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Serendipita indica: A Biostimulant Enhancing Low-Temperature Tolerance and Active Constituent Levels in *Polygonum cuspidatum*

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Abstract: *Polygonum cuspidatum* is a traditional medicinal plant enriched with resveratrol and polydatin. However, low temperatures reduce the medicinal component contents of *P. cuspidatum*, and prolonged low temperatures also affect the growth and survival of *P. cuspidatum* at the seedling stage. It is unclear whether a culturable endophytic fungus *Serendipita indica* is able to enhance *P. cuspidatum*'s low-temperature tolerance and medicinal components. The objective of this study was to examine the biomass, leaf gas exchange, antioxidant enzyme activity, proline levels, medicinal constituent levels, and the expression of the resveratrol synthase (*PcRS*) and resveratrol-forming stilbene synthase 11 (*PcRS11*) genes of potted *P. cuspidatum* plants inoculated with *S. indica* at low temperatures (10 °C/6 °C, 12 h/12 h, day/night temperature). The six-week low-temperature treatment significantly reduced the root fungal colonization, biomass production, and leaf gas exchange variables, whereas *S. indica* inoculation significantly increased shoot and root biomass, photosynthetic rate, stomatal conductance, and transpiration rate at low temperatures. *S. indica* inoculation significantly increased superoxide dismutase and catalase activity as well as proline levels in leaves at low temperatures. The magnitude of root chrysophanol, emodin, polydatin, and resveratrol levels decreased by low temperatures was greater in uninoculated plants than in inoculated plants. Inoculation of *S. indica*, on the other hand, significantly increased the four medicinal component levels in roots at low temperatures, with a greater magnitude rise in chrysophanol, polydatin, and resveratrol at low temperatures than at suitable temperatures. The low-temperature treatment down-regulated the expression of *PcRS* and *PcRS11* genes in roots, while *S. indica* up-regulated the expression of *PcRS* and *PcRS11* genes at low temperatures. This implies that *S. indica* acts as a powerful microbial stimulant on *P. cuspidatum* to promote low-temperature resistance and medicinal component levels.

Keywords: antioxidant enzyme; medicinal plants; osmotlyte; polydatin; resveratrol; temperature stress

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1. Introduction

Polygonum cuspidatum Sieb. et Zucc., a well-known Chinese medicinal plant, is a perennial herb of the Polygonaceae family, and its dried rhizomes and roots contain compounds such as stilbenes, anthraquinones, flavonoids, proanthocyanidins, and others that are widely used in the treatment of inflammation, hepatitis, tumors, bacterial infections, and neurodegenerative disorders [1,2]. Among them, *P. cuspidatum* is rich in resveratrol and polydatin, with *P. cuspidatum* having the highest resveratrol content among all plants [2]. *P. cuspidatum* has been listed as one of the five special herbs in Hubei Province, China, with a planting area of 4600 ha in the province and an annual output of 100,000 tons. *P. cuspidatum* have ovate-oval leaves, erect stems with scattered red or purplish-red spots, unisexual flowers, and ellipsoid achenes, preferring to grow at altitudes of 140–2000 m.

Resveratrol is non-flavonoid polyphenolic compound with a variety of biological activities, which is a kind of plant antitoxin in response to external stimuli; polydatin is

a product of the combination of resveratrol and glucose, with three phenolic hydroxyl groups, which has good antioxidant protective effects [3]. With increasing market demand, *P. cuspidatum*'s wild resources have been heavily depleted, and artificial cultivation has steadily become a major source of these medical herbs. The environment has a significant impact on the yield and quality of *P. cuspidatum* [4]. In recent years, frequent low temperatures at the ecological farming sites of *P. cuspidatum* have limited its growth and medicinal component levels [5]. Based on the findings of Zhao et al. [6], resveratrol and total tannin contents were positively correlated with the temperature of the habitat of *P. cuspidatum*. Among them, the lowest temperature of the coldest month reached 3.3% in the contribution rate of ecological factors affecting the distribution of *P. cuspidatum*, ranking fifth among 23 ecological factors [4]. High temperature seasonality in the wild habitat is an important factor driving the elevated resveratrol content of *P. cuspidatum* [6]. In addition, the seedling stage of *P. cuspidatum* often suffers from prolonged low temperatures, which inhibits its growth and survival. Therefore, it is critical to improve *P. cuspidatum*'s low-temperature resistance.

