horticulturae

MDPI

Article

Piriformospora indica Enhances Resistance to Fusarium wilt in Strawberry by Increasing the Activity of Superoxide Dismutase, Peroxidase, and Catalase, While Reducing the Content of Malondialdehyde in the Roots

Yuji Huang [1,2,†], Jinman Li [1,†], Chaocui Nong [1], Tong Lin [1], Li Fang [3], Xu Feng [1], Yiting Chen [4], Yuling Lin [1], Zhongxiong Lai [1,*] and Lixiang Miao [1,*]

[1] College of Horticultural, Fujian Agriculture and Forestry University, Fuzhou 350002, China; yjhuang2004@163.com (Y.H.); linyuling@fafu.edu.cn (Y.L.)
[2] Fujian Universities and Colleges Engineering Research Center of Modern Facility Agriculture, Fuqing 350300, China
[3] Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
[4] Fruit Research Institute, Fujian Academy of Agricultural Sciences, Fuzhou 350003, China
* Correspondence: laizx01@163.com (Z.L.); mlx.123@163.com (L.M.)
† These authors contributed equally to this study.

Abstract: Strawberry Fusarium wilt, mainly caused by *Fusarium oxysoporum* f. sp. *Fragariae* (*Fof*), seriously threatens the yield and quality of strawberry. *Piriformospora indica* is an endophytic fungus that can colonise the roots of a wide range of plants, promoting plant growth and enhancing plant resistance. Against this background, the positive effects of *P. indica* on the growth of the daughter plants of 'Benihoppe' strawberry (*Fragaria* × *ananassa* Duch.) under *Fof* stress were investigated in this study. The study began by examining the inhibitory effect of *P. indica* on *Fof* growth through dual culture on agar plates. Subsequently, a symbiotic system between *P. indica* and strawberry plantlets was established, and the impact of *P. indica* on Fusarium wilt resistance and related physiological and biochemical indexes of the plantlets were evaluated. The results indicate that fungus colonization with *P. indica* significantly enhances the growth indices of strawberries, including plant height, petiole length, petiole diameter, and leaf area. Additionally, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in the leaves of *P. indica* were increased, and the content of malondialdehyde (MDA) was decreased compared to those without colonization. Under the stress from *Fof*, the growth indexes of plant height, stem diameter, leaf area, petiole diameter, and root length of strawberry plants colonization with *P. indica* were significantly higher than those without colonization and the symptoms of wilting were relatively mild. The activities of SOD, POD, and CAT in roots and leaves of plants colonized with *P. indica* were significantly increased compared to those without colonization. Furthermore, the content of MDA in roots was decreased. These results suggested that *P. indica* could increase resistance to Fusarium wilt in strawberry by increasing the activity of antioxidant enzymes and reducing the content of MDA.

Keywords: strawberry; *Piriformospora indica*; Fusarium wilt; Benihoppe

Citation: Huang, Y.; Li, J.; Nong, C.; Lin, T.; Fang, L.; Feng, X.; Chen, Y.; Lin, Y.; Lai, Z.; Miao, L. *Piriformospora indica* Enhances Resistance to Fusarium wilt in Strawberry by Increasing the Activity of Superoxide Dismutase, Peroxidase, and Catalase, While Reducing the Content of Malondialdehyde in the Roots. *Horticulturae* **2024**, *10*, 240. https://doi.org/10.3390/horticulturae10030240

Academic Editor: Boqiang Li

Received: 9 January 2024
Revised: 2 February 2024
Accepted: 27 February 2024
Published: 29 February 2024

1. Introduction

The strawberry (*Fragaria ananassa* Duch.) is a perennial evergreen herbaceous plant belonging to the *Fragaria* genus of the Rosaceae family. It is one of the seven largest fruits in the world and is favored by consumers and businesses due to its sweet fruit taste, unique nutritional value, short growth cycle, early results, and high yield. Therefore, it, is commonly referred to as the 'Fruit Queen' in China [1]. In 1978, the total area of strawberry cultivation in China was less than 300 square hectometers, and the yield was less than

2000 tons. By 2018, the total area of strawberry cultivation had increased to 173,333 square hectometers, with a yield of 5,000,000 tons. This represents the largest increase in strawberry production among all crops [2]. However, the continuous expansion of strawberry planting areas has led to an increase in soil-borne diseases, such as strawberry wilt, a major disease in continuous cropping that may occur throughout the whole cultivation process, from seedling to harvest. Its incidence is about 20% in the main strawberry producing areas of China, and more than 80% in severe cases. This results in a significant decline in strawberry yield and quality. The growth and stability of the strawberry industry are hindered by the presence of *F. oxysoporum* f. sp. *Fragariae* (*Fof*), a soilborne pathogen that causes strawberry wilt [3]. It was first discovered in Australia in 1962 [4].

Under suitable environmental conditions, the *Fof* germinates and invades through root wounds or young roots of strawberries. It then propagates and grows in the vascular bundle of root tissue and stem, forming small conidia. The *Fof* moves and proliferates in the catheter, eventually blocking the vascular bundle and secreting a large amount of toxins. This can affect plant growth and even result in the death of the entire plant [5]. Currently, methods for controlling strawberry wilt include agricultural, physical, chemical, and biological means. Biological control involves the use of organisms or their metabolites to reduce harm to plants and is an effective and sustainable method for controlling plant diseases [4,6–10]. This control method has been widely accepted by farmers, aligning with the concept of environmentally friendly plant disease and pest control in China. It offers a new approach to disease control.

During the growth process, plants interact with some microorganisms in their roots. This relationship can confer stress tolerance or resistance on host plants. It is important to note that this interaction is not always beneficial and can have negative effects on plant growth [11]. *P. indica* is an endophytic fungus capable of colonizing mature root areas of many plants [12,13], with a wide range of hosts. It has been confirmed that *P. indica* can colonize over 200 plant species, including more than 30 families, such as grasses, legumes, solanaceae, umbelliferaceae, asteraceae, and cruciferae [14]. Numerous studies have demonstrated that *P. indica* can promote host plant growth, enhance stress resistance in unfavorable external environment, and improve disease resistance against pathogens [15–19]. Studies have shown that *P. indica* can increase resistance to *F. pseudograminearum* in wheat by inducing the phenylpropanoid pathway [15]. The colonization of *P. indica* in gerbera roots can enhance resistance to root rot by increasing antioxidant enzyme activity [20]. Cultivating *P. indica* can improve the content of K, Fe, soluble sugar, and titrable acidity in bananas infected by BBrMV [21]. Furthermore, it has been discovered that *P. indica*, which colonizes the roots, can enhance the resistance of dendrobium officinale against Cymbidium mosaic virus (CymMV) [22] and the resistance of tomato to *verticillium* wilt caused by *Verticillium dahliae* [23]. In the present study, Benihoppe strawberry plantlets were used as test materials to explore the mechanism by which *P. indica* induces strawberry resistance to Fusarium wilt. The study begins by testing for growth inhibition between *P. indica* and *Fof* on petri dishes. Following successful inoculation with *P. indica*, strawberry plantlets were then reinoculated with *Fof*, revealing that *P. indica* was able to induce resistance to *Fof*. The aim is to provide a theoretical basis for the effective prevention and control of strawberry Fusarium wilt in production.

