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Article

The Mechanism behind Arbuscular Mycorrhizal Fungi Aiding Growth and Development and Inhibiting Root Rot in *Lycium barbarum*

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Abstracts: To investigate the potential role of arbuscular mycorrhizal fungi (AMF) in the resistance of *Lycium barbarum* to disease stress, two types of AMF, *Funneliformis mosseae* and *Rhizophagus intraradices*, were selected as materials. *Lycium barbarum* was inoculated with AMF and pathogenic bacteria in potting soil under controlled conditions, and we analyzed the antioxidant capacity of the fungi against root rot and changes in disease-process-related protein activities. In addition, we performed transcriptome analysis to explore the physiological and molecular changes in AMF in the prevention and control of root rot in *L. barbarum* 'Ningqi No.1' cultivar. The results show that AMF can promote the growth and development of *L. barbarum* plants while also increasing antioxidant enzyme and disease-resistant protease activity. The 'Ningqi No.1'-AMF symbiont triggered several key biological pathways, including the peroxisomal signaling pathway, after *F. oxysporum* infestation. In conclusion, AMF can prevent root rot in *L. barbarum*, providing valuable evidence that AMF symbiotically improves the ability of *L. barbarum* to resist root rot through its molecular mechanism.

Keywords: root rot in *Lycium barbarum*; arbuscular mycorrhizal fungi; biological control; transcriptomics

1. Introduction

Lycium is a Solanaceae plant and a perennial deciduous shrub [1,2]. There are about 80 species, which show a discrete distribution globally, occurring in South and North America, Australia, Eurasia, the Pacific Islands, and South Africa. [3]. Goji berries are nutritionally dense and medicinally valuable, serving as a cornerstone in traditional Chinese medicine and modern health industries, with significant potential in global functional food and nutraceutical markets[4]. However, with the increasing market demand for wolfberries from the native Chinese plant *Lycium barbarum* (*L. barbarum*), and the continuing development of this industry, long-term cultivation with current intensive cropping patterns could cause problems such as anthracnose, root rot, and powdery mildew in *L. barbarum* and other plants. This seriously affects the yield and quality of *L. barbarum* and limits the sustainable development of the *L. barbarum* industry [5–7].

Root rot in *L. barbarum* is difficult to eradicate and has been called a plant cancer. The leaves of *L. barbarum* plants affected by root rot are yellow and wilted, and occasionally, half of the plant is affected. The other half can grow new branches. The rot in the rhizomes is reddish brown, the xylem is soft and spongy, and most of the inside of the rhizomes turns reddish brown [1]. According to research, the disease is severe in the field, with contiguous patches where columns of plants die, and specimens replanted after the diseased plants have been dug up usually also develop the disease. *L. barbarum* root rot is caused by a variety of pathogenic *Fusarium* spp.: *F. solani*, *F. oxysporum*, *F. concolor*, *F. acuminatum*, and *F. moniliforme* are the most common pathogens, with *F. oxysporum* being the most pathogenic [9]. Currently, the main observed bioprophylactic factors include bacteria, fungi, and actinomycetes [10–12].

Arbuscular mycorrhizal fungi (AMF) comprise a group of ancient endophytic fungi able to colonize more than 80% of terrestrial plant bodies. They play an important role in regulating plant growth, development, and disease resistance [13]. By forming a clumped branching structure within the surface layer of the plant root system, AMF significantly improve plant water uptake, promote plant growth, and enhance host plant resistance [14,15]. Moreover, AMF compete with pathogenic bacteria for loci to improve the disease resistance of host plants, such as eggplant and tomato, and reduce or delay pathogen attacks on plants [16]. AMF are also able to induce regulation of antioxidant enzyme activity in host plants, which has high potential for preventative applications, the control of soil-borne diseases, and enhancing the resistance of plants to diseases [17]. Ma [18] found that inoculation with a clumping mycorrhizal fungus could induce changes in plant root secretion, regulate the inter-root microbial community, and inhibit the growth of pathogenic bacteria, thus improving the disease resistance of plants. Sheng Min [19] found that *L. barbarum* had an extremely high infestation rate in his study of mycorrhizal-type plants in saline-alkaline land in Ningxia. Meanwhile, a diversity of soil fungal communities in the inter-root system of *L. barbarum* was determined via high-throughput sequencing. Guo Huan found that *L. barbarum* could form clumped mycorrhizae with *Glomeromycota*. Different species of AMF have different effects on the control of soil-borne diseases [20] and also appear to have different effects on host plant resistance. However, the mechanism behind the action of AMF on root rot in *L. barbarum* is still unclear.

Therefore, this study took the goji berry cultivar 'Ningqi No.1' as the research subject and analyzed its physiological changes through inoculation with AMF and pathogenic bacteria. Through the use of transcriptomics, we analyzed the physiological and molecular regulatory mechanisms behind the mycorrhizalization of *L. barbarum* seedlings in terms of resistance to root rot, providing a scientific basis and technical support for this approach within the search for safe and effective biological measures to prevent and control *L. barbarum* root rot disease.

2. Materials and Methods

2.1. Test Materials

The goji berry 'Ningqi No.1' was harvested from the Tsaidam Basin in Qinghai.

AMF *Glomus mosseae* and *Glomus intraradices* were provided by the Institute of Microbiology, Guangxi Academy of Agriculture and Forestry.

The *L. barbarum* growth medium was mixed with pre-sieved sand and soil (1:3, W/W) and sieved through a 2 mm sieve; the sand and soil were purchased from the Shandong, Jinan flower market (River sand: grass peat soil = 3:1). The growth medium set was autoclaved at 121°C for 2 h, twice in 3 days.

A two-stage experiment was established in a light incubator to examine the interactions among *L. barbarum*, AMF, and pathogens. Seeds were sterilized and inoculated in different groups in AMF growth medium and incubated under light at 28°C for 96 h. After the shoots had grown to 1-2 cm, one *L. barbarum* seedling was set in each pot. Before transplanting the seedlings, inter-root soil (20 g) containing AMF was sown in layers within the growth medium. In treatments that did not receive AMF, we replaced the AMF with an equal amount of sterilized soil substrate (Figure 1).

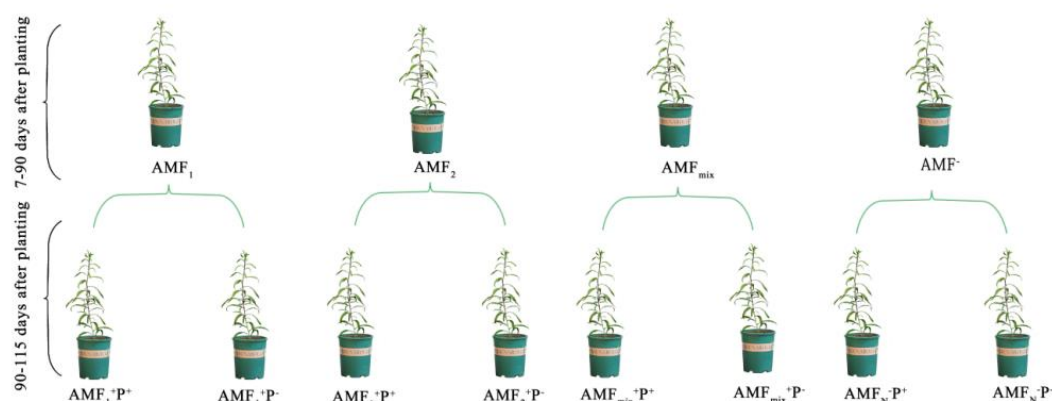


Figure 1. Technical roadmap.