Serendipita indica is a root-associated endophytic fungus with pear-shaped spores discovered in the roots of shrubs in India's Thar Desert [7]. *S. indica* colonizes the roots of about 150 commercially monocotyledonous and dicotyledonous plants [8,9]. This fungus has functional features comparable to arbuscular mycorrhizal fungi, such as improving plant growth, promoting nutrient uptake, and enhancing stress resistance [9,10]. Unlike arbuscular mycorrhizal fungi, *S. indica* can be culturable and proliferated on synthetic media without the need of a host [11]. Therefore, *S. indica* has a high potential for use as a biostimulant. Inoculation of *S. indica* is known to enhance host resistance to abiotic stresses such as low temperature, drought, salt stress, flooding stress, heavy metal contamination, and so on [8–12]. The capacity of *S. indica* to grow in plants at −30 °C to 4 °C demonstrates its adaptability to low temperatures [13]. The root colonization of *S. indica* has been demonstrated to promote the production of new proteins and increase the level of soluble sugars in passion plants to adapt to low-temperature stress [14]. In mung bean, *S. indica* also activated the plant's antioxidant enzyme defense system to enhance resistance to low-temperature stress [15]. These imply that *S. indica* has a positive effect on host resistance to low temperatures.

Earlier studies showed that *S. indica* could colonize the roots of *P. cuspidatum* and promote plant growth and nutrient acquisition as well as resveratrol levels, accompanied by the up-regulation of *chalcone synthase 1* (*PcCHS1*) and *PcCHS2* gene expression [2,16]. This shows that *S. indica* has good potential for enhancing the medicinal constituents of *P. cuspidatum*. However, it is unclear whether and how *S. indica* can enhance *P. cuspidatum*'s low-temperature resistance and medicinal constituent levels. The objective of this study was to analyze the effects of *S. indica* inoculation on the growth, photosynthetic characteristics, antioxidant enzyme activities, proline levels, and medicinal component levels of *P. cuspidatum* under low-temperature stress, as well as changes in the expression of *resveratrol synthase* (*PcRS*) and *resveratrol-forming stilbene synthase 11* (*PcRS11*) genes.

2. Materials and Methods

2.1. Preparation of the Fungal Inoculum

S. indica strain DSM 11827 was provided by the Institute of Root Biology, Yangtze University, and preserved at −70 °C. The inoculum, prepared as a solid medium with mycelium, was cut into 0.5 × 0.5 cm pieces, which were placed in potato dextrose liquid medium, and incubated in a shaker (180× *g*/min) at 28 °C for 7 d. The active pieces of mycelium were inoculated into potato dextrose liquid medium at 28 °C and 180× *g*/min for 7 d before being combined with sterile water at a 1:20 ratio, crushed, and prepared for use.

2.2. Plant Culture, Fungal Inoculation, and Low-Temperature Treatment

Seeds of *P. cuspidatum* (identification: WUK 0310891) were provided by Shiyan Academy of Agricultural Sciences. After surface disinfection in 70% alcohol for 7 min, seeds were placed in an autoclaved mixture of peat and sand for germination. After approximately 52 days, *P. cuspidatum* seedlings with three leaves were transplanted into a 2.6-L plastic container pre-filled with a mixture of autoclaved vermiculite, sand, and soil as growth substrate.

Fungal inoculation treatments were performed at the time of seedling transplanting. The 60 mL of spore suspension was inoculated around the roots as the inoculated treatment (+*Si*), while the uninoculated treatment received 60 mL of autoclaved spore suspension as the uninoculated treatment (−*Si*). Following 2 weeks of fungal inoculation, 30 mL of the spore suspension was continued to be injected around the roots of inoculated seedlings to increase root colonization.

The transplanted seedlings were placed in a light incubator (LC-GZX-180F, Shanghai Lichen Instrument Technology Co., Ltd., Shanghai, China) with suitable temperature (28 °C/18 °C, 12 h/12 h, day/night temperature), light intensity of 4000 Lux, and relative humidity of 55% for 6 weeks. Based on the ecological regionalization of *P. cuspidatum* in China [4], 10 °C/6 °C (12 h/12 h, day/night temperature) was chosen for the low-temperature treatment. Following that, half of the seedlings continued to be cultured under the condition of suitable temperature, while the other half were shifted to low-temperature conditions, i.e., 10 °C/6 °C (12 h/12 h, day/night temperature), along with same light intensity and relative humidity. The seedlings were collected 6 weeks after being subjected to such a low-temperature treatment.