2. Materials and Methods

2.1. Experimental Materials

In this study, daughter plantlets of 'Benihoppe', one of the most widely grown fresh strawberry varieties in China, were used as experimental materials. The *P. indica* used in the experiment was kept in the Institute of Horticultural Plant Bioengineering of Fujian Agriculture and Forestry University. The *Fof* was presented by the Institute of Plant Protection and Microbiology Zhejiang Academy of Agricultural Sciences.

2.2. Experimental Design

Suspensions of *P. indica* and *Fof* were prepared according to the methods of Cheng [24] and Song [25], respectively. The spore concentration was determined using a blood cell counting plate, and the final spore content was adjusted to 1×10^5 spores/mL and 1×10^7 spores/mL, respectively. Four groups were established in the experiment: non-colonization (CK), colonization with *P. indica* (P), inoculation with *Fof* (F) and colonization with *P. indica* followed by inoculation with *Fof* (PF). Strawberry plantlets of equal growth were selected for the experimental treatment. Each group consisted of 45 plants. For the colonization of *P. indica*: 100 mL of spore suspension was poured near the root of strawberry plantlets in P and PF. To increase the colonization percentage, the strawberry plants were irrigated with *P. indica* solution every third day a total of three times. One month after *P. indica* inoculation, roots of *P. indica* colonized and non-colonized plants were collected and the Varma method [26] was used to determine whether *P. indica* had successfully colonized the strawberry roots. Strawberry plantlets colonized with *P. indica* were divided into two groups, group P and group PF, which were inoculated with *Fof*. A total of 100 mL of *Fof* spore suspension was inoculated near the root soil of strawberry plantlets in F and PF. The remaining strawberry plantlets were watered with PDB solution diluted in the same ratio proportion, while *P. indica* and *Fof* strawberries were watered. Management was carried out at the Smart Agriculture Teaching Practice base at the Horticulture College of Fujian Agriculture and Forestry University. Phenotypes were observed and photographed 30 d after the onset of strawberry disease. At the same time, strawberry leaves and roots were collected, frozen in liquid nitrogen, and stored at −80 °C for later determination.

2.3. Dual Culture of *P. indica* and *Fof*

The inhibitory effect of *P. indica* on *Fof* growth was examined via the dual culture of *P. indica* and *Fof* on agar plates, following the method described by Cheng et al. [24]. With reference to the previous methods, four groups of experiments were carried out, as follows: (1) One or two *P. indica* plugs with 0.5 cm diameter and one or two *Fof* plugs of the same size were simultaneously inoculated at the opposite one-third positions of a PDA plate (Figure 1A,B). The plates were incubated at 28 °C in the dark. Photographs were taken at 1, 3, 5, and 9 days post co-cultivation. (2) One *Fof* plug with a diameter of 0.5 cm was inoculated for four days, followed by the placement of one *P. indica* plug at the opposite side of the plate for investigation of the inhibitory effect of *P. indica* on Fof growth (Figure 1C). The plates were incubated at 28 °C in the dark. Photographs were taken at 0, 1, 2, and 3 days after the *P. indica* plug was placed. (3) One plug of *P. indica* with a diameter of 0.5 cm was inoculated for four days. Following this, four *Fof* plugs were placed equidistantly around the *P. indica* plug (Figure 1D). Photographs were taken at 0, 2, 4, and 6 days after the *Fof* plug was placed. Three replicates were performed for each type of dual culture. PDA plates only inoculated with *P. indica* or *Fof* were used as controls.

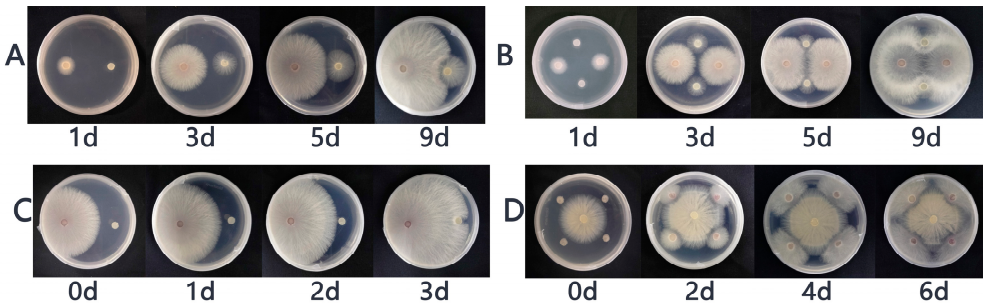

Figure 1. Antibiosis assay for antibiotic secretion and growth inhibition effects of *P. indica* and *Fof*. (**A**): one plug each of *P. indica* (right) and *Fof* (left) were placed on PDA a plate; (**B**): two plugs of *P. indica* (up and down) and *Fof* (left and right) were placed on PDA plate at equal distance; (**C**): one plug of *P. indica* (right) was placed beside *Fof* (left); (**D**): four plugs of *Fof* were placed around *P. indica* corner at equal distance.

2.4. Calculation of Disease Index

After 30 d of strawberry wilt inoculation, the incidence of strawberry wilt was observed and recorded; the disease classification standard referred to the method of Cao [27] and the disease index was calculated. Severity symptoms on individual leaves were rated on a scale of 0–4 according to the percentage of the area of decayed foliage according to Cao et al. (2016): 0: no yellow leaves; 1: less than 25% leaves yellow and withered; 2: 25–50% leaves yellow and withered; 3: 50–75% leaves yellow and withered; 4: more than 75% leaves yellow and withered. The severity of the disease affecting the whole plantlet was recorded as the disease index (*DI*), which was calculated as follows: $DI = [\sum(S_i \times X_i)/(S_{max} \times N)] \times 100$, where S_i is the severity rating, X_i is the number of strawberry leaves with the corresponding severity rating, S_{max} is the maximum value of disease grade ($S_{max} = 4$), and N is the total number of leaves on the investigated strawberry plant.

2.5. Determination of Strawberry Growth, and Physiological and Biochemical Indexes

The methods used to measure each index were as follows. Plant height was measured as the natural vertical distance from the base of the root of strawberry plantlets' root to its highest point. Leaf area was calculated by measuring the maximum width of the central lobule of the third outward spreading central leaf and multiplying it by the length from the depression of the raw petiole to the tip of the strawberry leaf, using the formula leaf area = length × width × 0.73 [28]. The stem diameter was measured using a vernier caliper at the surface of the root. The petiole length of the third leaf was measured with the center leaf spreading outward. The petiole diameter of the third leaf was measured using a vernier caliper with the center leaf spreading outward. The root length was measured from the base of the stem to the tip of the root. Physiological and biochemical indices, including SOD, POD, CAT, and MDA, were determined using the kit produced by Sangon Biotech (Shanghai, China) Co., Ltd., The instructions provided by the manufacturer were followed.