2.2. Disease Treatment

F. oxysporum was used as the test pathogen and was donated by the Beijing Beinachuanglian Biotechnology Research Institute.

F. oxysporum was activated and made into a bacterial suspension. On the 45th day after the pot test was established, five root samples (1 cm long) were collected from each group of *L. barbarum* plants, cleaned, and stained, and the colonization of AMF was examined via ink and vinegar staining (Yang Mu et al.). On the 90th day after the potting test was set up, 15 pots of robust 'Ningqi No.1' were selected in each group to be inoculated with the pathogen of *L. barbarum* root rot, and 20 ml of bacterial suspension was added to the roots.

Four AMF treatments were set up in this experiment: the control without inoculation (C), *F. mosseae* (M), *R. intraradices* (R), and *F. mosseae*, and *R. intraradices*, mixed homogeneously at a mass ratio of 1:1 with mixed AMF (H).. Two pathogen treatments were set up: inoculation with pathogenic bacteria (G) and without pathogenic bacteria (N). There were eight treatments in total (HG, RG, MG, CG, HN, RN, MN, and CN), with 15 replicate pots per treatment, for a total of 120 pots.

The incidence and severity of root rot in *L. barbarum* were determined on the 15th day after inoculation with the pathogen. The incidence rate was expressed as the percentage of diseased leaves. Disease severity was estimated using the disease index (DI) calculated according to the disease scale 0-5 [21], utilizing the following formula:

$$DI = \frac{\text{self-employed} * \text{Sum of leaf grades}}{\text{Maximum condition score} * \text{Number of sample leaves}} * 100\%$$

The single-leaf rating in the formula is the disease rating of each leaf of *L. barbarum*.

The experiment was conducted in a light incubator space with light amplitude during the growth period and an average temperature of 28°C (daytime) and 22°C (night-time).

2.3. Plant Harvest and Measurement of Samples

Total RNA was extracted from 0.1 g of fresh leaves randomly sampled from each pot on the 0, 1, 3, 5, 7, 9, 11, 13, and 15th day, immediately snap-frozen in liquid nitrogen species for 15 min, and stored at -80°C. In addition, 0.5 g of fresh leaves were sampled from each pot as described previously, and enzyme activities, including POD, CAT, and SOD, were measured using a Solarbio kit. We conducted measurements of disease-resistant protease activities including chitinase, polygalacturonase, β -1,3-glucan, and pectin esterase. Subsequently, five pots were randomly selected from the four treatment groups that were not inoculated with *F. oxysporum* for the measurement of plant height, ground diameter, aboveground fresh weight, belowground fresh weight, aboveground dry weight, and belowground dry weight. Three pots were randomly selected, and the complete root system of *L. barbarum* was separated from the soil by the water washing method after digging out the 'Ningqi No.1'–AMF symbiont in the substrate. After that, they were placed in the Epson Root Scanner (GXY-A, China) to be scanned. WinRHIZO Root Analysis System Software was used to obtain the

data for the root system of the specific annual symbiont, the total root length, root surface area, mean root diameter, and root volume, with three replicates for each treatment [22].

2.5. Differential Expression Analysis and Functional Enrichment

Differential expression analysis was performed using DESeq2 software, and the functional annotation of differential genes (DEGs) was performed by applying Diamond and ID mapping tools combined with information from the GO database. Through the application of the KEGG database, the genes were also categorized in this study based on the metabolic pathways and functions that they are involved in. The *p*-values were corrected using four multiple-testing methods (Bonferroni correction, Holm correction, Sidak correction, and False Discovery Rate (FDR) correction). KEGG PATHWAY enrichment analysis was performed using KOBAS (<http://kobas.cbi.pku.edu.cn/home.do>) and calculated on the same principle as GO function enrichment analysis. On this basis, genes closely related to the pathogenicity of pathogenic bacteria were screened and analyzed [23].

2.6. Statistical Analysis

All analyzed results are expressed as the mean $\pm$ standard deviation (SD) of three replicates. Multi-group comparisons were performed in IBM SPSS 20 using one-way ANOVA, with *P* < 0.05 being considered a significant difference. Data were visualized using GraphPad Prism 8.0.2 and SigmaPlot software.

2.4. Transcriptome Analysis of Plant Leaves

While measuring enzyme activity, leaves from day 15 of each treatment (HG, RG, MG, CG, HN, RN, MN, and CN) were selected, and total RNA was isolated using the kit, which was repeated three times for each treatment. The concentration and purity of each RNA sample was determined using a Nanodrop 2000, and RNA integrity was examined by 1.0% denaturing agarose gel electrophoresis.

The sequencing of eukaryotic mRNA was performed by Shanghai Meiji Biotechnology Co. The generated raw sequence dataset has been submitted to the National Center for Biotechnology Information Short Read Archive (SRA) database under the registration number.

3. Results

3.1. AMF Colonization in the Root System of *L. Barbarum*

On the 45th day of plant establishment in the potting test, *F. mosseae* and *R. intraradices* successfully infested the root system of one-year-old 'Ningqi No.1' goji berry and formed structures such as vesicles, hyphae, and mycelium (Figure 2).