The present study consisted of four treatments, including (i) inoculation with *S. indica* at suitable temperatures (St + *Si*), (ii) inoculation without *S. indica* at suitable temperatures (St − *Si*), (iii) inoculation with *S. indica* at low temperatures (Lt + *Si*), and (iv) inoculation without *S. indica* at low temperatures (Lt − *Si*). Each treatment was replicated eight times in a total of 32 pots.

2.3. Determination of Plant Biomass and Leaf Gas Exchange

Plants were harvested and separated into shoots and roots, and their biomass was weighed. One day before harvest, the gas exchange in the second leaf at the top was measured using an Li-6400 photosynthesizer (Li-COR Inc., Lincoln, NE, USA), along with an external buffer bottle to control CO_2 concentration and red and blue light source (1200 μmol/m^2/s). The temperature was 20–25 °C, the concentration of CO_2 was 385 μmol/mol the flow rate was 500 μmol/s, and leaf area was 6 cm^2. After stabilizing the data, the parameters including photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (Gs) were recorded.

2.4. Determination of Proline Levels in Leaves

Proline levels in leaves were determined according to the protocol described by Bates et al. [17]. A 0.25 g fresh leaf sample was added to 6 mL of pre-cooled 30 g/L sulfosalicylic acid solution at 100 °C for 10 min. Following filtering, a reaction solution including 1 mL of the filtrate and 3 mL of acidic ninhydrin was incubated for 30 min at 100 °C. After cooling, 2 mL of toluene was added, and the absorbance value of the upper phase was determined at 520 nm, using proline as a standard.

2.5. Determination of the Activity of Three Antioxidant Enzymes in Leaves

The 0.3 g leaf samples were ground with 5 mL of 0.05 mol/L phosphate buffer (pH 7.8) containing 0.1% (*w*/*v*) polyvinylpolypyrrolidone (PVPP) and centrifuged at 5000× *g*/min for 15 min. The supernatant was used for the determination of three antioxidant enzyme activity. Superoxide dismutase (SOD) activity was determined by inhibition of nitroblue tetrazolium (NBT) reduction [18], where a 3 mL reaction solution consisting of 0.05 mol/L phosphate buffer, 750 μmol/L NBT, 130 mmol/L methionine, 100 μmol/L EDTA-Na_2,

20 mmol/L riboflavin, and 0.05 mL supernatant was used. Catalase (CAT) activity was determined by a colorimetric assay [19], in which 100 mmol/L hydrogen peroxide was added to the supernatant for the reaction, and the change in absorbance value was measured at 240 nm for 1 min. Peroxidase (POD) activity was determined by the guaiacol method [20], where a 3 mL reaction solution consisted of 0.1 mL of supernatant and 0.05 mol/L phosphate buffer, 2% hydrogen peroxide, and 0.05 mmol/L guaiacol. The enzyme solution at 100 °C for 3 min was used as a control and the change in absorbance value was measured at 470 nm for 1 min.

2.6. Determination of the Levels of Four Medicinal Substances in Roots

Four medicinal substances including chrysophanol, emodin, polydatin, and resveratrol from the roots were extracted with 80% of methanol using the method described by Sun et al. [2]. The extract obtained was passed through a microporous filter membrane (0.45 μm size) and then used for HPLC analysis. The HPLC instrument was a Shimadzu LC-10A (Kyoto, Japan), with an SPD-M10Avp photodiode array detector and a phenomenex C18 column (4.0 mm × 150.0 mm, 5 μm). The mobile phase was methanol: ddH_2O solution (40:60) at a flow rate of 0.8 mL/min at 30 °C. Standards of chrysophanol (0.015 mg/mL), emodin (0.016 mg/mL), polydatin (0.020 mg/mL), and resveratrol (0.017 mg/mL) were used to establish standard curves in the HPLC assay.

2.7. Determinations of PcRS and PcRS11 Gene Expression in Roots

Total RNA in roots was extracted with Tiangen RNAprep Pure Plant kit (Tiangen Biotech (Beijing) Co., Ltd., Beijing, China), and the concentration and quality of total RNA were detected by electrophoresis after DNase I digestion. The first single-stranded cDNA was synthesized using a reverse transcription kit and stored at −80 °C. According to the results of Sun et al. [16] and Liu [21], *PcRS* and *PcRS11* genes were involved in the resveratrol biosynthesis of *P. cuspidatum*. As a result, the two *PcRS* genes were chosen, and their specific primers in qRT-PCR were designed (Supplementary Material Table S1). The PCR reaction procedures were as follows: pre-denaturation at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s for 30 cycles, and extension at 72 °C for 10 min. *PcActin* gene was used as the internal reference gene. Four biological replicates were used for each treatment. The $2^{-\Delta\Delta Ct}$ method [22] was used to calculate the relative expression of target genes, which was normalized by the St − *Si* treatment.