2.6. Statistical Analysis

All raw data and experiments are expressed as the means of three independent replicates. Microsoft Excel 2010 was used to statistically organize the experimental data, and IBM® SPSS® statistical software version 26.0 (IBM Corp., Armonk, NY, USA) was used to analyze the significance of differences. The experimental data between various treatments were analyzed by one-way analysis of variance (ANOVA) and Pearson's correlation tests at a threshold of $p \leq 0.05$, and GraphPad Prism 8.0.1 was used to draw the figures.

3. Results

3.1. The Results of the Plate Confrontation between P. indica and Fof

Figure 1A,B show the results of simultaneously inoculating one or two pieces of *P. indica* and *Fof* on the PDA plate. The growth rate of *Fof* was significantly faster than that of *P. indica*. On the 5th day, the two strains came into contact, and *Fof* invaded *P. indica*, inhibiting its growth and causing a gradual decrease in the colony area of *P. indica*. On the 4th day, *Fof* was inoculated at the edge of the *P. indica* colony while *Fof* was in a dominant position, resulting in the complete inhibition of *P. indica* growth (Figure 1C). Four *Fof* plugs were then inoculated equidistantly around *P. indica*, after it had grown for four days. *P. indica* growth was dominant, and *Fof* growth was inhibited to some extent. However, *Fof* was eventually able to invade *P. indica*, and there was no inhibition zone during the process (Figure 1D).

3.2. Detection of P. indica Colonization in the Roots of Strawberry Plantlets

After one month of inoculation with *P. indica*, 45 strawberry plantlets were selected for colonization by the fungus. Upon trypan blue staining, *P. indica* colonization was observed under a microscope. The chlamydospore was round, subpear-shaped, and oval

(Figure 2), indicating that *P. indica* successfully colonized strawberry roots with a 100% colonization rate.

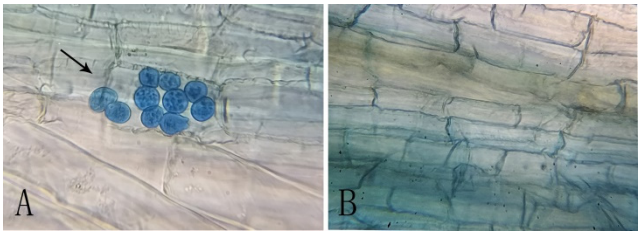

Figure 2. Roots stained with trypan blue (arrow show the chlamydospores of *P. indica*). (**A**): The roots of strawberry were colonized by *P. indica*; (**B**): control group strawberry root.

3.3. Calculation DI

The disease incidence (*DI*) reflects the degree of damage to the plant. Following inoculation of the strawberry plantlets with *Fof*, the investigation of the *DI* revealed that all strawberry plantlets in F had the disease, with a disease index of 63. In contrast, those in PF had an incidence of 80% and a *DI* of 46 (Table 1). The results showed that *P. indica* could improve the resistance of strawberry to *Fof*, and reduce the onset symptoms and the *DI*, and the control effect was 27% (Table 1).

Table 1. The disease rate and *DI* of strawberry inoculated with *Fof*.

Treatment	Disease Rate	*DI*	Efficiency
F	100%	63	-
PF	80%	46	27%

3.4. Effects of P. indica on Strawberry Phenotypes under Fof Stress

The colonization of *P. indica* by strawberry root had an impact on its phenotypic growth. The above-ground and root growth of strawberry in P were significantly better than that in CK (Figure 3A,B). After 30 days of inoculation with *Fof*, the typical symptoms of strawberry wilt were more pronounced in F, with leaf wilt, brown petioles, and weak plant growth (Figure 3A). The underground roots were also shorter and brown (Figure 3B). PF had milder symptoms and a better growth status. The stem base's longitudinal section revealed evident brown lesions in F, while PF's stem base grew well (Figure 3C). The results indicate that *P. indica* can alleviate strawberry wilt symptoms.

Figure 3. Effect of *P. indica* on plant phenotype of strawberry under *Fof* stress. (**A**): Shoot growth phenotype; (**B**): underground growth phenotype; (**C**): F and PF stem base contrast.

3.5. Effects of P. indica on Strawberry Growth under Fof Stress

Figure 4 shows the growth index results of strawberry plantlets after colonization by *P. indica* and *Fof*. The height of strawberry plantlets in treatment P was 1.07 times higher than that in CK (Figure 4A). In contrast, the plant height of strawberry plantlets in treatment F was significantly lower than in CK, measuring only 92% of the height in CK. However, the height of strawberry plantlets in treatment PF was significantly higher than that in F,

measuring 1.09 times the height in F. There was no significant difference in plantlet height between PF and CK treatment.

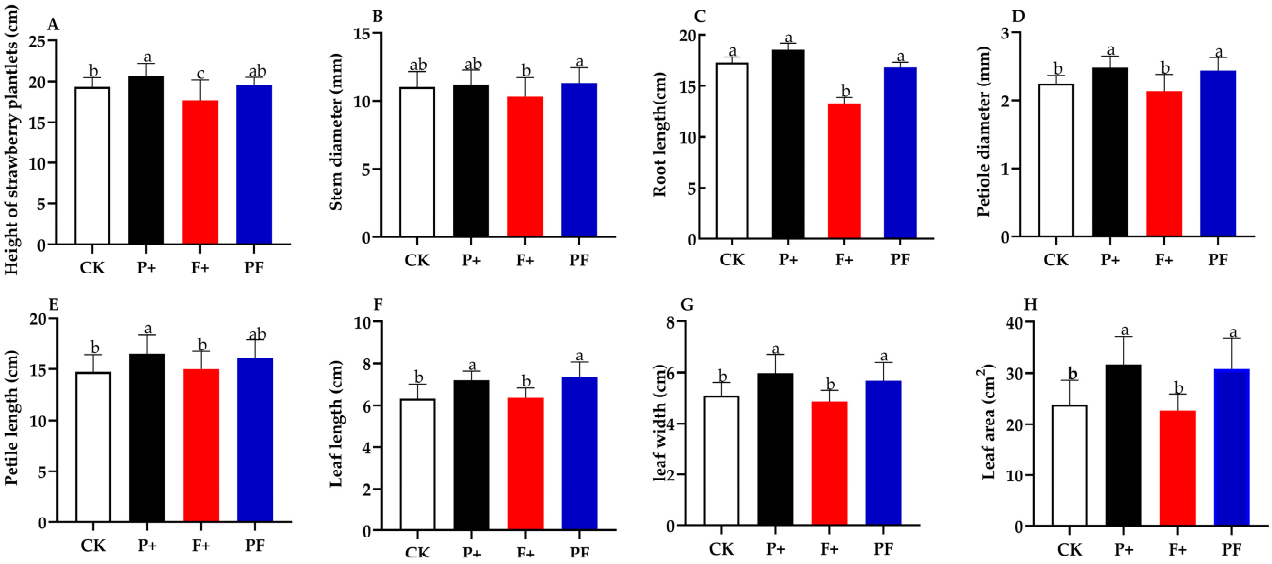

Figure 4. Effect of *P. indica* treatment on growth characteristics of strawberry plantlets under *Fof* stress. (**A**): height; (**B**): stem diameter; (**C**): root length; (**D**): petiole diameter; (**E**): petile length; (**F**): leaf length; (**G**): leaf width; (**H**): leaf area. Data are represented as means ± standard error from $n = 15$ replicates, and different letters denote statistical variation (one-way ANOVA, $p < 0.05$).