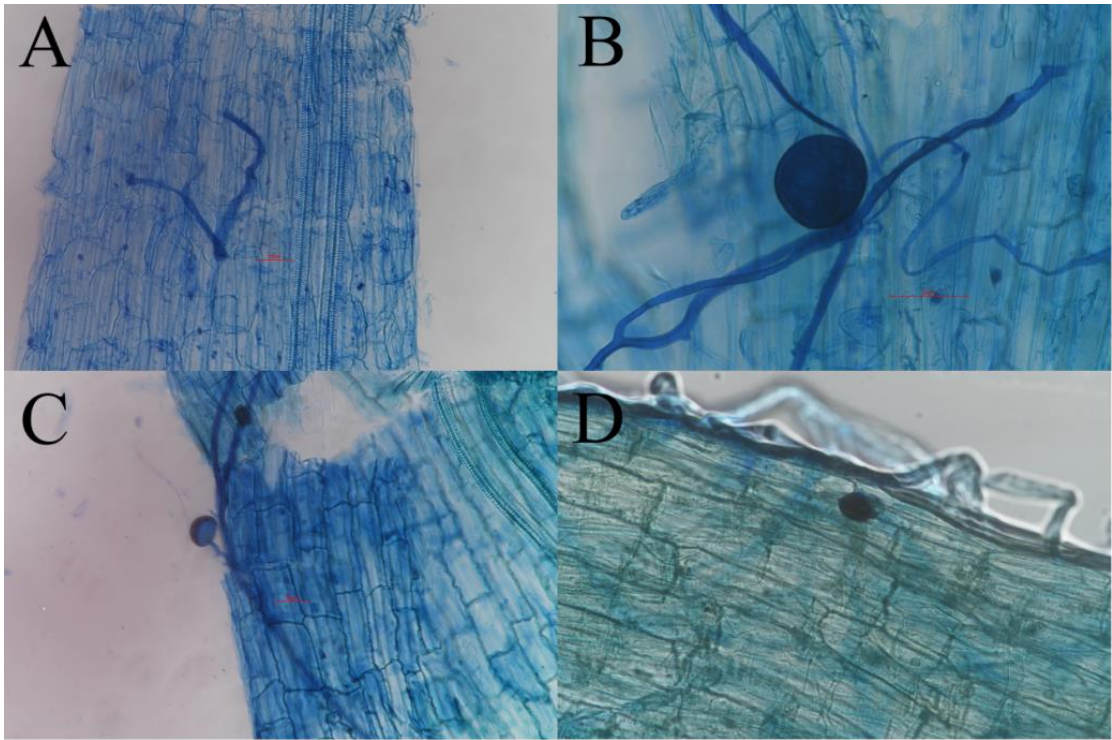


Figure 2. Colonization of the root system of *L. barbarum* by *Rhizoctonia solani* within the roots. A and B are mycelium, B, C and D are spores.

3.2. Effect of AMF on the Growth Characteristics of *L. Barbarum* Seedlings

3.2.1. Effect of Inoculation with AMF on Growth Indexes of *L. Barbarum*

Compared with the control group, other AMF-inoculated *L. barbarum* plants showed significant increases in plant height, diameter, aboveground fresh weight, belowground fresh weight, aboveground dry weight, and belowground dry weight, all of which were HN>RN>MN>CN, with no differences among the three AMF-inoculated treatments. Moreover, all three of these treatments differed significantly from the control. HN showed the best performance in each index (Table 1).

Table 1. Effect of different inoculation treatments on the growth parameters of *L.barbarum*. Different letters in the table indicate significant differences ($P<0.05$) among various indicators in ‘Ningqi No.1’ symbionts under different treatments.

Treatment	Height	Aboveground fresh weight	Belowground fresh weight	Stem diameter	Aboveground dry weight	Belowground dry weight
HN	57.00±15.43a	15.18±7.35a	7.89±6.40a	4.30±0.72a	3.90±1.95ab	5.20±3.65ab
RN	54.60±21.40a	11.93±5.24a	6.26±4.48a	4.01±0.65a	2.38±1.34ab	1.81±1.23b
MN	43.71±11.99a	8.46±3.94a	4.12±2.66ab	3.33±0.45b	2.41±0.32ab	2.36±1.18b
CN	27.09±17.43b	2.88±1.52b	0.76±0.65b	2.23±0.44c	0.93±0.48b	0.39±0.30b

3.2.2. Effect of AMF Inoculation on the Growth of *L. Barbarum*

Compared to the control group (CN), the growth of annual *L. barbarum* seedlings was significantly increased in all treatments at different periods, except at 40 d. At 10 d, the growth rate of MN was significantly higher than that of HN, RN, and CN. At 20 and 30 d, the growth rate of AMF-inoculated *L. barbarum* was much higher than that of the control group, and HN was the highest. At 40 d, the growth rate of the control group was not significantly different from that of RN

and MN, and it was significantly lower than that of HN. At 50 d, the growth rate of all AMF-inoculated groups was significantly higher than that of the control group (Figure 3).



Figure 3. Comparison of plant height of ‘Ningqi No.1’.

3.2.3. Trends in root system changes in ‘Ningqi No.1’–AMF symbiosis

Compared to the control group (CN), the total number of roots and total root projected area showed $RN > HN > MN > CN$; the total root length and number of root tips showed $RN > MN > HN > CN$; and the total root volume showed $HN > RN > MN > CN$ (Table 2). The number of root tips was significantly higher in the RN treatment compared to the other treatments, the total root projected area was significantly elevated in the HN and RN groups, and the mean diameter and total number of root tips was significantly higher in the RN treatment compared with the other treatments (Figure 4).

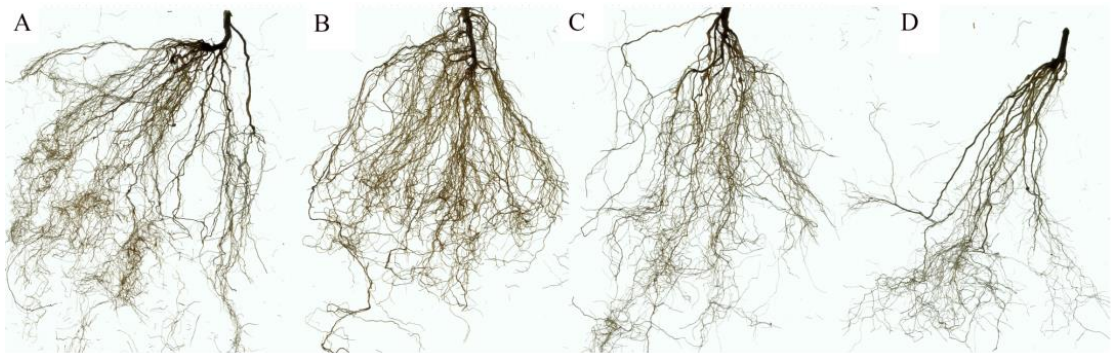


Figure 4. Scan of root systems in AMF–‘Ningqi No.1’ symbiosis. (a) Scan of the root system of the mixed group (HN); (b) scan of the root system of RN; (c) scan of the root system of MN; and (d) scan of the root system of CN.

Table 2. Parameters of root systems in AMF-‘Ningqi No.1’ symbiosis seedlings.

Treatment	Total number of roots	Number of root tips	Total root length	Root total projected area	Total root surface area	Total root volume	Average root diameter
CN	2746 ± 529 b	1734 ± 300 b	836.23 ± 91.19 b	4768.74 ± 701.41 b	14981.43 ± 2203.55 b	4350.30 ± 748.87 b	0.43 ± 0.01a
HN	5246 ± 869 a	2205 ± 532 ab	1528.67 ± 363.85 a	8666.90 ± 2353.95 a	27227.88 ± 7395.16 a	7753.69 ± 1275.35 a	0.46 ± 0.02a
MN	5077 ± 1050 a	2455 ± 546 ab	1634.84 ± 265.16 a	8482.65 ± 1427.42 ab	26649.04 ± 4484.36 ab	5633.69 ± 1059.65 a	0.43 ± 0.01a
RN	6338 ± 1150 a	3142 ± 498 a	1877.55 ± 361.88 a	10141.97 ± 2225.95 a	31861.93 ± 6993.01 a	7467.66 ± 2734.90 a b	0.44 ± 0.01a

3.3.1. Effect of AMF inoculation on the incidence and disease index of *L. barbarum* root rot.

Comparing HG, RG, and MG with CG, the incidence rate of *L. barbarum* root rot decreased by 53.81%, 52.8%, and 37.56%, the disease index decreased by 37.2%, 37.38%, and 27.28%, and the prevention and control effects were remarkable, reaching 80.97%, 81.35%, and 59.37%, respectively (Table 3).