2.8. Data Analysis

The experimental data were analyzed by one-way analysis of variance, and the significance of difference between treatments was analyzed by Duncan's multiple range tests at 0.05 levels. These data analyses were carried out using IBM SPSS 22.0 (Chicago, IL, USA). The Sigmaplot software (v10.0; Systat Software, San Jose, CA, USA) was utilized to generate graphs.

3. Results

3.1. Effect of Low Temperatures on the Root Fungal Colonization Rate

In the roots of *P. cuspidatum*, fungal colonization appeared only in seedlings inoculated with *S. indica*, with a considerable number of pear-shaped chlamydospore in the roots (Figure 1a). The root fungal colonization rate was 41.78% at low temperatures and 64.63% at suitable temperatures (Figure 1b). The low-temperature treatment significantly inhibited the root fungal colonization rate by 35.36%, compared with the suitable-temperature treatment.

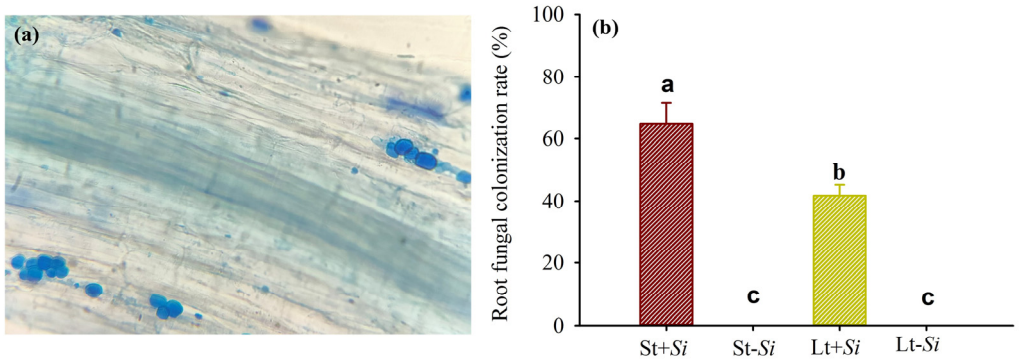

Figure 1. Root colonization (**a**) of *Serendipita indica* in roots of *Polygonum cuspidatum* seedlings and effect of low temperatures on root colonization rate (**b**). Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: St + *Si*, inoculation with *S. indica* under suitable temperature conditions; St − *Si*, inoculation without *S. indica* under suitable temperature conditions; Lt + *Si*, inoculation with *S. indica* under low temperature conditions; Lt − *Si*, inoculation without *S. indica* under low temperature conditions.

3.2. Effect of Low Temperatures and S. indica on Biomass Production

The low-temperature treatment significantly suppressed shoot and root biomass production by 22.83% and 29.42%% in uninoculated seedlings and 16.60% and 26.89% in inoculated seedlings, respectively, compared with the suitable-temperature treatment (Figure 2). However, *S. indica* colonization significantly increased shoot and root biomass production by 30.98% and 20.73% at suitable temperatures and 41.55% and 25.05% at low temperatures, respectively.

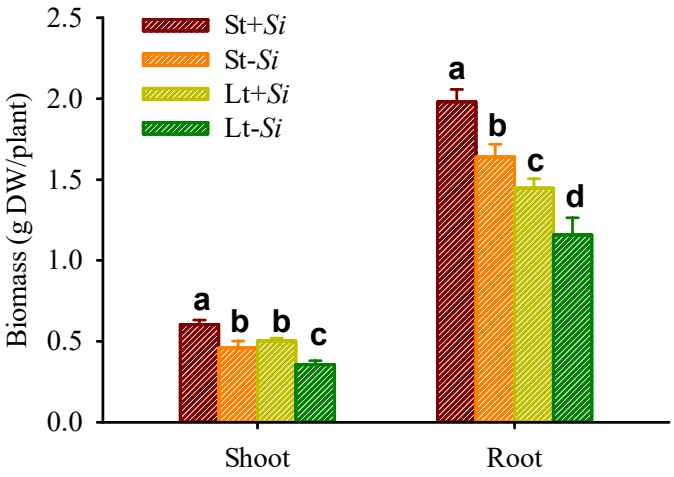

Figure 2. Effect of low temperatures and *Serendipita indica* on shoot and root biomass of *Polygonum cuspidatum* seedlings. Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($p < 0.05$) differences. See Figure 1 for abbreviations.