The stem diameter and root length of strawberries in group P did not differ significantly from those in group CK (Figure 4B,C). However, in group F, the stem diameter and root length of strawberries were significantly lower than those in group CK, measuring only 94% and 77% of those in CK, respectively. In contrast, in group PF, the stem diameter and root length of strawberries in PF were significantly higher than those in F, measuring 1.10 and 1.26 times those in group F, respectively. Nevertheless, there was no significant difference in stem diameter and root length between group PF and group CK.

The petiole diameter and petiole length of strawberries in treatment P were significantly higher than those in the control group (CK), by 1.10 and 1.12 times (Figure 4D,E), respectively. In treatment F, there were no significant differences in the petiole diameter and petiole length of strawberries compared to the control group. However, in treatment PF, the petiole diameter and petiole length of strawberries were significantly higher than those in treatment F, by 1.12 and 1.06 times, respectively. There was no significant difference in petiole length between PF and CK. However, the petiole diameter of PF was significantly higher than those of CK, being 1.06 times greater.

Figure 4F,G show that inoculation with *P. indica* significantly increased the length and width of strawberry leaves. The leaf area of strawberries in treatment P was significantly higher than those in the control group (CK), by 1.33 (Figure 4H). In treatment F, there were no significant differences in the leaf area of strawberries compared to the control group. However, in treatment PF, the leaf area was significantly higher than those in treatment F, by 1.35 times. However, the leaf area of PF was significantly higher than those of CK, being 1.29 times greater.

3.6. Effects of P. indica on the Antioxidant Oxidase Activity of Strawberry under Fof Stress

SOD is a metal enzyme that is widely present in organisms. It plays a crucial role in scavenging oxygen free radicals by catalyzing the disproportionation of superoxide anions to produce H_2O_2 and O_2. SOD is not only a superoxide anion-scavenging enzyme but also the main enzyme responsible for producing H_2O_2, which is an important component of the biological antioxidant system. The SOD activity in strawberry leaves was 1.14 times higher in P than in CK. The SOD activity of strawberry leaves in treatment F was significantly

lower than that in the control group (CK), with a decrease of 10%. In contrast, the SOD activity in strawberry leaves of treatment PF was significantly higher than that of treatment F, with an increase of 1.10 times, but no significant difference was found between treatment PF and CK (Figure 5A). The SOD activity of strawberry roots in treatment P was not significantly different from that in CK. However, after inoculation with *Fof*, the SOD activity of strawberry roots in treatment F was significantly lower than that in CK, with a decrease of 26%. The SOD activity in the roots of PF was 1.13 times higher than that of F, reaching a significant level (Figure 5B).

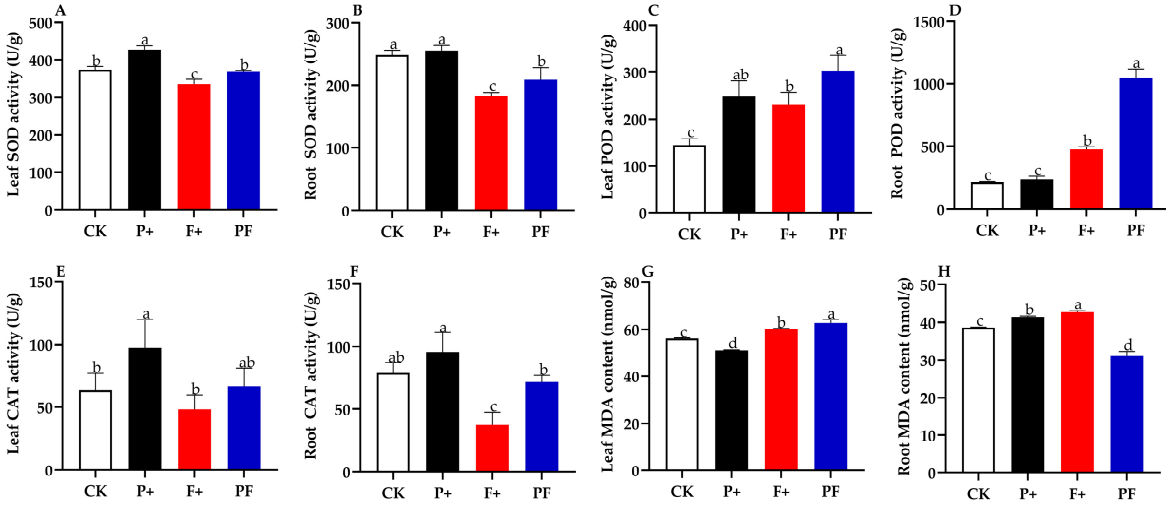

Figure 5. Effects of *P. indica* inoculation on the SOD, POD and CAT activities, and MDA content of strawberry roots and leaves under *Fof* stress. (**A**): leaf superoxide dismutase activity; (**B**): root superoxide dismutase activity; (**C**): leaf peroxidase activity; (**D**): root peroxidase activity; (**E**): leaf catalase activity; (**F**): root catalase activity; (**G**): malondialdehyde content in leaf; (**H**): malondialdehyde content in root. Data are represented as means ± standard error from n = 3 replicates, and different letters denote statistical variation (one-way ANOVA, $p < 0.05$).

POD is a widely distributed enzyme found in animals, plants, microorganisms, and cultured cells. Its function is to catalyze the oxidation of phenols and amines by hydrogen peroxide, while also eliminating their toxicity. The results of the determination of POD activity on strawberry roots and leaves are shown in Figure 5C,D. The POD activity of strawberry leaves in P, F, and PF was significantly higher than that in CK, with increases of 1.73, 1.60, and 2.10 times, respectively. There was no significant difference in the POD activity of strawberry leaves between the P and PF treatments. However, the POD activity of strawberry leaves in PF was 1.32 times higher than that in F, which was statistically significant (Figure 5C). The POD activity of strawberry roots in treatment P was slightly higher than that in CK, while the POD activity of strawberry roots in treatment F was 2.22 times higher than that in CK. The POD activity of strawberry roots in the PF group was significantly higher than that in the other three groups. Specifically, it was 4.89 times higher than in the CK group, 4.38 times higher than in the P group, and 2.20 times higher than in the F group (Figure 5D).

CAT is a ubiquitous enzyme found in animals, plants, microorganisms, and cultured cells. It plays a crucial role in scavenging hydrogen peroxide (H_2O_2) and is an essential component of the active oxygen-scavenging system. The CAT activity in strawberry leaves was 1.53 times higher in P than in CK. However, the CAT activity in strawberry leaves in F did not reach a significant level compared to CK. Additionally, the CAT activity in strawberry leaves in PF and other groups did not reach a significant difference (Figure 5E). There was no significant difference in CAT activity between P and CK. However, the CAT activity of strawberry roots in treatment group F was significantly reduced, measuring only 48% of that in CK, and 1.89 times that in F, reaching a significant level (Figure 5F).