Table 3. Incidence rate, disease index, and control effect of *L. barbarum* root rot under different inoculation treatments.

Treatment	Incidence rate	Disease index	Control effect
HG	13.75	8.75	80.97
RG	14.76	8.57	81.35
MG	30	18.67	59.37
CG	67.56	45.95	-
HN	-	-	-
RN	-	-	-
MN	-	-	-

3.3.2. Effect of *L. Barbarum* root rot on Antioxidant Enzyme Activities in Seedlings

The mean CAT activities of HN, RN, and MN were 240.5763, 182.2705, and 116.2026, respectively, within 15 days after inoculation with AMF (Figure 5A). On the 13th d after inoculation, it was found that HG, RG, and MG increased by 838.12%, 682.23%, and 587.24% compared to the CN group. The mean POD activities of HN, RN, and MN increased by 74.69%, 54.95%, and 43.52%, compared to that of CN, in 15 days (Figure 5B). Compared to the CN group, HG, RG and MG increased by 87.23 %, 69.72 % and 58.87 %, respectively. After 13 days of inoculation with AMF treatment, the SOD activity of *L. barbarum* plants (HG, RG, and MG) inoculated with pathogenic bacteria increased by 34.68%, 27.48%, and 28.06% compared to CN (Figure 5C). The SOD activity of HN, RN, and MN increased by 14.98%, 23.41%, and 9.39% compared to CN (Figure 5C).

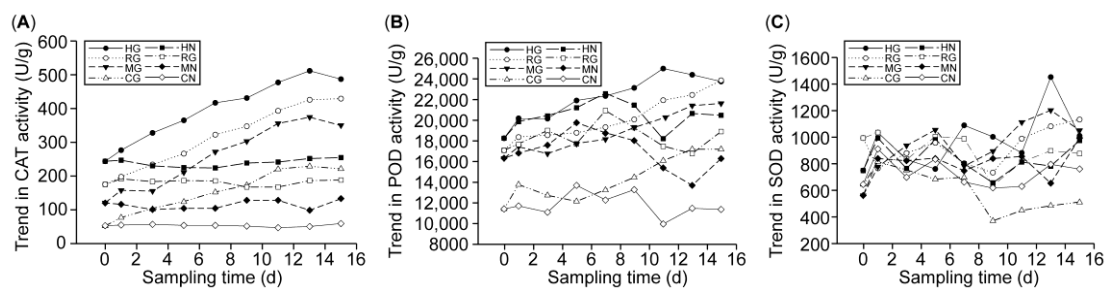


Figure 5. Effect of *L. barbarum* root rot on antioxidant enzyme activities in *L. barbarum* seedlings. (A) Trends in CAT activity; (B) trends in POD activity; (C) trends in SOD activity.

3.3.3. Effect of *L. Barbarum* Root rot on Disease-Resistant Protease Activity in Seedlings

The PE activity of HG, RG, and MG increased by 29.70%, 16.23%, and 13.39% compared to CN. HN, RN, and MN were not significantly different from CN and increased by 9.67%, 6.15%, and 6.89%, respectively. Compared to the control, the PG activity of HG, RG, and MG increased by 74.73%, 64.59%, and 56.18% (Figure 6A). Compared to CN, the activities of HN, RN, and MN increased by 18.41%, 19.80%, and 15.18%, respectively. In addition, compared to CN, β -1-3 glucoamylase in HG, RG, and MG increased by 59.64%, 46.18%, and 41.80%, respectively (Figure 6B). Compared to CN, the chitinase activities of HG, RG, and MG showed an increasing trend and increased by 54.27%, 51.49%, and 42.85%, respectively (Figure 6C). In comparison with CN, HN, RN, and MN were elevated by 26.62%, 25.65%, and 20.99%, respectively (Figure 6D).

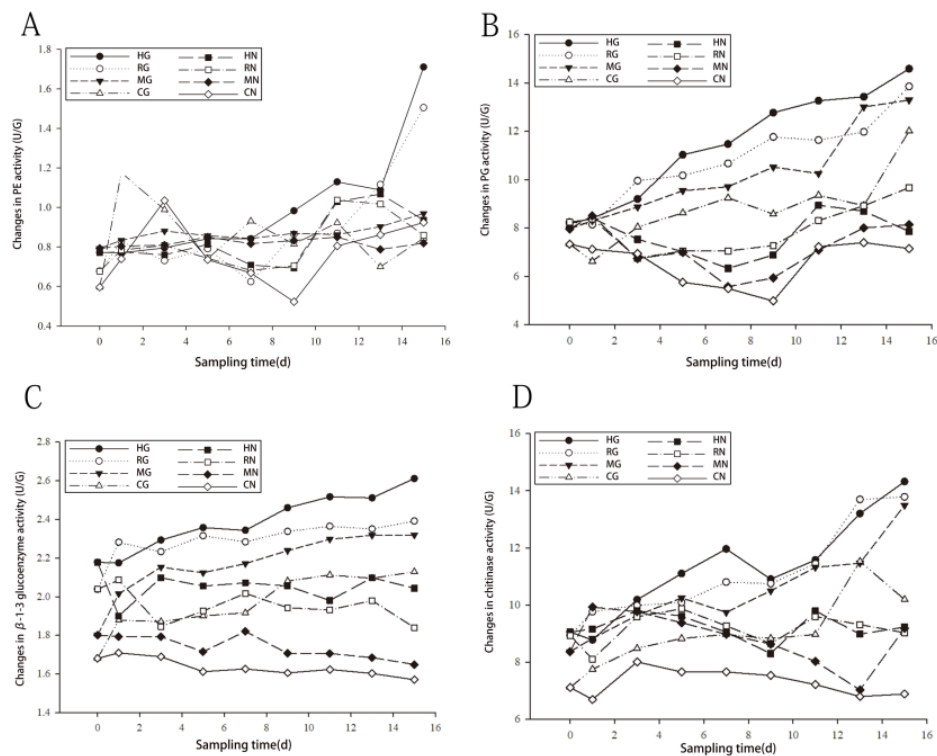


Figure 6. Effect of *L. barbarum* root rot on disease-resistant protease activity in *L. barbarum* seedlings. (A) Trends in PE activity; (B) trends in PG activity; (C) trends in β -1-3 glucoamylase activity; (D) trends in chitinase activity.