3.3. Effect of Low Temperatures and S. indica on Leaf Gas Exchange

The photosynthetic rate, E, and Gs in the leaves were significantly inhibited under low temperature versus suitable temperature conditions, as evidenced by the reduction of 37.77%, 42.14%, and 48.12%, respectively in uninoculated seedlings and 32.15%, 35.08%, and 49.24%, respectively in inoculated seedlings (Figure 3a–c). After inoculation of *S. indica*, the Pn, E, and Gs in the leaves were significantly increased by 35.47%, 22.38%, and 38.73%, respectively, at suitable temperatures, and 47.70%, 37.31%, and 35.75%, respectively, at low temperatures.

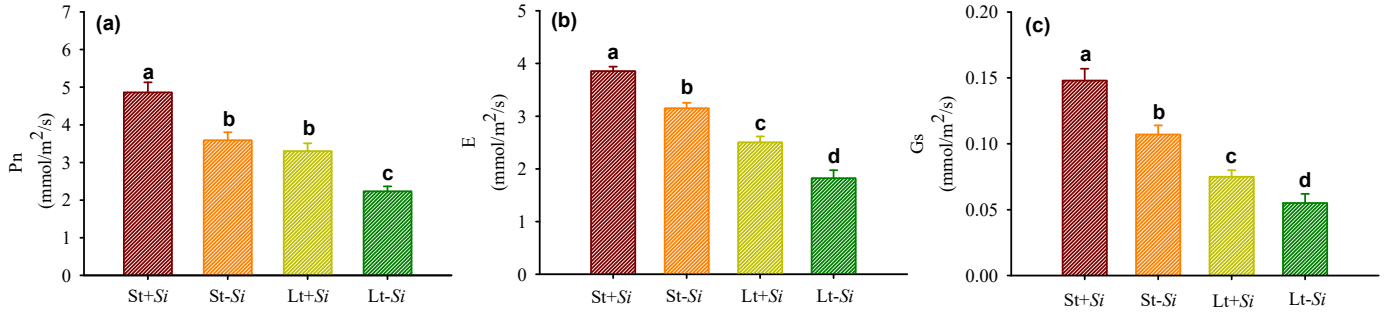

Figure 3. Effect of low temperatures and *Serendipita indica* on photosynthetic rate (Pn) (**a**), transpiration rate (E) (**b**), and stomatal conductance (Gs) (**c**) in leaves of *Polygonum cuspidatum* seedlings. Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($p < 0.05$) differences. See Figure 1 for abbreviations.

3.4. Effect of Low Temperatures and S. indica on Proline Levels in Roots

Proline levels in roots were affected by both low-temperature treatment and *S. indica* inoculation, showing an increasing trend (Figure 4). The proline levels of inoculated and uninoculated seedlings were significantly increased at low temperatures versus moderate temperatures by 47.23% and 26.34%, respectively. *S. indica* inoculation also significantly increased proline levels in roots by 47.43% and 71.81% at suitable and low temperatures, respectively, as compared with the uninoculated treatment.

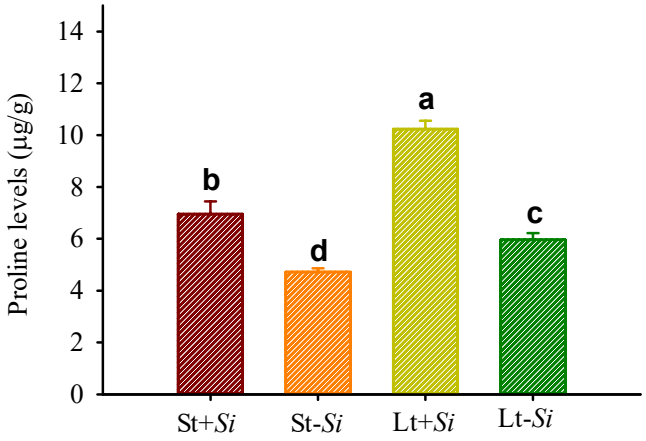

Figure 4. Effects of low temperatures and *Serendipita indica* on proline levels in the roots of *Polygonum cuspidatum* seedlings. Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($p < 0.05$) differences. See Figure 1 for abbreviations.