3.7. Effects of P. indica on the MDA Content of Strawberry under Fof Stress

Oxygen radicals react with the unsaturated fatty acids in lipids, resulting in the formation of lipid peroxides. These peroxides then break down into various compounds, including MDA. The level of lipid oxidation can be determined by measuring the amount of MDA that is present. Figure 5G,H illustrates significant differences in MDA content between the roots and leaves of the strawberry plantlet under different treatments. The MDA content in the leaves of P was decreased by 8.7% compared to CK. In contrast, the MDA content in the leaves of F was 1.07 times higher than the CK. The MDA content in the leaves of PF was significantly higher than that of CK and F, at 1.12 and 1.04 times, respectively. The MDA content in the roots of strawberry in P and F was significantly higher than that in CK, by 1.10 and 1.07 times, respectively. In contrast, the MDA content in the roots of strawberry in PF was significantly lower than that in CK and F, by 19% and 27%, respectively.

4. Discussion

The experiment revealed that the growth rate of *Fof* was significantly faster in the experiment between *P. indica* and *Fof*. Inoculating *P. indica* first slowed down the growth rate of *Fof* and inhibited it to some extent, but *Fof* still managed to invade. Conversely, when *Fof* was inoculated first, it completely inhibited the growth of *P. indica* and even overtook it. These results suggest that *P. indica* does not have significant antagonism towards *Fof* on the plate. Although *P. indica* does not have an inhibitory effect on *Fof* on the plate, previous studies have found that the enhanced resistance of plant roots colonized by *P. indica* to pathogens is not directly caused by *P. indica* [20]. Research has shown that *P. indica* does not have a significant antagonistic effect on banana wilt on the plate. The enhancement of banana's resistance to wilt may be achieved by inducing the banana to produce systematic disease resistance and enhancing its antioxidant enzyme activity.

In the dual culture of *P. indica*, the growth of banana wilt on the plate was not inhibited [24]. When grown in dual culture with *P. indica* and *Phytophthora cinnamomum* and *P. plurivora* on PDA medium, no inhibition zone was observed [29]. Although *P. indica* does not have a direct antagonistic effect on *P. cryptogea*, it can reduce the harm of root rot by increasing the SOD, CAT, and POD activities of gerbera and reducing the accumulation of H_2O_2, MDA, and Pro [20].Therefore, in this experiment, it was observed that *P. indica* did not have a significant inhibitory effect on *Fof* growth on the plate. The resistance of *P. indica* to *Fof* may be attributed to the enhanced activity of antioxidant enzymes and the reduced content of MDA.

Plants infected with the wilt fungus exhibit above-ground symptoms, including stunted growth, wilting leaves, crumpled leaf margins, and brown petioles. The symptoms of strawberry Fusarium wilt in this experiment were consistent with those mentioned above. *P. indica* is a beneficial fungus that promotes plant growth, induces plant resistance to biological stress, and reduces infection symptoms. The colonization of *P. indica* has been shown to reduce symptoms caused by banana bract mosaic virus (BBrMV) infection [21]. Inducing the phenylpropane pathway under the colonization of *P. indica* could improve the resistance of wheat seedlings to *F. pseudograminis*. The phenotype was less affected by Fusarium crown rot infection [15]. In this experiment, the phenotype of *P. indica* significantly reduced the severity of strawberry Fusarium wilt infestation, which is consistent with the results. In this research, the growth indexes of strawberry, including plant height, petiole length, leaf area, and root length were significantly higher in the P treatment compared to the CK treatment. This resulted in an improvement in the biomass of the strawberry plantlets and a noticeable growth promotion effect. These findings are consistent with a previous study that showed that *P. indica* can significantly increase the biomass of longan [30]. The plant height, stem diameter, leaf area, petiole length, and root length of strawberries in PF were significantly greater than those in F. This improved the promotion effect of *P. indica* on strawberry growth under *Fof* stress. Previous studies have shown that the colonization of *P. indica* can promote the uptake of phosphorus by

cyclamen [31] and anthurium [32]. Additionally, root colonization of *P. indica* can increase wheat yield, biomass and phosphorus content under both phosphorus deficiency and abundance conditions [33]. The application of *P. indica* significantly increased the nitrogen, phosphorus, and potassium contents of Zrnmgo plants [34]. The colonization of *P. indica* can significantly increase the activity of nitrate reductase in tobacco and Arabidopsis, and promote the nitrogen absorption of plants [35]. Therefore, it is speculated that the colonization of *P. indica* in strawberry roots establishes a mutualistic symbiosis with the host, resulting in increased root length and improved absorption of more mineral elements by the plant roots. This, in turn, promotes an increase in strawberry biomass. Research has demonstrated that the root system of strawberry plants becomes 25% shorter after *P. indica* inoculation [36]. However, the present study did not find a significant change in root length. On the other hand, the inoculation of *P. indica* followed by *Fof* resulted in both a significantly greater number of roots and longer root lengths compared to no *P. indica* inoculation. The experiment showed that the plant growth index of strawberries infected by *Fof* decreased compared to CK. However, the growth index of strawberries in PF was significantly higher than that in F, and the disease symptoms were mild. These results suggest that *P. indica* could alleviate the damage caused by *Fof* to strawberry growth to some extent.

Enzyme activity is a crucial indicator for measuring plant damage under stress, as it directly reflects the physiological and biochemical changes in plants. In times of adversity, the balance of reactive oxygen species (ROS) is disrupted, and a significant accumulation of ROS can harm plants [37]. POD, SOD, and CAT are the three main enzymes in the plant antioxidant enzyme system. When plants are under pathogen-induced stress, the antioxidant enzyme system will indirectly contribute to the plantlet's disease resistance response by eliminating reactive oxygen species. SOD is the primary active oxygen scavenger in plants under stress. It catalyzes the disproportionation reaction between active oxygen and free radicals, producing H_2O_2. POD and CAT enzymes can decompose H_2O_2 into H_2O and O_2, reducing the toxic effect of H_2O_2, and maintaining the REDOX balance of plant cells [38].

Currently, numerous studies have demonstrated that *P. indica* can enhance the disease resistance of host plants by improving their antioxidant activity. For instance, *P. indica* has been found to safeguard barley roots against the antioxidant oxidase loss caused by *F. culmorum* [19]. Additionally, the colonization of *P. indica* on onion has been shown to protect against leaf blight caused by *Stemphylium vesicarium,* increasing the activity of SOD, POD, and other enzymes [39]. The colonization of *P. indica* increased the activities of CAT and SOD enzymes in maize roots. Additionally, it may inhibit the colonization of *F. verticillium* [40]. *P. indica* can promote the growth of economically important chickpea plants and protects them against the pathogenic fungus *Botrytis cinerea* by improving the antioxidant system [41]. In rice, the colonization of *P. indica* increased SOD activity, delayed the infection process of sheath blight, and alleviated the symptoms caused by sheath blight [42].