3.3.4. Analysis of the Combined Effect of *L. Barbarum* Root rot Disease on the Activities of Antioxidant Enzymes and Disease-Resistant Proteins in the Symbiont

After *F. oxysporum* infestation, compared with CG, AMF infestation significantly increased antioxidant enzymes and disease-resistant protein activities in *L. barbarum* symbionts. Among these,

the HG group showed the best performance for all the indexes, especially the expression of CAT activity and chitinase activity. HG effectively activated the disease-resistance mechanism of *L. barbarum* and strengthened its defense ability against root rot disease. In contrast, CG showed the worst performance for these biological indexes (Figure 7).

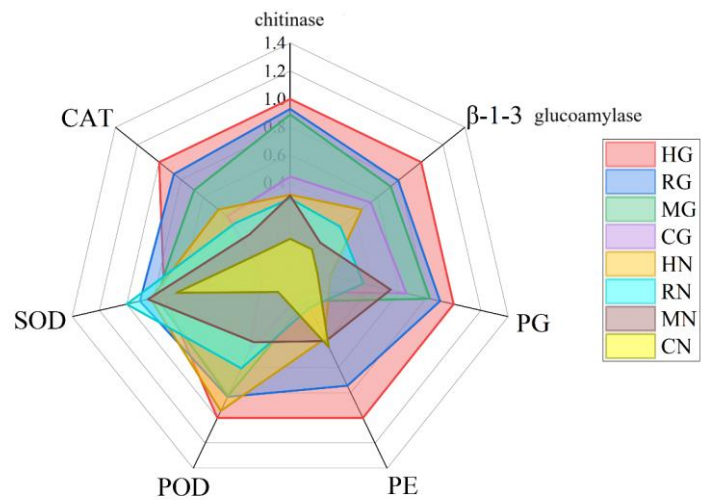


Figure 7. Radar diagram of antioxidant enzyme and pathogenesis-related protein activity levels in ‘Ningqi No.1’–AMF symbiotic associations post-infection with *F. oxysporum*.

3.4.1. Data Quality Control Analysis

A total of 344.83 Gb of clean data was obtained in this study, and 37,175 expressed genes were detected in terms of genome-wide expression analysis, including 27,407 known genes that have been annotated and 9,768 potential new genes. Moreover, a total of 69,230 transcripts were identified, consisting of 25,429 known transcripts and 43,801 novel transcripts (Table 4).

Table 4. Statistics of the transcriptome sequencing output.

GR	OU	P	HG	RG	M	G	CG	HN	RN	MN	CN
Ra	Pr	Cle	Cle	Q2	Q3	GC					
425	641	410	612	96.	94.	42.					
156	986	671	137	806	413	523					
94	979	38	490	666	333	333					
4	4	3	7	3	3	3					
462	698	449	669	96.	94.	42.					
802	831	102	424	786	376	613					
64	986	94	961	666	666	333					
4	4	9	7	7	7	3					
511	771	499	745	96.	94.	42.					
133	811	512	534	953	626	323					
39.	423	82.	101	333	666	333					
3	9	67	5	3	7	3					
490	740	473	706	95.	42.						
719	986	117	651	97.	013	453					
56	535	50.	591	21	333	333					
6	67	5	3	3	3	3					
556	840	536	800	97.	95.	42.					
581	438	475	931	216	043	316					
90	669	78.	997	666	333	666					
0	67	8	7	3	67						
515	778	504	752	96.	94.	42.					
544	471	242	092	816	436	403					
01.	460	63.	858	666	666	333					
3	1	3	3	7	7	3					
464	701	455	682	97.	42.						
752	776	822	512	126	94.	456					
74	637	56.	923	666	89	666					
4	67	9	7	7	67						
499	754	489	726	96.	94.	42.					
514	266	447	693	853	516	723					
56	985	20.	123	333	666	333					
6	7	3	3	7	7	3					

3.4.2. Transcript Matching Evaluation

The filtered sequences were aligned to the reference genome using HISAT2 software. As shown in Table 5, when the proportion of alignments to the reference genome (total mapped) was more than 70%, this indicated that the selected reference genome was appropriate and the alignment results had not been interfered with by the genomes of other species (Table 5).

Table 5. Transcriptional mapping information statistics.

Sample	Total reads	Total mapped	Multiply mapped	Uniquely mapped
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HG-1	40552074	38609423(95.21%)	1483136(3.66%)	37126287(91.55%)
HG-2	40436220	38470676(95.14%)	1412317(3.49%)	37058359(91.65%)
HG-3	42213120	40173019(95.17%)	1484422(3.52%)	38688597(91.65%)
RG-1	47432464	45038106(94.95%)	1828490(3.85%)	43209616(91.1%)
RG-2	46208982	43933057(95.07%)	1747784(3.78%)	42185273(91.29%)
RG-3	41089436	39073561(95.09%)	1574836(3.83%)	37498725(91.26%)
MG-1	48905964	46283124(94.64%)	1695796(3.47%)	44587328(91.17%)
MG-2	53053692	50118148(94.47%)	1849052(3.49%)	48269096(90.98%)
MG-3	47894192	45167590(94.31%)	1666538(3.48%)	43501052(90.83%)
CG-1	45863360	42098606(91.79%)	1558693(3.4%)	40539913(88.39%)
CG-2	50489018	46260846(91.63%)	1720050(3.41%)	44540796(88.22%)
CG-3	45582874	41720056(91.53%)	1553915(3.41%)	40166141(88.12%)
HN-1	51497724	48389092(93.96%)	1720144(3.34%)	46668948(90.62%)
HN-2	56842560	53333300(93.83%)	1914805(3.37%)	51418495(90.46%)
HN-3	52602452	49294176(93.71%)	1773999(3.37%)	47520177(90.34%)
RN-1	51755466	49113072(94.89%)	2142014(4.14%)	46971058(90.76%)
RN-2	48393240	45826708(94.7%)	1972168(4.08%)	43854540(90.62%)
RN-3	51124084	48350244(94.57%)	2066359(4.04%)	46283885(90.53%)
MN-1	44065952	41845056(94.96%)	1599851(3.63%)	40245205(91.33%)
MN-2	49442120	46871985(94.8%)	1791651(3.62%)	45080334(91.18%)
MN-3	43238698	40929798(94.66%)	1565924(3.62%)	39363874(91.04%)
CN-1	52580114	50462974(95.97%)	1903266(3.62%)	48559708(92.35%)
CN-2	48787804	46576392(95.47%)	1826575(3.74%)	44749817(91.72%)
CN-3	45466244	43419217(95.5%)	1679233(3.69%)	41739984(91.8%)

3.4.3. Differentially Expressed Gene Analysis

At 15 d of *F. oxysporum* infection in symbionts, a total of 11096 DEGs were identified from HG vs. CG, including 5,520 up-regulated differential genes and 5,576 down-regulated differential genes (Figure 8A). A total of 9205 differential genes were identified in RG vs. CG, including 5379 up-regulated differential genes and 3826 down-regulated differential genes (Figure 8B). A total of 4541 differential genes were identified in MG vs. CG, including 2734 up-regulated differential genes and 1907 down-regulated differential genes (Figure 8C).