3.5. Effect of Low Temperatures and S. indica on Antioxidant Enzyme Activity in Roots

The low-temperature treatment significantly reduced SOD and POD activities in roots, showing a decrease of 20.52% and 16.89% in uninoculated seedlings and 8.20% and 23.16% in inoculated seedlings, respectively, compared to the suitable-temperature treatment (Figure 5). However, CAT activity in roots was significantly increased at low temperatures versus moderate temperatures, as evidenced by an increase of 21.53% and 25.01% in inoculated and uninoculated seedlings, respectively. At suitable temperatures, *S. indica* inoculation did not significantly alter SOD and POD activity in roots, but significantly increased CAT activity in roots by 13.69%, compared to the uninoculated treatment; at low temperatures, *S. indica* inoculation did not alter POD activity in roots, but significantly increased SOD and CAT activity in roots by 22.14% and 10.52%, respectively.

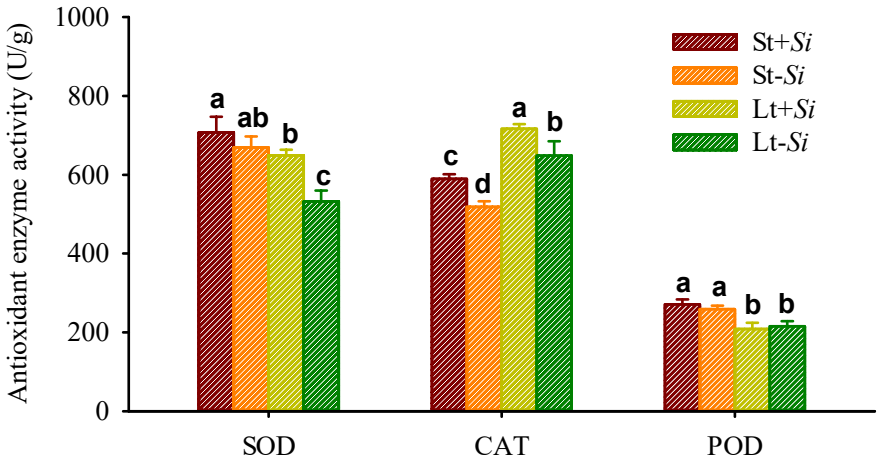

Figure 5. Effects of low temperatures and *Serendipita indica* on superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity in roots of *Polygonum cuspidatum* seedlings. Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($p < 0.05$) differences. See Figure 1 for abbreviations.

3.6. Changes in Medicinal Component Levels in Roots

The low-temperature treatment significantly decreased chrysophanol, emodin, polydatin, and resveratrol levels in roots by 45.03%, 13.19%, 20.13%, and 14.57%, respectively, in uninoculated seedlings, and by 42.04%, 16.21%, 17.76%, and 14.37%, respectively, in inoculated seedlings, as compared to the suitable-temperate treatment (Figure 6). Inoculation of *S. indica*, however, significantly increased medicinal component levels in roots at suitable and low temperatures: by 31.35% and 38.49% in chrysophanol, by 24.32% and 19.99% in emodin, by 22.64% and 26.28% in polydatin, and by 21.64% and 21.93% in resveratrol, respectively.

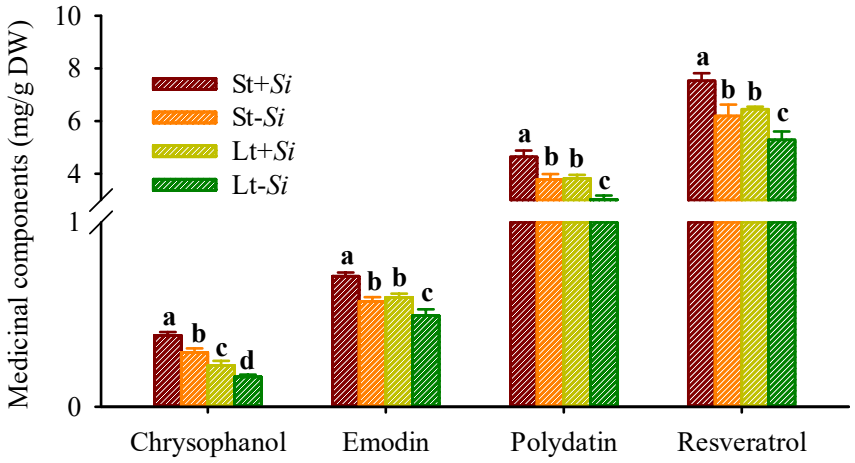

Figure 6. Effect of low temperatures and *Serendipita indica* on chrysophanol, emodin, polydatin, and resveratrol levels in roots of *Polygonum cuspidatum* seedlings. Data (means ± SD, n = 4) followed by different letters above the bars indicate significant ($p < 0.05$) differences. See Figure 1 for abbreviations.