The results of this study indicate that the colonization of *P. indica* can enhance the activities of POD, CAT, and SOD in strawberry leaves. However, there was no significant difference observed between the treatment and control groups in the root system. Additionally, *Fof* inoculation induced the POD activity of strawberry root and leaf cells to a certain extent, which is consistent with the study results of POD activity changes in the root system during the interaction between banana and wilt [24]. The colonization of *P. indica* led to an increase in the activities of SOD and POD in strawberries. The increase in POD activity in strawberry plants of F and PF indicated that the immune response induced by *Fof* infection was activated, thus alleviating the damage caused by *Fof*. The POD activity in PF was significantly higher than that in F, indicating that the strawberry roots colonized by *P. indica* had a stronger response to infection. This suggests that *P. indica* enhances the host's defense response. The changes in enzyme activity in plants help to balance reactive oxygen species and reduce damage to plant cells [43]. This is consistent with previous studies that have shown *P. indica* can improve disease-resistant enzyme activity in gerbera [20]

and banana [44]. Therefore, we hypothesize that the colonization of *P. indica* increases the activity of SOD, CAT, and POD, enhances the defense response of strawberry, and reduces the severity of infection by *Fof*.

MDA is a crucial indicator for determining the extent of membrane lipid peroxidation caused by stress in plants. Its content is frequently used as a marker to measure lipid peroxidation [45]. The study found that the roots and leaves of strawberry infected by *Fof* had a significantly higher MDA content than the control group. This suggests that the plant was under disease stress, which led to the destruction of the antioxidant defense system of cells, an increase in the content of reactive oxygen species, intensified membrane lipid peroxidation, destruction of the plasma membrane, and reduced stability. As a result, a large amount of MDA was accumulated. This was consistent with the change in MDA content in wheat leaves against root rot [46]. Additionally, the content of MDA in strawberry leaves colonized with *P. indica* was significantly lower compared to other groups. In this experiment, the MDA content of strawberry roots in PF was significantly reduced compared to other groups. This suggests that *P. indica* improved the antioxidant defense system of strawberry, reduced the content of reactive oxygen species, alleviated the peroxidation of membrane lipids, and improved the membrane stability. As a result, the resistance of strawberry to Fusarium wilt caused by *Fof* was improved.

The effects of *P. indica* on enhancing resistance to Fusarium wilt in strawberries, as outlined in the present study, require a critical examination of potential limitations and alternative explanations to ensure a comprehensive interpretation of the findings. Environmental conditions, a known determinant of plant–pathogen interactions, could significantly influence the outcome of the *P. indica-Fusarium oxysporum* f. sp. *Fragariae* (*Fof*) interaction. Variations in temperature, humidity, and soil composition can affect the symbiotic relationship between the endophytic fungus and the host plant, which may influence the observed changes in growth indices and antioxidant enzyme activities. It is also important to consider the specificity of strawberry cultivars in responding to *P. indica* and *Fof*, as different cultivars may exhibit distinct molecular and physiological responses. Furthermore, valuable insights could be gained by drawing parallels with studies on soil microbiological populations and Fusarium incidence in bananas in Colombia [47,48] and Venezuela [49,50]. It is important to understand the broader ecological context, particularly the dynamics of soil microbial communities, as this may reveal additional factors contributing to Fusarium resistance or susceptibility in strawberry plants. Therefore, to refine our understanding of the complex interplay between endophytes, pathogens, and environmental factors in plant disease resistance, future investigations should incorporate a more nuanced consideration of these variables.

5. Conclusions

This present study investigated the positive effects of *P. indica* on the growth of the daughter plants of 'Benihoppe' strawberry (*Fragaria* × *ananassa* Duch.) under *Fof* stress. Although the dual culture of *P. indica* and *Fof* did not show direct resistance between the two on the plates, strawberry plantlets inoculated with *P. indica* prior to *Fof* showed a better growth than those inoculated with only *Fof*. The symbiotic relationship between *P. indica* and strawberries can enhance the growth of strawberry plantlets under the stress of *Fof*, increase biomass, and reduce the symptoms of strawberry disease, with a control effect of 27%. The colonization of *P. indica* primarily stimulates the increase of SOD, POD, and CAT activities in strawberry leaves and roots, effectively alleviating the damage caused by superoxide anions on leaf and root cells, and reducing the content of MDA. This improves the resistance of strawberry to *Fof*. The results obtained in this study indicated that the beneficial effect of *P. indica* colonization on the *Fof* resistance of strawberry might be achieved, at least partly, through the regulation of antioxidant enzyme activities and MDA content in the roots.

Author Contributions: Conceptualization, Y.H. and L.M.; data curation, J.L.; formal analysis, J.L.; funding acquisition, Y.H. and L.M.; investigation, J.L.; methodology, J.L.; project administration, Z.L. and L.M.; resources, L.F. and Y.C.; software, C.N. and T.L.; supervision, Y.H. and L.M.; validation, Y.H. and J.L.; visualization, X.F. and Y.L.; writing—original draft, Y.H., J.L. and L.M.; writing—review and editing, Y.H. and L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation of Fujian Province (2023J01450), Training Funds for Core Young Scholar of Horticulture College, Fujian Agriculture and Forestry University (722022011), the Science and Technology Innovation Special Fund of Fujian Agriculture and Forestry University (KFb22027XA and CXZX2020026A), the National Natural Science Foundation of China (31701900), and Fujian Province Plateau Discipline Construction Fund Project (102/71201801101).

Data Availability Statement: Data supporting reported results can be requested by contacting the corresponding author. The data are not publicly available due to compliance with data protection regulations.