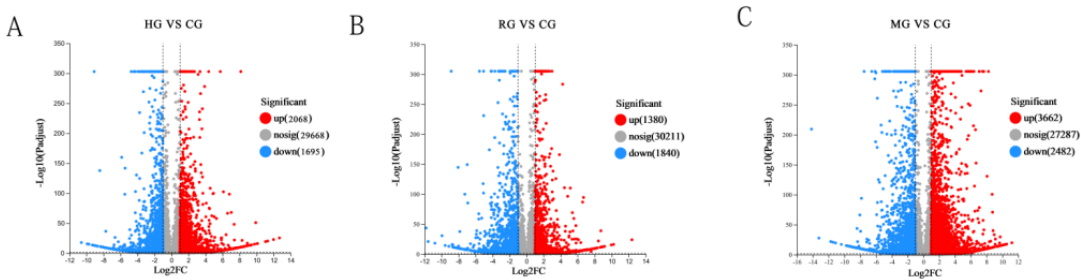


Figure 8. DIFFERENTIAL expression volcano plot.A is HG vs CG, B is RG vs CG, C is MG vs CG.

3.4.4. EggNOG Annotation Analysis of Differential Genes

Using the software to compare HG and CG with the EggNOG database, we found a total of 1,442 genes for information storage and processing, 1,825 genes for metabolism, and 2,084 genes for cellular

processes and signaling. Comparing RG and CG, we found 1,233 genes for information storage and processing, 1,499 genes for metabolism, and 1,716 genes for cellular processes and signaling. Comparing MG and CG, we found a total of 555 genes for information storage and processing, 1380 genes for metabolism, and 822 genes for cellular processes and signaling. HG had a higher number for each function than RG and MG (Figure 9).

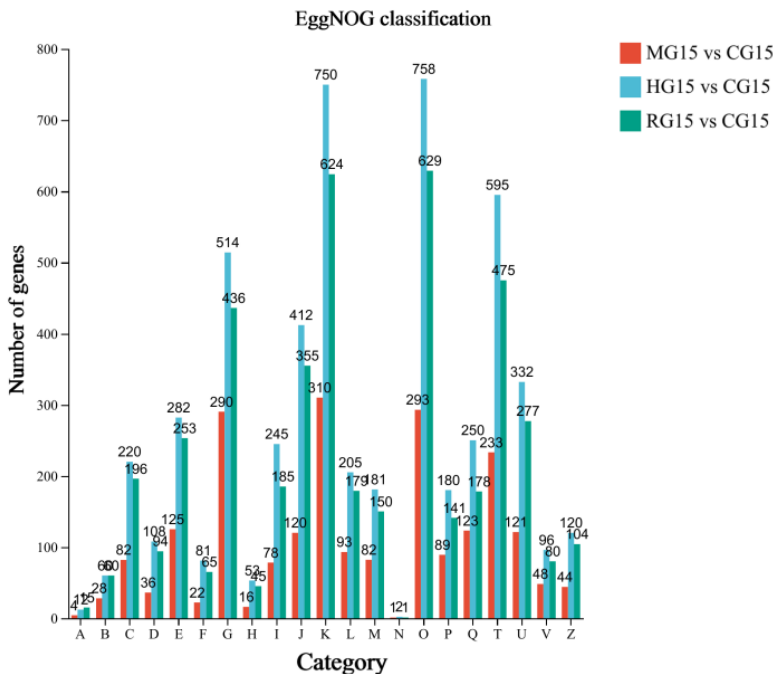


Figure 9. EggNOG classification summary bar chart.

3.4.5. Go Function Analysis of Differentially Expressed Genes

Analyzed at the Go functional enrichment Level 2 (Figure 10A,10B,10C)0), when *F. oxysporum* infested the symbionts HG, RG, and MG, in the biological process, DEGs were enriched to 51, 50, and 49 entries at 15 d, respectively. The numbers of genes enriched by HG were 1498, 3081, and 3541; the numbers of genes enriched by RG were 1249, 2575, and 2960; and the numbers of genes enriched by MG were 635, 1252, and 1401, respectively.

In the cellular component, DEGs were enriched in Go terms such as membrane part, organelle part, organelle, membrane, and cell part. The numbers of HG-enriched genes were 3425, 1379, 2650, 1260, and 4716, respectively. The numbers of genes enriched in RG were 2816, 1190, 2228, 994, and 3905, and the numbers of genes enriched in MG were 1476, 978, 523, and 1811.

In molecular function, there was differential gene enrichment in the Go entries for binding and catalytic activity. The numbers of genes enriched in Go entries for binding and catalytic activity were 4468 and 4330 for HG, 3698 and 3594 for RG, and 1815 and 1897 for MG, respectively.

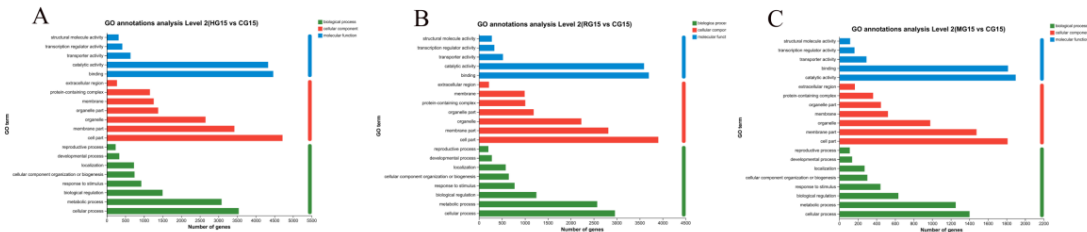


Figure 10. GO classification summary bar chart. A is HG vs CG, B is RG vs CG, C is MG vs CG.

3.4.6. KEGG Enrichment Analysis of DEGs

The KEGG enrichment analysis of differential genes in *L. barbarum* is shown in Figure 11A,11B,11C. When *F. oxysporum* infected *L. barbarum* in HG, RG, and MG, the differentially expressed genes were annotated to 135, 133, and 123 pathways, respectively. Among the 20 most significantly enriched pathway entries, 7 pathways had *P*-values <0.05 for HG, 11 pathways had *P*-values <0.05 for RG, and 12 pathways had *P*-values <0.05 for MG.

The top five HG *p*-values after *F. oxysporum* infestation were found for DNA replication (Rich factor=0.58), Citrate cycle (Rich factor=0.54), Steroid biosynthesis (Rich factor=0.58), Alpha-Linolenic acid metabolism (Rich factor=0.53), and Pyrimidine metabolism (Rich factor=0.51).