3.7. Changes in PcRS Gene Expression in Roots

The low-temperature treatment significantly suppressed the expression of both *PcRS* and *PcRS11* genes by 0.25- and 0.36-fold in uninoculated seedlings and 0.36- and 0.51-fold in inoculated seedlings, respectively, compared to the suitable-temperature treatment (Figure 7a). After *S. indica* inoculation, the expression of *PcRS* and *PcRS11* genes was significantly increased by 0.70- and 0.43-fold, respectively, at suitable temperatures, and 3.66- and 1.78-fold, respectively, at low temperatures. In addition, the expression of *PcRS*

and *PcRS11* in roots was significantly ($p < 0.01$) positively correlated with both polydatin (Figure 7b) and resveratrol (Figure 7c) levels in roots.

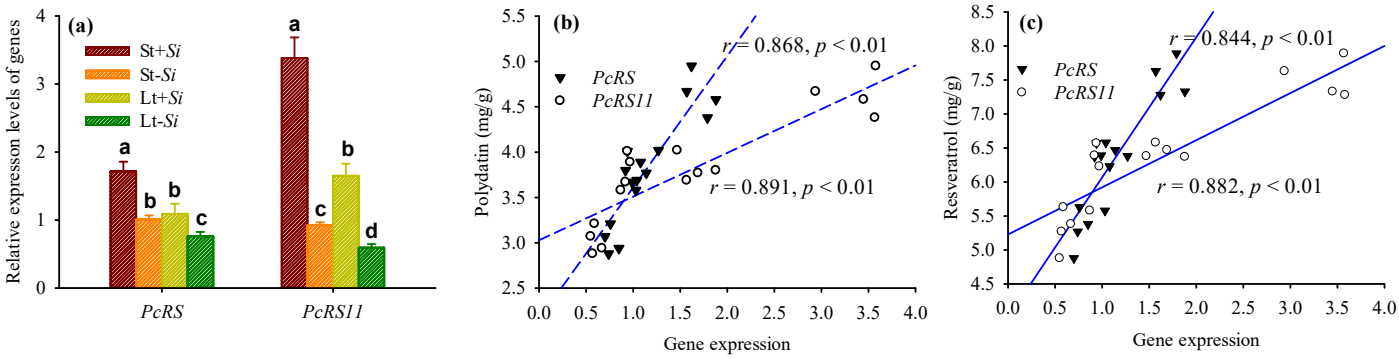

Figure 7. Changes in the expression of *resveratrol synthase* (*PcRS*) and *resveratrol-forming stilbene synthase 11* (*PcRS11*) genes in roots of *Polygonum cuspidatum* seedlings (**a**) and its correlation with root polydatin (**b**) and resveratrol levels (**c**). Data (means ± SD, $n = 4$) followed by different letters above the bars indicate significant ($p < 0.05$) differences. The r value was the Pearson's correlation coefficient. See Figure 1 for abbreviations.

4. Discussion

In the present study, the low-temperature treatment significantly inhibited the root colonization rate of *S. indica* in the roots of *P. cuspidatum* seedlings, suggesting that the colonization is temperature-dependent. This is consistent with the findings of Wu and Zou [23] in *Glomus mosseae*-colonized *Citrus tangerine* seedlings at low temperatures. At the same time, the low-temperature treatment also inhibited the biomass production of the shoots and roots of *P. cuspidatum*, because low-temperature stress reduced the photosynthetic capacity of the plants, resulting in the retarded growth and development of the plants [24]. Moreover, the trend of biomass inhibition by low temperatures was more pronounced in uninoculated seedlings than in inoculated seedlings, suggesting that the inoculated plants have a better tolerance of low temperatures than the uninoculated plants. The shoot and root biomass of *P. cuspidatum* was significantly boosted by inoculation with *S. indica*, and this enhancement was more pronounced at low temperatures than at suitable temperatures, implying that *S. indica* could mitigate the effects of low temperatures on the growth of *P. cuspidatum*. This is consistent with the results of Tian et al. [25] on cucumber inoculated with *Diversispora versiformis* under high-temperature stress and Murphy et al. [26] on barley inoculated with *S. indica* under low-temperature stress. The improvement in growth under *S. indica* colonization could be attributed to enhanced photosynthetic capacity, nutrient uptake, and auxin and cytokinin levels [27].