Acknowledgments: We gratefully acknowledge Yongchao Han (Institute of Industrial Crops, Hubei Academy of Agricultural Sciences, China) for his technical assistance.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Xu, C.; Zhang, X.; Liang, J.; Fu, Y.; Wang, J.; Jiang, M.; Pan, L. Cell wall and reactive oxygen metabolism responses of strawberry fruit during storage to low voltage electrostatic field treatment. *Postharvest Biol. Technol.* **2022**, *192*, 112017. [CrossRef]
2. Zhang, Y.; Lei, J.; Zhao, M.; Zhang, Y.; Wang, G.; Zhong, C.; Chang, L.; Ning, Z.; Sun, R.; Wang, B.; et al. Fruit scientific research in new China in the past 70 years: Strawberry. *J. Fruit Sci.* **2019**, *36*, 1441–1452.
3. Dilla-Ermita, C.J.; Goldman, P.H.; Jaime, J.H.; Ramos, G.; Pennerman, K.K.; Henry, P.M. First report of *Fusarium oxysporum* f. sp. *fragariae* race 2 causing fusarium wilt of strawberry (*Fragaria* × *ananassa*) in California. *Plant Dis.* **2023**, *107*, 2579–2897. [CrossRef] [PubMed]
4. Koike, S.T.; Gordon, T.R. Management of fusarium wilt of strawberry. *Crop Prot.* **2015**, *73*, 67–72. [CrossRef]
5. Nam, M.; Kang, Y.; Lee, I.; Kim, H.; Chun, C. Infection of daughter plants by *Fusarium oxysporum* f. sp. *fragariae* through runner propagation of strawberry. *Hortic. Sci. Technol.* **2011**, *29*, 273–277.
6. Wang, Y.; Zhao, X.; Yin, B.; Zhen, W.; Guo, J. BiochemicaL defenses induced by mycorrhizae fungi glomus mosseae in controlling strawberry fusarium wilt. *Open Biomed. Eng. J.* **2015**, *9*, 301–304. [CrossRef]
7. Subbarao, K.V.; Kabir, Z.; Martin, F.N.; Koike, S.T. Management of soilborne diseases in strawberry using vegetable rotations. *Plant Dis.* **2007**, *91*, 964–972. [CrossRef] [PubMed]
8. Njoroge, S.M.C.; Kabir, Z.; Martin, F.N.; Koike, S.T.; Subbarao, K.V. Comparison of crop rotation for verticillium wilt management and effect on pythium species in conventional and organic strawberry production. *Plant Dis.* **2009**, *93*, 519–527. [CrossRef]
9. Hernández-Muñiz, P.; Borrero, C.; Ordóñez-Martín, J.; Pastrana, A.M.; Avilés, M. Optimization of the use of industrial wastes in anaerobic soil disinfestation for the control of fusarium wilt in strawberry. *Plants* **2023**, *12*, 3185. [CrossRef]
10. Chen, Y.; Xu, Y.; Zhou, T.; Akkaya, M.S.; Wang, L.; Li, S.; Li, X. Biocontrol of fusarium wilt disease in strawberries using bioorganic fertilizer fortified with *Bacillus licheniformis* X-1 and *Bacillus methylotrophicus* Z-1. *3 Biotech* **2020**, *10*, 80. [CrossRef]
11. Li, D.; Bodjrenou, D.M.; Zhang, S.; Wang, B.; Pan, H.; Yeh, K.-W.; Lai, Z.; Cheng, C. The endophytic fungus *Piriformospora indica* reprograms banana to cold resistance. *Int. J. Mol. Sci.* **2021**, *22*, 4973. [CrossRef] [PubMed]
12. Waller, F.; Achatz, B.; Baltruschat, H.; Fodor, J.; Becker, K.; Fischer, M.; Heier, T.; Hückelhoven, R.; Neumann, C.; von Wettstein, D.; et al. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13386–13391. [CrossRef] [PubMed]
13. Rai, M.; Varma, A. Arbuscular mycorrhiza-like biotechnological potential of *Piriformospora indica*, which promotes the growth of *Adhatoda vasica* Nees. *Electron. J. Biotechnol.* **2005**, *8*, 107–112. [CrossRef]
14. Mensah, R.A.; Li, D.; Liu, F.; Tian, N.; Sun, X.; Hao, X.; Lai, Z.; Cheng, C. Versatile *Piriformospora indica* and its potential applications in horticultural crops. *Hortic. Plant J.* **2020**, *6*, 111–121. [CrossRef]
15. Li, L.; Hao, R.; Yang, X.; Feng, Y.; Bi, Z. *Piriformospora indica* increases resistance to *Fusarium pseudograminearum* in wheat by inducing phenylpropanoid pathway. *Int. J. Mol. Sci.* **2023**, *24*, 8797. [CrossRef]
16. Li, L.; Guo, N.; Feng, Y.; Duan, M.; Li, C. Effect of *Piriformospora indica*-induced systemic resistance and basal immunity against *Rhizoctonia cerealis* and *Fusarium graminearum* in wheat. *Front. Plant Sci.* **2022**, *13*, 836940. [CrossRef] [PubMed]
17. Rabiey, M.; Shaw, M.W. *Piriformospora indica* reduces fusarium head blight disease severity and mycotoxin DON contamination in wheat under UK weather conditions. *Plant Pathol.* **2016**, *65*, 940–952. [CrossRef]
18. Rabiey, M.; Ullah, I.; Shaw, M.W. The endophytic fungus *Piriformospora indica* protects wheat from fusarium crown rot disease in simulated UK autumn conditions. *Plant Pathol.* **2015**, *64*, 1029–1040. [CrossRef]