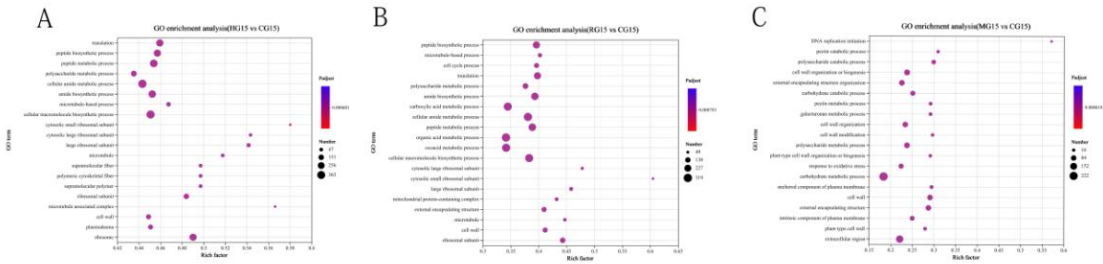


Figure 11. KEGG enrichment analysis result chart.A is the HGvsCG enrichment analysis, B is the RGvsCG enrichment analysis, and C is the MGvsCG enrichment analysis.

Ribosome (Rich factor=0.49), Citrate cycle (Rich factor=0.54), Steroid biosynthesis (Rich factor=0.60), Phagosome (Rich factor=0.47), and Taurine and hypotaurine metabolism (Rich factor=0.71) were the top five results in terms of *P*-values in RG after *F. oxysporum* infection.

After *F. oxysporum* infection in MG, the top five *P*-values were found for Valine, leucine and isoleucine degradation (Rich factor=0.33), Tyrosine metabolism (Rich factor=0.29), Nitrogen metabolism (Rich factor=0.37), Isoquinoline alkaloid biosynthesis (Rich factor=0.32), and Propanoate metabolism (Rich factor=0.32).

3.4.7. Analysis of DEGs in Defense-Related Pathways in *L. Barbarum*

In the peroxisome (map04146) signaling pathway (Figure 12A), 17 DEGs were identified in HG vs. control, and 51 DEGs were identified in HG vs. CG The up-regulation of the genes may also promote the enhanced activity of the antioxidant enzyme system in the plant and the up-regulation of the membrane-anchored protein (PEX3) gene Pex19p, the CAT-related Lba07g01760 genes, and the SOD-related Lba06g00182, Lba08g00496, Lba09g01319, Lba09g01375, Lba10g01332, and Lba10g02180 genes .

Sixty-nine DEGs were identified in the plant–pathogen interaction pathway for HG versus control (Figure 12B). In the PT1 system, related genes such as cyclic nucleotide-gated channels (CNGCs) and calcium-dependent protein kinase [24–26] (CDPK) were up-regulated and expressed at 15 d, and related genes such as plant respiratory burst oxidase homologue (Rboh) were down-regulated. Upon the recognition of calcium ions by cyclic nucleotide-gated channel proteins in the plant cell wall, calcium-dependent protein kinase was up-regulated, and plant respiratory burst oxidase was inhibited, stimulating an increase in cellular reactive oxygen species secretion and an increase in the expression of related defense genes. Cellular flagellin (flg22) was recognized by receptor proteins (FLS2), and genes such as Lba12g01077 associated with FLS2 were up-regulated, which transmitted the information to the interior of the cell through cellular endocytosis, activated the production of reactive oxygen species (ROS), and triggered the effects of cell wall reinforcement, the superoxide response, and the enhancement of antioxidant enzyme activities. In addition, FLS2 activated downstream mitogen-activated protein kinase (MEKK), and genes such as Lba01g00615, which is related to MPK3/MPK6, were down-regulated. These signals were transmitted into the interior of the nucleus, and after transmission to transcription factors, Lba03g02584 genes related to the glycerol kinase gene (NHO) were down-regulated.

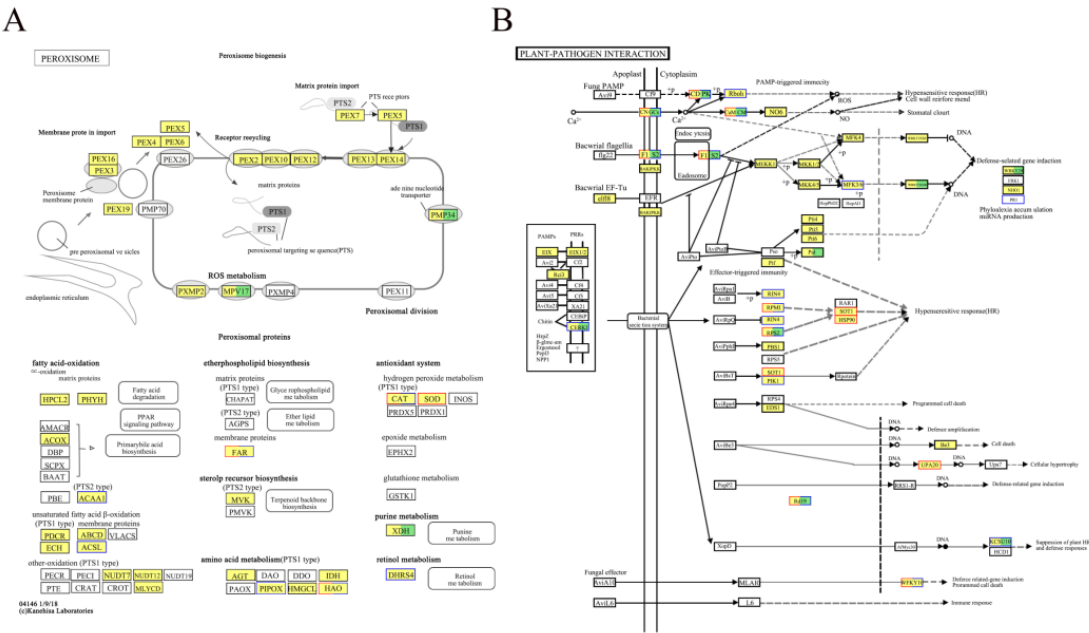


Figure 12. Signaling pathway diagram. A is map04146 signaling pathway diagram, B is map04626 signaling pathway diagram.

In the ET1 system, genes such as the SGT1 gene (suppressor of the G2 allele of SKP1) and the HSP90 gene were up-regulated at 15 d. The RPM1 gene and the RPS2 gene were both up- and down-regulated. The expression of HSP90 and SGT1 genes in the symbiont was significantly increased after induction by *L. barbarum* root rot, which promoted the hypersensitive response (HR) of the plant .

4. Discussion

In this study, we investigated the growth characteristics of one-year-old ‘Ningqi No.1’ seedlings inoculated with different AMFs and found that AMFs had a significant promoting effect on the growth of *L. barbarum* seedlings. The experimental results demonstrate that compared with the control group not inoculated with AMF, the AMF-inoculated goji berry seedlings exhibited significant growth in plant height, diameter, above-ground biomass, and below-ground biomass, as well as root characteristics, which is in agreement with Zeng’s findings on cypress [27].

