaves showed a decreasing trend at all determination times. Moreover, when the determination time was (>24 h), it showed a downward trend even with the increasing Mn^{2+} concentration. Therefore, the *POD27* expression was the highest when the determination treatment time was 12 h and the Mn^{2+} concentration was 50 μM.

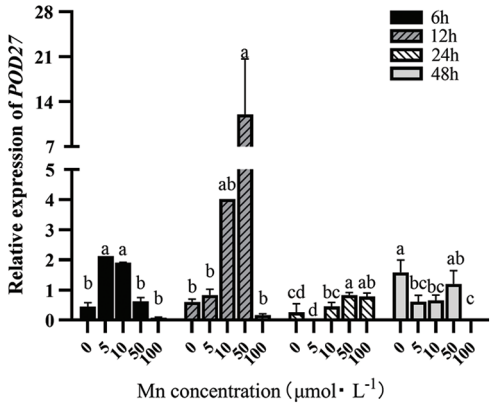

Figure 2: Effects of different treatment concentrations and determination time on the relative expression of *POD27* in "Hong yang" leaves. Data were expressed as mean ± SD of three replicates. Values designated over the bars in different letter are significant differences at $p < 0.05$

With the increasing Mn^{2+} concentration, the *POD64* expression showed an "M" shaped trend. At the same Mn^{2+} concentration, it first increased and then decreased with the increasing determination time. However, at 12 h and 50 μM, the *POD64* expression level was the highest (see Fig. 3).

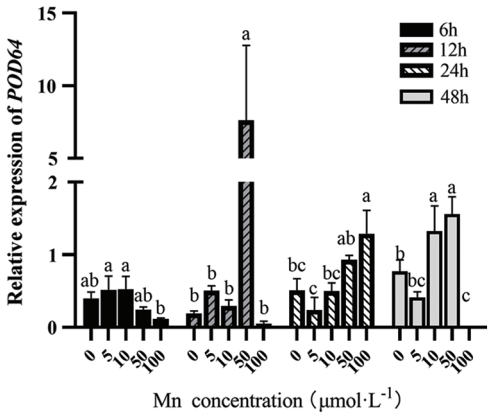

Figure 3: Effects of different treatment concentrations and determination time on the relative expression of *POD64* in "Hong yang" leaves. Data were expressed as mean ± SD of three replicates. Values designated over the bars in different letter are significant differences at $p < 0.05$

4 Discussion

Plants have a powerful enzymatic antioxidant system, including SOD, POD, and CAT, which coordinate with each other and play an important defense role [24]. They can effectively remove excess ROS, reduce the associated toxicity, and maintain the metabolic balance in plants [25]. They also provide abiotic stress resistance. SOD is an important antioxidant enzyme in plants, and the higher the activity of SOD, the stronger the resistance of plants. The results showed that the activities of SOD, CAT, POD, and APX in the leaves of "Hong yang" seedlings first increased and then decreased with the increasing Mn^{2+} concentration, which indicated that excess $MnCl_2{\cdot}4H_2O$ would cause toxicity, thereby reducing the activities of SOD, POD, CAT, and APX and the GSH content in the "Hong yang" seedlings. The contents of MDA and H_2O_2 decreased first and then increased with the increase of Mn^{2+} concentration. One reason is that the SOD protection system is not activated at low Mn concentration [26]. The other is that with the increase of Mn concentration, SOD activity gradually increases, and the maximum value appears at the concentration of 50 μM. The results of this study were consistent with previous results [27], which indicated that the rate of cell oxidation could be effectively reduced when suitable Mn concentration was sprayed on kiwifruit seedlings [28]. However, when $MnCl_2{\cdot}4H_2O$ is sprayed with high concentration, lipid composition in membrane and membrane structure will be changed, and the damage of membrane lipid will be aggravated, which is consistent with the results of previous studies [29,30,31]. When different concentrations of $MnCl_2{\cdot}4H_2O$ were sprayed on the leaf surface of "Hong yang" seedlings, the expressions of antioxidant system-related genes like *Mn-SOD* and *POD27* first increased and then decreased with the increasing Mn^{2+} concentration. The reason is that Mn is a cofactor of SOD [32]. Under appropriate concentration, Mn can not only stimulate the activity of plant growth-related enzymes, but also promote plant growth and development, improve the activities of POD and CAT, and produce certain resistance to various abiotic stresses affecting plant growth, playing an important role in defense. At the same time, with higher antioxidant enzyme activity and antioxidant gene expression, lower plasma membrane permeability [33], which plays a crucial role in plant metabolism [34].

In the experiment, it was also found that the activities of SOD, POD, GSH, APX and CAT contents of "Hong yang" seedlings increased first and then decreased with the passing of time, and the maximum value

appeared at 12 h, but the overall difference was not significant. The expression level of antioxidant system related gene *POD64* changed significantly with the passage of Mn^{2+} determination time, indicating that Mn would accumulate continuously in kiwifruit seedlings over time, resulting in increased accumulation of ROS *in vivo*, leading to impaired enzyme activity [35]. It is further verified that when the concentration of Mn in plants is higher than the optimal content in cells, it will have a destructive effect on plants and reduce the vitality of plant cells [36]. It can also cause manganese poisoning [37], which reduces plant quality and, in severe cases, leads to the death of affected plants [38].

Currently, there are very few studies on the effects of Mn treatment on the antioxidant system of plants and their temporal response. This study can provide a theoretical basis for later studies on the effects of trace metal elements on the antioxidant system of plants at different times. However, there are still some shortcomings. Whether these antioxidant activity changes of kiwifruit post Mn^{2+} treatment can improve the fruit quality, improve plant stress resistance and prevent the invasion of pathogens needs to be studied further.

5 Conclusion

In this study, different $MnCl_2{\cdot}4H_2O$ concentrations were sprayed on the leaf surface of "Hong yang" seedlings. The results showed that although low concentration of $MnCl_2{\cdot}4H_2O$ could improve the activities of SOD, POD, and CAT in the leaves, high concentrations could also cause certain damage to the "Hong yang" seedlings. Furthermore, the activities of SOD, POD, and CAT were increased for determination times up to 12 h post spraying, with the expressions of *Mn-SOD, POD27*, and *POD64* related genes in the leaves also being promoted. Therefore, when the exogenous Mn acts on "Hong yang" kiwifruit, the optimal treatment concentration formula of antioxidant enzyme in leaves is 50 μM, which can improve the antioxidant enzyme activity and regulate the physiological and biochemical functions of the plant itself.

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