19. Harrach, B.D.; Baltruschat, H.; Barna, B.; Fodor, J.; Kogel, K.-H. The mutualistic fungus *Piriformospora indica* protects barley roots from a loss of antioxidant capacity caused by the necrotrophic pathogen *Fusarium culmorum*. *Mol. Plant-Microbe Interact.* **2013**, *26*, 599–605. [CrossRef]
20. Wu, H.; Wang, B.; Hao, X.; Zhang, Y.; Wang, T.; Lu, Z.; Lai, Z.; Cheng, C. *Piriformospora indica* promotes the growth and enhances the root rot disease resistance of gerbera. *Sci. Hortic.* **2022**, *297*, 110946. [CrossRef]
21. Sinijadas, K.; Amitha, P.; Radhika, N.S.; Manju, R.V.; Joy Michal, J. Beneficial fungal root endophyte *Piriformospora indica* diminishes yield loss without compromising quality of banana fruits due to banana bract mosaic virus infection through better soil nutrient mobilization. *Int. J. Plant Soil Sci.* **2023**, *35*, 1397–1415. [CrossRef]
22. Safeer, M.M.; Susha, S.T. Integrated management of Cymbidium mosaic disease in commercial Dendrobium *orchids* using root endophytic fungi *Piriformospora indica*. *Cogent Food Agric.* **2022**, *8*, 2139848. [CrossRef]
23. Fakhro, A.; Andrade-Linares, D.R.; von Bargen, S.; Bandte, M.; Buettner, C.; Grosch, R.; Schwarz, D.; Franken, P. Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. *Mycorrhiza* **2010**, *20*, 191–200. [CrossRef]
24. Cheng, C.; Li, D.; Qi, Q.; Sun, X.; Anue, M.R.; David, B.M.; Zhang, Y.; Hao, X.; Zhang, Z.; Lai, Z. The root endophytic fungus *Serendipita indica* improves resistance of banana to *Fusarium oxysporum* f. sp. *cubense* tropical race 4. *Eur. J. Plant Pathol.* **2020**, *156*, 87–100. [CrossRef]
25. Song, X.; Mei, F.z.; LI, X.; Zhu, Y.; Shi, F.; Liu, J. Screening and control effect of antagonistic bacteria against strawberry fusarium wilt. *Chin. J. Biol. Control* **2022**, *38*, 653–661.
26. Varma, A.; Verma, S.; Sudha; Sahay, N.; Bütehorn, B.; Franken, P. *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Appl. Environ. Microbiol.* **1999**, *65*, 2741–2744. [CrossRef]
27. Cao, Z.; Fan, T.; Bi, Y.; Tian, G.; Zhang, L. Potassium deficiency and root exudates reduce root growth and increase *Fusarium oxysporum* growth and disease incidence in continuously cultivated strawberry. *N. Z. J. Crop Hortic. Sci.* **2016**, *44*, 58–68. [CrossRef]
28. Zhang, X.; Zhou, Z.; Li, X.; Li, H. The effect of potassium treatment on the growth and fruit quality of 'Yanli' strawberry. *China Fruits* **2022**, *222*, 29–33.
29. Trzewik, A.; Maciorowski, R.; Klocke, E.; Orlikowska, T. The influence of *Piriformospora indica* on the resistance of two rhododendron cultivars to *Phytophthora cinnamomi* and *P. plurivora*. *Biol. Control* **2020**, *140*, 104121. [CrossRef]
30. Cheng, C.; Li, D.; Wang, B.; Liao, B.; Qu, P.; Liu, W.; Zhang, Y.; Lü, P. *Piriformospora indica* colonization promotes the root growth of *Dimocarpus longan* seedlings. *Sci. Hortic.* **2022**, *301*, 111137. [CrossRef]
31. Ghanem, G.; Ewald, A.; Zerche, S.; Hennig, F. Effect of root colonization with *Piriformospora indica* and phosphate availability on the growth and reproductive biology of a *Cyclamen persicum* cultivar. *Sci. Hortic.* **2014**, *172*, 233–241. [CrossRef]
32. Lin, H.; Xiong, J.; Zhou, H.; Chen, C.; Lin, F.; Xu, X.; Oelmueller, R.; Xu, W.; Yeh, K. Growth promotion and disease resistance induced in *Anthurium* colonized by the beneficial root endophyte *Piriformospora indica*. *BMC Plant Biol.* **2019**, *19*, 40. [CrossRef]
33. Taghinasab, M.; Imani, J.; Steffens, D.; Glaeser, S.P.; Kogel, K.-H. The root endophytes *Trametes versicolor* and *Piriformospora indica* increase grain yield and P content in wheat. *Plant Soil* **2018**, *426*, 339–348. [CrossRef]
34. Kumar, V.; Sarma, M.V.R.K.; Saharan, K.; Srivastava, R.; Kumar, L.; Sahai, V.; Bisaria, V.S.; Sharma, A.K. Effect of formulated root endophytic fungus *Piriformospora indica* and plant growth promoting rhizobacteria fluorescent pseudomonads R62 and R81 on *Vigna mungo*. *World J. Microbiol. Biotechnol.* **2012**, *28*, 595–603. [CrossRef] [PubMed]
35. Sherameti, I.; Shahollari, B.; Venus, Y.; Altschmied, L.; Varma, A.; Oelmüller, R. The endophytic fungus *Piriformospora* indica stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. *J. Biol. Chem.* **2005**, *280*, 26241–26247. [CrossRef]
36. Liu, W.; Tan, M.; Qu, P.; Huo, C.; Liang, W.; Li, R.; Jia, Y.; Fan, X.; Cheng, C. Use of *Piriformospora indica* to promote growth of strawberry daughter plants. *Horticulturae* **2022**, *8*, 370. [CrossRef]
37. Parida, A.K.; Das, A.B.; Mohanty, P. Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: Differential changes of isoforms of some antioxidative enzymes. *J. Plant Physiol.* **2004**, *161*, 531–542. [CrossRef] [PubMed]
38. Khaleghi, A.; Naderi, R.; Brunetti, C.; Maserti, B.E.; Salami, S.A.; Babalar, M. Morphological, physiochemical and antioxidant responses of *Maclura pomifera* to drought stress. *Sci. Rep.* **2019**, *9*, 19250. [CrossRef]
39. Roylawar, P.; Khandagale, K.; Randive, P.; Shinde, B.; Murumkar, C.; Ade, A.; Singh, M.; Gawande, S.; Morelli, M. *Piriformospora indica* primes onion response against stemphylium leaf blight disease. *Pathogens* **2021**, *10*, 1085. [CrossRef] [PubMed]
40. Kumar, M.; Yadav, V.; Tuteja, N.; Johri, A.K. Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. *Microbiology* **2009**, *155*, 780–790. [CrossRef] [PubMed]
41. Narayan, O.P.; Verma, N.; Singh, A.K.; Oelmüller, R.; Kumar, M.; Prasad, D.; Kapoor, R.; Dua, M.; Johri, A.K. Antioxidant enzymes in chickpea colonized by *Piriformospora indica* participate in defense against the pathogen *Botrytis cinerea*. *Sci. Rep.* **2017**, *7*, 13553. [CrossRef] [PubMed]
42. Nassimi, Z.; Taheri, P. Endophytic fungus *Piriformospora indica* induced systemic resistance against rice sheath blight via affecting hydrogen peroxide and antioxidants. *Biocontrol Sci. Technol.* **2017**, *27*, 252–267. [CrossRef]
43. Sun, C.; Johnson, J.; Cai, D.; Sherameti, I.; Oelmueller, R.; Lou, B. *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *J. Plant Physiol.* **2010**, *167*, 1009–1017. [CrossRef]

44. Dan, L.; Mensah, R.A.; Fan, L.; Na, T.; Quan, Q.; Yeha, K.; Xu, X.; Chen, C.; Lai, Z. Effects of *Piriformospora indica* on rooting and growth of tissue-cultured banana (*Musa acuminata* cv. Tianbaojiao) seedlings. *Sci. Hortic.* **2019**, *257*, 108649. [CrossRef]
45. Yaghoubian, Y.; Goltapeh, E.M.; Pirdashti, H.; Esfandiari, E.; Feiziasl, V.; Dolatabadi, H.K.; Varma, A.; Hassim, M.H. Effect of *Glomus mosseae* and *Piriformospora indica* on growth and antioxidant defense responses of wheat plants under drought stress. *Agric. Res.* **2014**, *3*, 239–245. [CrossRef]
46. Hao, R.; Li, L.; Yang, X.; Ma, H.; Shi, S.; Feng, Y. Endophytic fungus *Piriformospora indica* induces wheats against root rot. *Acta Microbiol. Sin.* **2023**, *63*, 3292–3309. [CrossRef]
47. Rodríguez-Yzquierdo, G.; Olivares, B.O.; González-Ulloa, A.; León-Pacheco, R.; Gómez-Correa, J.C.; Yacomelo-Hernández, M.; Carrascal-Pérez, F.; Florez-Cordero, E.; Soto-Suárez, M.; Dita, M.; et al. Soil predisposing factors to *Fusarium oxysporum* f.sp *Cubense* tropical race 4 on banana crops of La Guajira, Colombia. *Agronomy* **2023**, *13*, 2588. [CrossRef]
48. Rodríguez-Yzquierdo, G.; Olivares, B.O.; Silva-Escobar, O.; González-Ulloa, A.; Soto-Suarez, M.; Betancourt-Vásquez, M. Mapping of the susceptibility of colombian musaceae lands to a deadly disease: *Fusarium oxysporum* f. sp. *cubense* tropical race 4. *Horticulturae* **2023**, *9*, 757. [CrossRef]
49. Mejías Herrera, R.; Hernández, Y.; Magdama, F.; Mostert, D.; Bothma, S.; Paredes Salgado, E.M.; Terán, D.; González, E.; Angulo, R.; Angel, L.; et al. First Report of fusarium wilt of cavendish bananas caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in Venezuela. *Plant Dis.* **2023**, *107*, 3297. [CrossRef]
50. Olivares Campos, B.O. Evaluation of the incidence of banana wilt and its relationship with soil properties. In *Banana Production in Venezuela: Novel Solutions to Productivity and Plant Health*; Olivares Campos, B.O., Ed.; Springer Nature: Cham, Switzerland, 2023; pp. 95–117. [CrossRef]