In addition, the results of the present study are in agreement with those of Richa's study on *Glycine max* (L.) Merr., which showed that inoculation with AMF had a tremendous growth-promoting effect on plant height, stem thickness, fresh mass, and dry mass in *Glycine max* (L.) Merr. Seedlings[28]. Meanwhile, the present study is also in agreement with the results of Zheng on *Salvia miltiorrhiza* Bunge, in which AMF significantly increased the aboveground dry weight, number of leaves, and number of roots of *Salvia miltiorrhiza* Bunge 0.24~0.65 fold, and the contents of active ingredients in the aboveground and belowground fractions were also significantly increased by 0.44~1.78 fold, respectively [28–30]. Moreover, the present study found that inoculation with *F. mosseae* promoted the germination of *L. barbarum* seeds, which is consistent with the findings of Edgar Omar Rueda-Puente's study on *Capsicum annuum* L. [31]. Studies have shown that inoculation with AMF promotes the germination rate of host plants, with significant increases in plant height, root length, fresh weight, and dry weight.

AMF inoculation remarkably increased the total number of roots, the number of root tips, the total length of roots, the total projected area of roots, the total surface area of roots, and the total volume of roots of *L. barbarum* seedlings, indicating that AMF can effectively promote the development of the *L. barbarum* root system, thus enhancing the water and nutrient absorption capacity of seedlings and promoting their growth and development. In the next stage, AMF inoculation also increased the aboveground and belowground biomass of *L. barbarum* seedlings, indicating that the symbiotic effect of AMF was not limited to root growth but also promoted the

growth of the whole plant. A scanning analysis of the root system further demonstrated the positive effects of AMF inoculation on root development in *L. barbarum* seedlings, enhancing the root expansion capacity and biomass accumulation. These improved root system characteristics are essential in enhancing plant adaptability and survival, especially in drought or nutrient-poor environments. This is consistent with the findings of Yuan [32] on *Ipomoea batatas* L., and those of El-Mesbahi [33] on *Zea mays* L., showing that inoculation with AMF can promote an increase in root tip number and increase the root projection area, root volume, and root surface area. AMF can promote the growth of plant root systems and enhance the accumulation of host plant biomass [34–36].

Our study showed that the inoculation of AMF promotes the growth of goji berry seedlings and improves their ability to adapt to the environment. *L. barbarum* root rot is the most widely spread soil-borne disease affecting the production of *L. barbarum* and seriously affects yield and quality. Currently, the main control measures for goji berry root rot include selecting and breeding new varieties and chemical control. Although new, disease-resistant varieties have been introduced, such as 'Ningqi No.7', 'Ningqi No.1' is still the most widely planted variety. Chemical means of control, although effective, cause problems such as pesticide residues, environmental pollution, and resistance in pathogenic bacteria [37]. In HG, RG, and MG, the incidence rate and disease index of *L. barbarum* root rot were notably reduced. Compared with CG, the incidence of *L. barbarum* root rot decreased by 53.81%, 52.8%, and 37.56%, the disease index decreased by 37.2%, 37.38%, and 27.28%, and the prevention and control effects reached 80.97%, 81.35%, and 59.37% in HG, RG, and MG, respectively.

The SOD, POD, and CAT activities of *L. barbarum* seedlings were significantly increased by AMF inoculation. The SOD, POD, and CAT activities of HG were significantly enhanced compared with CG. AMF inoculation significantly activated the antioxidant system of *L. barbarum* seedlings and improved their ability to scavenge ROS, thus enhancing plant resilience. This is consistent with the findings of Huang on *Robinia pseudoacacia* L. and Wang on *Sorghum bicolor* [38,39]. The AMF-inoculated *L. barbarum* seedlings were also significantly elevated in disease-related protein activities and were able to stimulate the disease defense response in *L. barbarum* seedlings and enhance the expression of disease process-related proteins in the plant, thereby improving the resistance of *L. barbarum* to root rot. This is consistent with the results of Pu's study on *Salvia miltiorrhiza* [28], in which the inoculation of *G. versiforme* was able to protect *Salvia miltiorrhiza* from the effects of *Salvia* wilt, and the disease-resistant proteases, such as chitodextrinase and β -1,3-glucanases, were significantly increased, which, in turn, protected *Salvia miltiorrhiza* against *F. oxysporum*.

By analyzing the transcriptome of *L. barbarum* leaves inoculated with different AMFs after *F. oxysporum* infestation, this study yielded 344.83 Gb of clean data, and the analysis of DEGs showed that 11,096, 9205, and 4,641 DEGs were identified in HG, RG, and MG, respectively, compared to CG. Between HG and CG, there was a total of 5,520 up-regulated and 5576 down-regulated differential genes; between RG and CG, there were 5379 up-regulated and 3826 down-regulated differential genes; and between MG and CG, there were 2734 up-regulated and 1907 down-regulated differential genes.

GO functional enrichment analysis further revealed that differentially expressed genes were mainly enriched in key biological processes such as bioregulation, metabolic processes, and cellular processes. KEGG pathway enrichment analysis highlighted several important pathways, including DNA replication, the citric acid cycle, and steroid biosynthesis. These pathways were differently enriched in the HG, RG, and MG groups. This is consistent with the findings of Gao in *Citrus sinensis*, where genes related to disease-resistant proteases and α -linolenic acid metabolic pathways were up-regulated after inoculation with *G. versiforme*, which promoted plant growth and resistance against diseases [40,41].

5. Conclusions

We have concluded the following main findings from our study:

(1) Inoculation with AMF significantly promoted the growth and development of *L. barbarum* seedlings. Compared with the control group, *L. barbarum* plants inoculated with AMF significantly

increased in growth indexes such as plant height, diameter, aboveground biomass, and belowground biomass.

(2) Inoculation with AMF tremendously increased the activities of antioxidant enzymes and disease-resistant proteases in *L. barbarum* seedlings, and the incidence rate and disease index of root rot decreased by 53.81% and 37.2%, respectively.

(3) Transcriptomic analysis illustrated that AMF inoculation had a significant effect on the genes of antioxidant enzyme metabolic pathways and enhanced the disease resistance and overall physiological state of *L. barbarum* through a variety of molecular mechanisms.

Author Contributions: MY and GD conceived the idea and designed the experiments. MY collected the data and wrote the manuscript. GD edited and made suggestions to improve the manuscript. ZX, SJ, TL, and ZW reviewed the manuscript and contributed to the discussion. All authors contributed to the article and approved the submitted version.

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Conflicts of Interest Statement: The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Abbreviations

D-S, mixed diseased soil sample; H-S, mixed healthy soil sample; L-D, diseased plants (from *L. barbarum*); L-H, healthy plants (from *L. barbarum*); OTU, operational taxonomic unit; NMDS, non-metric multidimensional scaling; LEfSe, linear discriminant analysis effect size; RDA, redundancy analysis; VPA, variation partitioning analysis; P, Pathotroph (in FUNGuild analysis); Sa, Saprotroph (in FUNGuild analysis); Sy, Symbiotroph (in FUNGuild analysis); C/N, carbon/nitrogen ratio; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, alkali-hydrolyzable nitrogen; AK, available potassium; AP, available phosphorus; SOM, soil organic matter; PCR, polymerase chain reaction; ITS2, Internal Transcribed Spacer 2; PEAR, Paired-End reAd merger software; QIIME2, Quantitative Insights Into Microbial Ecology 2; SPSS, Statistical Package for the Social Sciences.

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