Usually, low temperatures limit respiration and photosynthesis in plants and thus lead to the slow growth and development of plants [28]. We also found that Pn, E, and Gs in the leaves of *P. cuspidatum* seedlings were suppressed after the low-temperature treatment. However, the inoculation of *S. indica* significantly mitigated the inhibitory effect of low temperatures on leaf gas exchange variables. Wang et al. [29] also reported that the inoculation of *S. indica* on trifoliate orange under soil drought conditions increased Pn and water use efficiency. Thus, *S. indica* promotes photosynthesis of the host at low temperatures, which leads to dry matter accumulation increased, thus improving the growth of the host.

Low temperatures usually accelerate the level of reactive oxygen species (ROS) in plants, leading to oxidative damage; however, plants have evolved an efficient antioxidant enzyme defense system to reduce ROS levels [30,31]. In the present study, the low-temperature treatment distinctly inhibited SOD and POD activity in roots but increased CAT activity in roots, indicating that CAT in *P. cuspidatum* seedlings can be activated by low temperatures to reduce ROS (e.g., hydrogen peroxide) levels. Also, *S. indica* inoculation

significantly increased SOD and CAT activity in roots at low temperatures, implying that inoculated plants were at a relatively lower level of ROS than uninoculated plants at low temperatures. This is consistent with earlier results of *S. indica* inoculation on drought-stressed trifoliate orange seedlings [32]. *S. indica* is known to activate the ascorbate-glutathione cycle as well as the expression of genes encoding antioxidant enzymes, thereby allowing the host to maintain a low level of oxidative damage under abiotic stress [20,32,33].

Earlier studies have shown that *S. indica* inoculation can alter the medicinal constituent levels of some plants [34]. For example, the inoculation of potted *Ocimum gratissimum* with *S. indica* reduced the levels of ursolic acid and oleanolic acid in leaves and stems [35]. *S. indica* colonization resulted in higher concentrations of artemisinin in *Artemisia annua* plants [36]. The present study showed that the level of resveratrol was significantly higher than that of polydatin in the roots of *P. cuspidatum* seedlings, which is consistent with the results reported by Li et al. [37]. In addition, the level of chrysophanol, emodin, polydatin, and resveratrol levels in the roots *P. cuspidatum* seedlings was significantly increased after *S. indica* inoculation, with the increased trend in polydatin and resveratrol levels being higher at lower temperatures than at suitable temperatures. This shows that the endophytic fungus *S. indica* promoted the content of the major medicinal constituents of *S. indica*, which was more prominent at low temperatures. Interestingly, Liu et al. [38] successfully isolated six strains of endophytic fungi from *P. cuspidatum* seedlings, all of which had the ability to convert resveratrol glycosides into resveratrol. The present study also showed that *S. indica* inoculation significantly up-regulated the expression levels of *PcRS* and *PcRS11* in roots. This is consistent with the results of Sun et al. [39] in which *P. cuspidatum* seedlings were subjected to arbuscular mycorrhizal fungi and additional P supply. Correlation analysis showed that the levels of polydatin and resveratrol were significantly and positively correlated with the expression of *PcRS* and *PcRS11* in roots. This confirms that *S. indica* inoculation accelerated polydatin and resveratrol levels by up-regulating the expression of *PcRS* and *PcRS11*. The resveratrol and flavonoids of *P. cuspidatum* are derived from the phenylpropanoid metabolic pathway [40]. How *S. indica* inoculation mediates the phenylpropanoid metabolic pathway and thus facilitates the production of medicinal constituents in *P. cuspidatum* remains to be studied.

5. Conclusions

The low-temperature treatment significantly inhibited the growth, physiological activity, and levels of the medicinal components of *P. cuspidatum* seedlings, whereas inoculation with *S. indica* significantly promoted plant biomass production, the antioxidant enzyme defense system, gas exchange, and the medicinal components in *P. cuspidatum* plants at low-temperature stress. As a result, *S. indica* can be used as an effective microbial stimulant to improve *P. cuspidatum*'s low-temperature tolerance and the content of the medicinal components. It is critical to clarify at which stage to inoculate *S. indica* in the field cultivation of *P. cuspidatum*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14010007/s1, Table S1: Primer sequences of the genes used in this study for qRT-PCR.

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